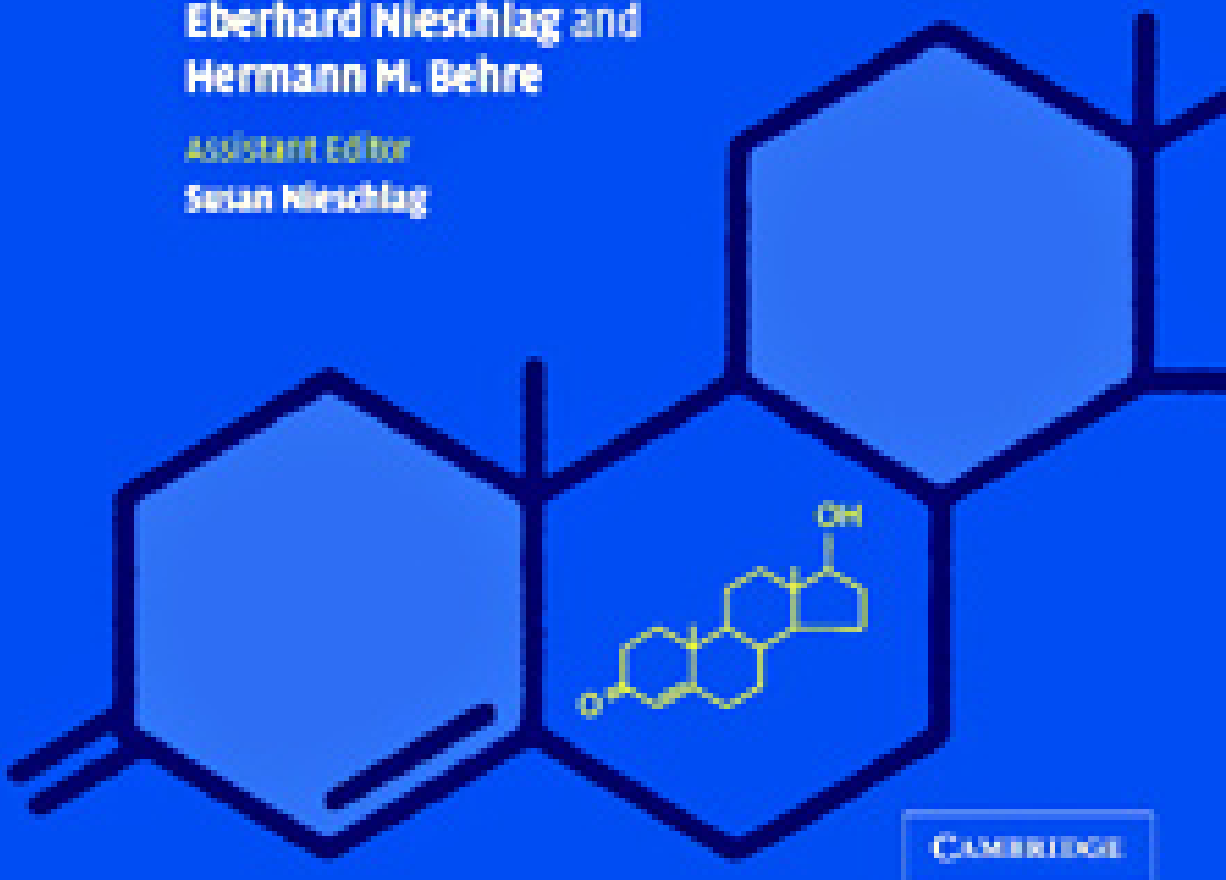
THIRD EDITION

Testosterone

Action · Deficiency · Substitution

Edited by
Eberhard Nieschlag and
Hermann M. Behre

Assistant Editor
Susan Nieschlag



CAMBRIDGE

CAMBRIDGE www.cambridge.org/9780521833806

This page intentionally left blank

Testosterone

Action, Deficiency, Substitution

Third edition

This book provides the most comprehensive and up-to-date source of information on testosterone and other androgens, and their role in human physiology and pathology. It covers biosynthesis and mechanisms of action and reviews their effects on brain and behaviour, spermatogenesis, hair growth, bones, muscles, erythropoiesis, the cardiovascular system and lipids, erection, and the prostate. Therapeutic uses of testosterone preparations are carefully evaluated, including use in women, the aging male, and its abuse and detection in sport. The book reviews applications in male contraception, the role of 5α-reductase inhibitors and the controversial use of DHEA.

For this book the editors have assembled the world leaders in testosterone research and clinical andrology and endocrinology. A special feature of the book is the fact that its 24 chapters were submitted simultaneously to ensure rapid publication. This revised and significantly expanded edition will serve as the standard source of reference for many years.

Testosterone

Action, Deficiency, Substitution

Third Edition

Edited by

E. Nieschlag
University of Münster

H. M. Behre
University of Halle

Assistant Editor
S. Nieschlag

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo

Cambridge University Press
The Edinburgh Building, Cambridge CB2 2RU, UK

Published in the United States of America by Cambridge University Press, New York

www.cambridge.org
Information on this title: www.cambridge.org/9780521833806

First published in print format 2004

ISBN-13 978-0-511-19467-2 eBook (EBL)
ISBN-10 0-511-19467-6 eBook (EBL)

ISBN-13 978-0-521-83380-6 hardback
ISBN-10 0-521-83380-9 hardback

Contents

Contributors

Allolio, Bruno
Department of Medicine
University of Würzburg
Josef-Schneider-Str. 2
97080 Würzburg, Germany

Arlt, Wiebke
DFG Senior Clinical Fellow
Division of Medical Sciences
University of Birmingham
Birmingham B15 2TH, United Kingdom

Artaza, Jorge
Division of Endocrinology, Metabolism and Molecular Medicine
Charles A. Drew University of Medicine and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Behre, Hermann M.
Andrology Unit
Department of Urology
University of Halle
Ernst-Grube-Strasse 40
06120 Halle, Germany

Bhasin, Shalender
Division of Endocrinology, Metabolism and Molecular Medicine
Charles A. Drew University of Medicine and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Burger, Natalie
Department of Obstetrics and Gynecology
University of Vermont
Burlington, Vermont, USA 05401

Bursi, Roberta
Department of Pharmacology
Male HRT/Contraception
N. V. Organon RE 2140.1
Molenstraat 110
5340 BH Oss, The Netherlands

Casson, Peter R.
Vermont Center for Reproductive Medicine
Women's Health Care Service FAHC
1 South Prospect Street
Burlington, VT 05401, USA

Cato, Andrew C. B.
Karlsruhe Research Center
Institute of Toxicology and Genetics
P. O. Box 3640
76021 Karlsruhe, Germany

Christiansen, Kerrin
Institute for Human Biology of the University
Martin-Luther-King-Platz 3
20146 Hamburg, Germany

Gonzalez-Cadavid, Nestor
Division of Endocrinology, Metabolism and Molecular Medicine
Charles A. Drew University of Medicine and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

De Gooyer, Marcel
Dept. of Pharmacology
Male HRT/Contraception
N. V. Organon RE 2140.1
Molenstraat 110
5340 BH Oss, The Netherlands

Gromoll, Jörg
Institute of Reproductive Medicine
University of Münster
Domagkstr. 11
48129 Münster, Germany

Grootenhuis, Arijan
Dept. of Pharmacology
Male HRT/Contraception
N. V. Organon RE 2140.1
Molenstraat 110
5340 BH Oss, The Netherlands

Handelsman, David J.
ANZAC Research Institute &
Dept. of Andrology, Concord Hospital
University of Sydney
Sydney NSW 2139, Australia

Hiort, Olaf
Department of Pediatric Endocrinology and Diabetology
Pediatric and Adolescent Medicine
Ratzeburger Allee 160
23538 Lübeck, Germany

Isaacs, John T.
Breast and Prostate Cancer Programs
The Sidney Kimmel Comprehensive Cancer
Center at Johns Hopkins
1650 Orleans St – CRB 1M40
Baltimore, MD 21231-1000, USA

Jasuja, Ravi
Division of Endocrinology, Metabolism and Molecular Medicine
Charles A. Drew University of Medicine and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Kamischke, Axel
Institute of Reproductive Medicine
University of Münster
Domagkstr. 11
48129 Münster, Germany

Kaufman, Jean Marc
Department of Endocrinology
Academish Ziekenhuis
De Pintelaan 185
9000 Gent, Belgium

Kaufman, Keith D.
Merck & Co., Inc.
P.O. Box 2000
Rahway NJ 07065-0900, USA

Klocker, Helmut
Department of Urology
University of Innsbruck
Anichstrasse 35
A-6020 Innsbruck, Austria

Leysen, Dirk
Department of Pharmacology
Male HRT/Contraception
N. V. Organon RE 2140.1
Molenstraat 110
5340 BH Oss, The Netherlands

Liu, Peter
Endocrine Research Unit, 5-194 Jo
Mayo Clinic
200 First St SW
Rochester, MN 55905, USA

Niehaus, Michael
Covance Laboratories
Kesselfeld 29
48163 Münster, Germany

Nieschlag, Eberhard
Institute of Reproductive Medicine
University of Münster
Domagkstr. 11
48129 Münster, Germany

Obendorf, Maik
Jenapharm GmbH & Co. KG
Preclinical Research
Department of Endocrinology and
Pharmacology
Otto-Schott-Str. 15
07745 Jena, Germany

Randall, Valerie Anne
Department of Biomedical Sciences
Richmond Building
University of Bradford
Richmond Road
Bradford BD7 1DP, United Kingdom

Rommerts, Focko F. G.
Department of Endocrinology and
Reproduction
Erasmus University Rotterdam
P.O. Box 1738
3000 DR Rotterdam, The Netherlands

Schänzer, Wilhelm
Institute of Biochemistry
German Sports University Köln
Carl-Diem-Weg 6
50933 Köln, Germany

Simoni, Manuela
Institute of Reproductive Medicine
University of Münster
Domagkstr. 11
48129 Münster, Germany

Singh, Atam B.
Division of Endocrinology, Metabolism
and Molecular Medicine
Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Singh, Rajar
Division of Endocrinology, Metabolism
and Molecular Medicine
Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Sinha-Hikim, Indrani
Division of Endocrinology, Metabolism
and Molecular Medicine
Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Storer, Thomas W.
Division of Endocrinology, Metabolism
and Molecular Medicine

Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Taylor, Wayne E.
Division of Endocrinology, Metabolism
and Molecular Medicine
Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

T'Sjoen, Guy
Department of Internal Medicine
Section Endocrinology
Ghent University Hospital, Belgium

Van der Louw, Jaap
Department of Pharmacology
Male HRT/Contraception
N. V. Organon RE 2140.1
Molenstraat 110
5340 BH Oss, The Netherlands

Vermeulen, Alex
Department of Internal Medicine
Section Endocrinology
Ghent University Hospital, Belgium

von Eckardstein, Arnold
Institute for Clinical Chemistry
University Hospital
Raemistr. 100
8091 Zürich, Switzerland

Wang, Christina C. L.
General Clinical Research Center
Harbor-UCLA Medical Center
1000 West Carson Street
Torrance, CA 90509, USA

Weinbauer, Gerhard F.
Covance Laboratories
Kesselfeld 29
48163 Münster, Germany

Wolf, Siegmund S.
Jenapharm GmbH & Co. KG
Preclinical Research
Dept. of Endocrinology and
Pharmacology
Otto-Schott-Str. 15
07745 Jena, Germany

Woodhouse, Linda
Division of Endocrinology, Metabolism
and Molecular Medicine
Charles A. Drew University of Medicine
and Science
1731 East 120th Street
Los Angeles, CA 90059, USA

Wu, Frederick C. W.
Central Manchester Healthcare NHS Trust
Department of Endocrinology
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL, United Kingdom

Zitzmann, Michael
Institute of Reproductive Medicine
University of Münster
Domagkstr. 11
48129 Münster, Germany

Preface

Testosterone, the hormone that turns males into men, has enjoyed continually growing interest among clinicians and scientists and has gained public attention over the past decades. This heightened interest is matched by a similarly increasing body of research and knowledge about male physiology and testosterone's biological and molecular action, about clinical symptoms and syndromes caused by testosterone deficiency and about the modalities for its use. The number of preparations available for clinical use has also multiplied impressively over recent years.

Proper diagnosis and treatment of testosterone deficiency require a profound understanding of the underlying science. The editors and authors have distilled current knowledge about action, deficiency and substitution of testosterone into this volume which follows two previous editions (1990 and 1998). Commensurate with the expanding knowledge about testosterone the current third edition has grown substantially in volume. Some previous chapters have been completely rewritten, some were combined and condensed in order to make room for new topics.

In order to synchronize the writing of the various chapters the editors and authors met at Castle Elmau in the splendid isolation of the Bavarian Alps at the end of September 2003 for final editing of the manuscripts previously submitted. This guaranteed that all chapters were concluded simultaneously and precisely reflect the current state of knowledge. This coordinated effort of all contributors ensures a long half-life of this book as an up-to-date reference source.

Peter Silver from Cambridge University Press encouraged this book project and we appreciate his help. We wish to thank the authors of the various chapters for their excellent compliance and timely submission of manuscripts. Our thanks go to Maria Schalkowski and Jasmin Oenning, our secretaries, who processed the manuscripts expediently. Futher thanks to Anne Olerink and Joachim Esselmann who dedicated their efforts to organizing a memorable meeting at Castle Elmau. Finally, the project and the meeting would not have been possible without the greatly appreciated financial support from Deutsche Forschungsgemeinschaft (DFG), Schering AG, Dr Kade / Besins Pharma GmbH, Dr August Wolff GmbH & Co., Ferring Arzneimittel GmbH, Jenapharm GmbH, and Organon GmbH.

1

Testosterone: an overview of biosynthesis, transport, metabolism and non-genomic actions

F.F.G. Rommerts

Contents

1.1 Introduction

Androgens in the male are essential for the development and maintenance of specific reproductive tissues such as testis, prostate, epididymis, seminal vesicles and penis, as well as other characteristic male properties such as increased muscle strength, hair growth, etc. (Mooradian *et al.* 1987). In order to maintain the androgen

concentration at appropriate levels, the production rates of androgens must be in balance with the metabolic clearance and excretion. The action of androgens in target cells depends on the amount of steroid which can penetrate into the cells, the extent of metabolic conversions within the cells, the interactions with the receptor proteins and finally, upon the action of the androgen receptors at the genomic level.

The biochemical aspects of production, metabolism, transport and action of androgens will be discussed in separate sections. Where possible, data obtained from human tissues will be emphasized. This chapter will deal with only the major and general aspects. A more extensive description of these topics and can be found in the book "The Leydig Cell" edited by Payne *et al.* (1996).

1.2 Biosynthetic pathways

1.2.1 General

In the human male, testosterone is the major circulating androgen. More than 95% is secreted by the testis, which produces approximately 6–7 mg per day (Coffey 1988). The metabolic steps required for the conversion of cholesterol into androgens take place in approximately 500 million Leydig cells that constitute only a few percent of the total testicular volume. Although Leydig cells are of major importance for the generation of circulating androgenic hormones, the adrenal cortex also contributes to this production. The production of steroids is not limited to endocrine glands but very small amounts, mainly pregnane derivatives, can also be produced in brain cells (Baulieu 1997). Although the contribution of cells in the nervous system to circulating hormones is very small, local production of steroids can be physiologically very important (King *et al.* 2002) especially when transport and clearance are low.

Since Leydig cells are most important for the production of androgens, the steroidogenic pathways in these cells will be described in some detail. The enzymes and intermediates involved in this reaction cascade are depicted in Figure 1.1. The pathways for biosynthesis of androgens and the regulation thereof have been reviewed extensively and the reader is referred to these reviews for detailed information (Rommerts and Brinkmann 1981; Ewing and Zirkin 1983; Rommerts and Cooke 1988; Hall 1988; Rommerts and van der Molen 1989; Saez 1989; 1994; Stocco and Clark 1996; Payne and O'Shaughnessy 1996).

The source for the synthesis of steroids is cholesterol. This substrate may be synthesized *de novo* from acetate but it may also be taken up from plasma lipoproteins. For human Leydig cells the LDL lipoprotein fraction seems to be the predominant extracellular store of cholesterol (Freeman and Rommerts 1996). In addition, intracellular lipid droplets which contain cholesterol esters may function as intracellular

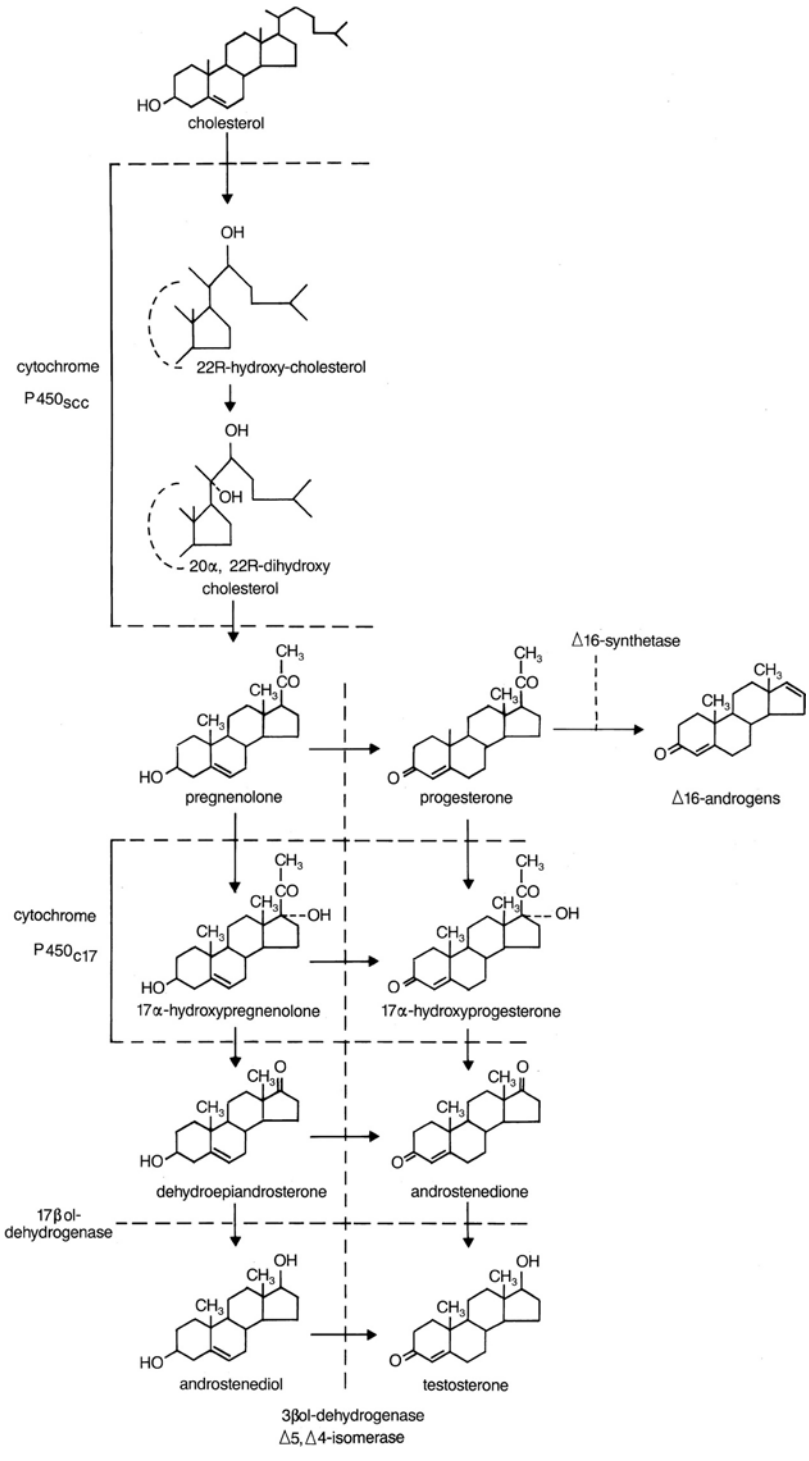


Fig. 1.1 Steroidogenic pathways in the human testis.

stores of cholesterol. The relative contribution of synthesis and cholesterol supply from lipoproteins or lipid droplets depends on the species and the extent of stimulation of steroid production. For high steroidogenic activity an ample supply of cholesterol is essential and sufficient hormone-sensitive lipase and enzyme activity for uptake of cholesterol (esters) must be present (Rao *et al.* 2003). For Leydig cells it appears that the cholesterol in the plasma membranes acts as the main and most readily available pool of cholesterol. A vesicle-mediated transport system involving an endosomal/lysosomal network seems to act as the conveyer belt for intracellular cholesterol transport to the mitochondria. The supply of cholesterol to the outer membrane of the mitochondria also requires transfer proteins and for this process sterol carrier protein$_2$ (SCP_2) could play an important role (van Noort *et al.* 1988). This protein could facilitate cholesterol trafficking inside the cell in conjunction with the cytoskeleton and the vesicular system, but although many suggestions have been made in this direction, there is still insufficient proof for this model. An important question in this respect is whether changes in intracellular cholesterol trafficking under the influence of LH are a consequence of utilization of cholesterol at the mitochondrial level followed by a re-equilibration process or whether LH actively directs cholesterol movement to the mitochondria.

Whatever mechanisms operate, the ultimate result of the coupled intracellular transport mechanisms is regulation of the availability of cholesterol at the level of the mitochondria for production of pregnenolone (C_{21}) from cholesterol (C_{27}). Cleavage of the side chain of cholesterol and the formation of pregnenolone inside the mitochondria is the start of the steroidogenic cascade. Subsequently, pregnenolone is converted to a variety of C_{19}-steroids by enzymes in the endoplasmic reticulum. The biosynthesis of the biologically active androgens is thus the result of a stepwise degradation of biologically inactive pregnenolone. This process is catalyzed by oxidative enzymes, many of which are members of a group of heme-containing proteins called cytochromes P450. As can be seen in Figure 1.1, the specific steroidogenic P450 enzymes can catalyse different although related reactions. The precise pathways which are utilized for the formation of testosterone most probably depend on the properties and amounts of the various enzymes as well as on the composition of the membrane into which these steroid-converting enzymes are integrated. Under normal conditions the total capacity of the pregnenolone-converting enzyme system in humans is insufficient to convert all available pregnenolone into testosterone. As a result many intermediates in the form of progesterone derivatives leak out of the Leydig cells. This illustrates that the rate-*limiting* step for the production of testosterone is localized at the level of the endoplasmic reticulum, whereas the rate-*determining* step for steroidogenesis (short term regulated by LH) is at the level of the cholesterol side chain cleavage activity in the mitochondria (van Haren *et al.* 1989).

1.2.2 Steroids other than testosterone

Some specific intermediates of the steroidogenic cascade are worth mentioning when testosterone substitution is practised. In the human, as well as in the pig, testicular pregnenolone and progesterone can also be oxidized to steroids other than testosterone, such as 16-androgens which can be further metabolised to androstenone (5α-androst-16-en-3-one) and androstenol (5α-androst-16-en-3β- or 3α-ol) in sweat glands (Weusten *et al.* 1987a). Although these steroids are not recognized as biologically active steroids in a classical sense, they clearly act as pheromones in pigs. Humans can also perceive these pheromones but there are less convincing data about the ultimate responses (Comfort 1971; Cowley and Brooksbank 1991). Another specific testicular metabolite derived from progesterone is 3α-hydroxy-4-pregnen-20-one, produced by immature Sertoli cells from rats. This steroid is reported to specifically suppress FSH secretion by the pituitary cells (Wiebe 1997). The biological effects of these metabolites of progesterone could be of interest since alternative receptors for these metabolites have recently been reported (see section 1.6). The formation and function of other testosterone metabolites such as 17β-oestradiol and 5α-dihydrotestosterone will be discussed later. Before describing the cascade of events regulated by luteinizing hormone (LH), more details about the steroidogenic reactions in the mitochondria and endoplasmic reticulum will be given.

1.2.3 Regulation of cholesterol side chain cleavage activity

The cholesterol side chain cleavage enzyme ($P450_{scc}$) responsible for the initiation of the steroidogenic process is located in the inner membrane of the mitochondria. This inner mitochondrial membrane contains small amounts of cholesterol. The availability of cholesterol in the inner membrane is thus at least one of the rate-limiting factors for the generation of pregnenolone from cholesterol. Other factors that are of importance are the amount of oxygen, the activity of the $P450_{scc}$ enzyme and the capacity for delivering reducing equivalents from NADPH to the $P450_{scc}$ via flavoproteins and iron containing proteins (see Figure 1.2). The capacity of the electron transport system appears not to be rate-limiting for steroid production since more than 10fold higher rates of pregnenolone production can be obtained in Leydig cells when, instead of cholesterol, the more soluble intermediate 22R-hydroxycholesterol is provided. The various steps in the side chain cleavage process i.e. the hydroxylations at C_{22} and C_{20} followed by cleavage of the bond between C_{20} and C_{22} are catalysed by one enzyme ($P450_{scc}$). The affinity of the enzyme for the intermediates and the conversion rates are high and no significant amounts of intermediates can be measured in mitochondria. Under normal physiological conditions the generation of steroids from cholesterol mainly depends on the supply of substrate (cholesterol) to the enzyme $P450_{scc}$ and on the amount of $P450_{scc}$ in

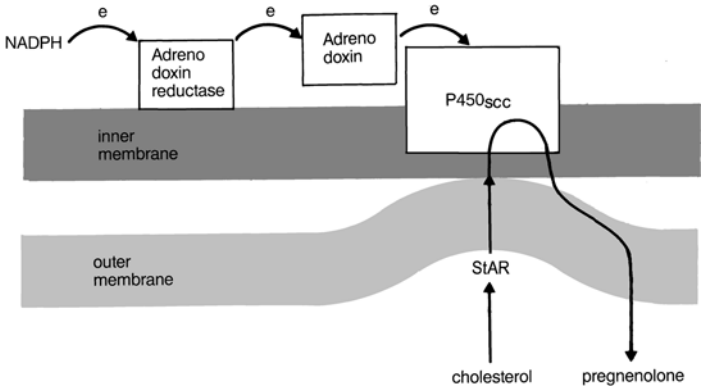


Fig. 1.2 The cholesterol side-chain cleavage system in mitochondria.

the mitochondria. The amount of the $P450_{scc}$ enzyme is clearly regulated by LH, especially at puberty, but after this induction period the enzyme expression is fairly constant. There are no indications that the $P450_{scc}$ protein can be regulated directly by hormone-dependent phosphorylation. During short-term regulation of steroidogenesis (when the amount of $P450_{scc}$ is constant), the rate-determining step is the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane that is deficient in cholesterol. For a long time it was known that hormone-controlled intra-mitochondrial cholesterol trafficking was dependent on the presence of one or more proteins with rapid turnover. Several years ago it was shown that the steroidogenesis activator protein (StAR) fulfils the criteria for this labile protein. This does not only hold true for Leydig cells, but applies to all cells in the adrenal and ovary that are active in hormone-dependent synthesis of steroids (Stocco 2001).

The transcription of the StAR gene during embryonal development is regulated by the transcription regulator SF-1, an orphan receptor, which also regulates the expression of the genes for the P450 enzymes (Rice *et al.* 1991; Clark *et al.* 1995; Parker and Schimmer 1997). It thus appears that all the essential elements for hormone-dependent steroidogenesis are regulated in a coordinated fashion. The rate of transcription of the StAR gene is controlled by hormones, but under normal conditions there is always enough messenger RNA to sustain a steady state production of a 37 kDa protein precursor. This 37 kDa protein can be transported to the mitochondria where it interacts with proteins on the outer mitochondrial membrane. As a result of transient interactions "contact sites" are formed between the outer and inner mitochondrial membrane. These bridges allow cholesterol transfer from the outer membrane to the inner membrane. Since StAR is continuously processed and inactivated, persistent synthesis and probably also hormone-dependent

phosphorylation of StAR are required to maintain hormone activated steroid production.

A very important observation in favour of the important role of StAR in the control of steroidogenesis came from studies on the disease lipoid congenital adrenal hyperplasia. This disease is characterised by an accumulation of cholesterol within Leydig cells and adrenal cells and an inability of the patients to synthesize enough steroids. It could be shown that mutations in the StAR gene which caused truncation and inactivation of the StAR protein were the cause of this disease (Stocco 2002). These clinical data further support the physiological importance of StAR for activated steroid production. However, it is known that limited amounts of steroids can also be produced in tissues without StAR, such as the placenta Although the production per cell is limited, together the many cells of the placenta can produce substantial amounts of steroids. Also Leydig cells in StAR knockout mice retain some capacity for androgen synthesis without the possibility for rapid hormonal regulation (Hasegawa *et al.* 2000). These observations indicate that other proteins, such as the peripheral type of the benzodiazepine receptor in the mitochondria, can also assist in cholesterol transport (Papadopoulos 1993). However, it is now firmly established that StAR is essential for the rapid regulation of steroid production.

1.2.4 Regulation of pregnenolone metabolism

The first product of the cholesterol side chain process, pregnenolone, which is biologically inactive, is further metabolised by enzymes present in the endoplasmic reticulum. Much has been learned about the primary structure and the biosynthesis of various P450 enzymes after application of new techniques such as protein chemistry and molecular biology (reviewed by Miller 1988). This can be illustrated for enzyme activities that convert C_{21}-pregnenolone to C_{19}-steroids. It was previously thought that 17-hydroxylase and $C_{17,20}$-lyase activity reside in separate enzymes which could be differentially regulated by hormones with predominant 17-hydroxylation in the adrenal and $C_{17,20}$-lyase activity in the testis (Smals *et al.* 1980; Rommerts and Brinkmann 1981). However, both enzyme activities occur in a single protein, $P450_{C17}$, coded by one gene CYP 17 (Zuber *et al.* 1986) and the differential expression of enzyme activities in the testis and the adrenal depends on the micro-environment of the enzyme in the endoplasmic reticulum. Mutations in the $P450_{C17}$ protein can also favour a particular enzyme activity (van den Akker *et al.* 2002). The formation of androgens in the testis is caused by relatively high reduction power owing to high levels of P450 reductase and cytochrome b_5 (Hall 1991). Protein phosphatase activities may play a role in the physiological regulation of the $P450_{C17}$ activity (Zhang *et al.* 1995; Pandey *et al.* 2003).

In the testis, the synthesis of $P450_{C17}$ is under the control of LH, via cAMP stimulation of CYP17 gene expression (Payne and O'Shauhgnessy 1996). This protein

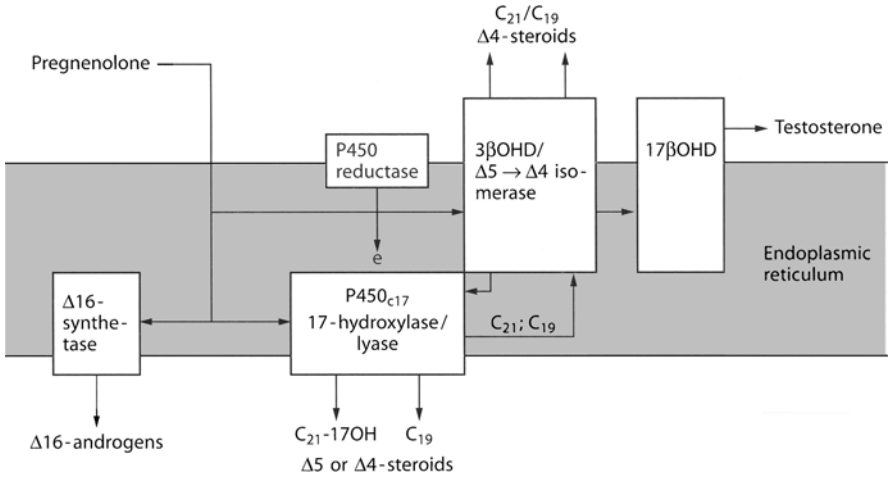


Fig. 1.3 Metabolism of pregnenolone in endoplasmic reticulum.

kinase A-mediated synthesis of $P450_{C17}$ can be inhibited by antimüllerian hormone (AMH) (Laurich *et al.* 2002). Deficiency of $P450_{C17}$ is rare but a few cases of individuals with female phenotypes have been reported (Monno *et al.* 1993; van den Akker *et al.* 2002). The degradation of the enzyme can be enhanced in the presence of elevated levels of steroids by oxygen-mediated damage. Although this steroid-mediated inactivation has been shown to occur in vitro, it is unknown whether this process plays a role in the regulation of enzyme activity in vivo at low oxygen tension (Payne *et al.* 1985).

The presence of many steroid-converting enzymes allows many different pathways to convert pregnenolone into testosterone (see Figure 1.1). Depending on whether pregnenolone is converted initially by the 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase complex or the $P450_{C17}$ enzyme, a Δ4- or Δ5-pathway predominates. In the human testis most of the steroids are formed via the Δ5-pathway with dehydroepiandrosterone (DHEA) as the first C_{19} intermediate (Weusten *et al.* 1987b) (see Figure 1.3). The enzyme 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3ßHSD) catalyses the conversion of Δ5-3β-hydroxysteroids to Δ4-3-ketosteroids, an essential step in the biosynthetic pathway. The dehydrogenase and isomerase activities are catalysed by one protein coded by one gene (Lachane *et al.* 1990). Although the two enzyme activities are carried out by one single protein, separate sites on the molecule mediate the specific enzyme activities (Luu-The *et al.* 1991). Different isoforms of this enzyme are expressed in steroidogenic tissues but also in non-steroidogenic tissues. In the human testis the type II iso-enzyme is expressed with almost equal affinity for dehydroepiandrosterone and pregnenolone. Several point mutations of the gene that affect

intracellular location and affinity for the substrate have been identified (Rhéaume *et al.* 1995).

The final step in the biosynthetic pathway of testosterone is the reduction of the 17-keto-group by the 17β-hydroxysteroid dehydrogenase (17βHSD). This enzyme activity is represented by five different isoforms that are ubiquitously present in many tissues (Andersson and Moghrabi 1997). An interesting feature of 17βHSD type 2 is that the enzyme also possesses 20αHSD activity. In the testis the type 3 isoform is present, mainly in the Leydig cells. Although generally present in the body, deficiency of the testicular activity of 17βHSD accounts for most defects in testosterone biosynthesis in the human (Geissler *et al.* 1994; Labrie *et al.* 1997). When all steroid-converting activities are taken together, the pregnenolone-converting enzymes present in the smooth endoplasmic reticulum function in close cooperation and act as a metabolic trap for pregnenolone, released by the mitochondria.

This enzyme system is not capable of converting all pregnenolone into testosterone. Therefore it acts as the rate-limiting step for the ultimate production of androgens. Since the enzyme activities are differentially regulated, they play an important role in determining the output of testosterone, especially during development. Thus the normal testis produces many intermediates in addition to testosterone. Although these steroids are not androgens, they represent secretion products and they may have alternative functions. This must be kept in mind when only testosterone substitution therapy is applied for treatment of hypogonadism.

1.3 Regulation of androgen synthesis by LH

1.3.1 General

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are required for the development and maintenance of testicular functions. LH is the most important hormone for control of Leydig cell functions, but other hormones and locally produced factors also play a role. Hormones regulate steroid production by controlling the metabolic activities in existing cells, but they also control the size of the Leydig cell population via control of proliferation and differentiation (Chemes 1996). In the foetal period around 14 weeks of gestation there is a sharp increase in the number and activity of Leydig cells. In this developmental period maternal hCG plays an important role for the regulation of Leydig cell activities. It is less clear what controls Leydig cell development before this period, but there is good evidence that early Leydig cell development and onset of steroid production take place without gonadotropin stimulation. During postnatal development of the human testis major changes occur in the Leydig cell population. In the early neonatal period gonadotropins stimulate the development and activity of foetal Leydig cells to such an extent that during the first three months of life peripheral and testicular levels of

testosterone are similar, as during puberty. In the next period of the first year these foetal Leydig cells regress via ill-defined mechanisms and a dormant phase remains until puberty. During puberty a second wave of proliferation and differentiation occurs under the influence of rising plasma LH levels. This ultimately leads to the adult population of Leydig cells (Chemes 1996; Saez 1994)

Many studies on the short-term effects of LH on steroid production have been carried out with isolated cells from rats or mice. These in vitro systems are less suitable for investigations on long-term or trophic effects of hormones, since isolated cells change their phenotypic properties after prolonged culture periods owing to the absence or abnormal composition of local growth factors. Tumour cell lines, such as the MA-10 mouse tumour cell line, are much more constant in their functional properties and are thus a better choice for these investigations of trophic effects. However, the regulatory systems in tumour cells and normal cells are not always the same.

Basically, regulation of Leydig cell function is the same as for other somatic cells. Via their receptors protein hormones and growth factors control phosphorylation of important cellular proteins, either via direct activation of kinases or indirectly via elevations in the level of intracellular second messengers. The covalently modified or newly synthesized proteins can then affect a variety of activities in discrete subcellular structures (plasma membrane, mitochondria, cytoskeleton, nuclei, etc.). As a result of a complex interplay between these intracellular activities, different physiological responses are generated (steroid production, energy production, cell growth, protein secretion, etc.). Since one hormone often stimulates multiple transducing systems (pleiotypic response) that may show different response kinetics, it is not easy to link specific transducing pathways to particular responses (see Figure 1.4).

1.3.2 Stimulatory actions of LH

LH is the most important hormone for regulation of Leydig cell number and functions. LH acts on Leydig cells via LH receptors of which the structure has been known since 1989 (McFarland *et al.* 1989; Loosfelt *et al.* 1989). Much about the importance of the LH receptor, its functional properties and LH-dependent receptor activation has been learned from studies dealing with receptor mutations (reviewed by Themmen and Huhtaniemi 2000; Ascoli *et al.* 2002). Activating mutations cause precocious puberty as a consequence of increased androgen production during the foetal and postnatal period, even in the absence of gonadotropic stimulation. The clinical manifestations depend on the severity of the LH receptor mutations. As can be expected, inactivating mutations of the LH receptor give rise to male pseudohermaphrodites caused by Leydig cell hypoplasia. Again, depending on the type of the mutation, receptors can be completely resistant or can still show a diminished

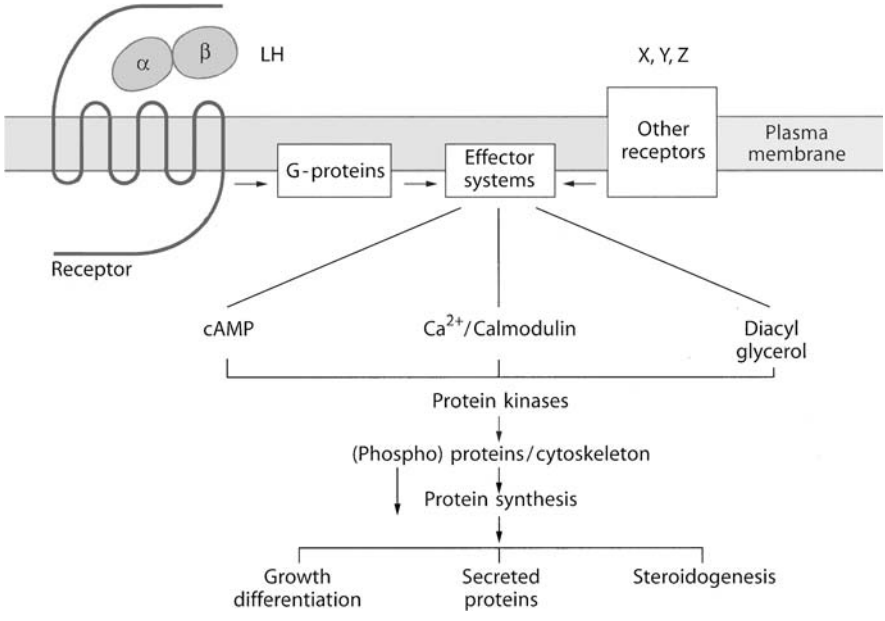


Fig. 1.4 Model for pleiotropic regulation of Leydig cell functions by LH and other local signalling molecules (X, Y, Z).

response. See Ascoli *et al.* (2002), for detailed information about structure-function relationships of the LH-receptor mutants. These observations indicate that the functional properties of the Leydig cells in these patients mainly depend on the gonadotropic stimulation of the (mutated) LH receptor and that paracrine systems within the testis cannot or can only partly compensate for the lack of LH receptor stimulation.

The natural ligand for the LH receptor is LH but also human choriogonadotropin (hCG) can equally well activate the LH receptor because both hormones show many structural similarities. hCG is isolated and purified from urine and this preparation has been used for decades in most basic and clinical studies on LH receptor stimulation. Today a variety of recombinant gonadotropins are available. Although hCG and LH are equipotent in stimulating the LH receptor, their binding properties are very different. The current notion of a strong and stabile binding as a prerequisite for LH receptor stimulation as derived from many studies with hCG, is not applicable to interactions between LH and the LH receptor, since tight binding of LH could not be demonstrated under normal physiological conditions (Combarnous *et al.* 1986). Similar findings have been made for interactions between FSH and the FSH receptor (van Loenen *et al.* 1994). More information on the actual mechanisms responsible for activation of gonadotropin receptors may be gained from recently developed low molecular weight agonists that are very specific in receptor

stimulation but that do not possess the large 3D surface of the 30 kD glycoproteins which are thought to be essential for receptor stimulation. Moreover. since these compounds are active after oral administration, in a clinical setting they are good candidates for replacing the recombinant hormones that are only active after injections (van Straten *et al.* 2002).

Activated LH receptors stimulate adenylyl cyclase via GTP binding proteins and this results in increased production of cyclic AMP, but other products may also be formed as a consequence of LH receptor activation (Rommerts and Cooke 1988; Saez 1994; Cooke 1996; Wang *et al.* 2000). Although cAMP can increase steroid production, there has been doubt as to whether cAMP is the only second messenger of the action of LH. Low concentrations of LH stimulate steroidogenesis without detectable changes in intracellular cAMP levels. Specific intercellular pools of cAMP have been postulated to explain these observations. Since rapid changes in intracellular calcium ion levels have been detected after administration of hormones (sometimes oscillations occur; Berridge and Galione 1988), calcium also appears to play an important role in signal transduction. For the Leydig cells calcium ions and calmodulin are also essential for full steroidogenic activities but it is less clear at which level calcium plays a role. Phospholipids, specific phospholipases and products of phospholipid metabolism such as arachidonic acid are of paramount importance in signal transduction of many types. These compounds have also been detected in rat Leydig cells and it has been shown that they are essential for the effects of LH on steroidogenesis (Wang *et al.* 2000). However, it is unknown to what extent these products are essential in human Leydig cells.

The activation of various signal transduction pathways in mouse or rat Leydig cells causes activation of different classes of protein kinases and kinase-linked pathways (Richards 2001). A major part of these kinase-linked pathways are directed to the nucleus where kinases or nuclear localized phosphoproteins can mediate the trophic effects of LH by regulation of gene expression. Transcription regulation of the steroidogenic enzymes has been investigated in great detail. The results of these studies show that the protein kinase A pathway is of predominant importance for controlling the promoter regions of most of these genes. However, only a limited number of cAMP responsive elements have been shown and regulation of transcription of steroidogenic enzymes clearly depends on a complex interaction between different transcription factors. Other phosphoproteins may be connected with the cytoskeleton that probably play a role in the intracellular transfer of cholesterol. As discussed previously, ongoing transcription of the StAR gene protein synthesis and protein phosphorylation is required for controlling the availability of cholesterol in the mitochondria via the protein StAR. This is considered the rapid control of steroidogenesis. Trophic control of steroidogenic activities in mitochondria and the smooth endoplasmic reticulum is mainly exerted by regulation of the biosynthesis

of the steroidogenic enzymes via increased levels of mRNAs. No activation of these enzymes by phosphorylation has been shown so far. Regulation of the amount of these proteins is a relatively slow process and it can take several hours before enzymatic activities change after stimulation with LH. The steroid-transforming activities of the enzymes in the endoplasmic reticulum are also affected by the levels of endogenous steroid precursors and endproducts and product inhibition has been shown to occur. Since the pattern of accumulated intermediates often depends on external conditions, it is difficult to make general conclusions on this regulatory aspect of steroidogenesis. For specific information the reader is referred to a review by Gower and Cooke, 1983. Although the most important Leydig cell products under the influence of LH are androgens, Leydig cells also produce protein products like IGF-1 and other growth factors. These products are mainly important for paracrine or autocrine regulatory events within the testis and will be discussed later.

1.3.3 Adaptation of Leydig cells

Most of the initial effects of LH or hCG on steroid production are stimulatory, as can be seen from the rapid increase of the testosterone concentration in plasma. In rats this response is much faster and more pronounced than in man (Huhtaniemi *et al.* 1983; Saez 1989). Although studies with isolated cells from human testis have shown that a small proportion of the human cells can respond more or less similarly to those in rats, it is unknown why the magnitude of the steroidogenic response of the intact human testis in vivo is small (Simpson *et al.* 1987). In both species the period of stimulated steroid production after hCG administration in vivo is followed by a period of diminished output of testosterone. This transient steroidogenic desensitization is the result of four different phenomena:

1) the coupling between the LH receptors and the adenylate cyclase is diminished, probably as a result of receptor phosphorylation or direct binding of arrestin (Hunzicker-Dunn *et al.* 2002);
2) the number of LH receptors is also decreased owing to an increased rate of receptor internalisation;
3) at the same time the mRNA level for the LH receptors has also decreased owing to a higher turnover of this mRNA pool;
4) the activities of the steroidogenic activities in the endoplasmic reticulum are lower.

In different species these LH-induced adaptive responses do not always occur to the same extent. This drop in androgen production, which occurs in rats 24–36 h after hCG administration, is accompanied by a rise in the secretion of 17αOH-progesterone, indicating a partial block at the level of C_{17-20}-lyase activity. In the same period the production of 17β-estradiol increases. Estrogens have therefore

been implicated as a causal factor in the development of steroidogenic lesions (Saez 1989). But other mechanisms are possible (Brinkmann *et al.* 1982). Following a period of diminished androgen production, plasma testosterone levels rise over the next period of 3–4 days, whereas 17α-hydroxyprogesterone levels diminish. This biphasic response of steroid production depends on the stage of development of the Leydig cells. In prepubertal children and in hypogonadal adult men, one injection of hCG induces a sustained rise in testosterone without significant changes in 17α-hydroxyprogesterone and 17ß-estradiol plasma levels. After long-term treatment with hCG, an adult pattern of response is observed in both groups (reviewed by Forest 1989). It therefore appears that the long-term steroidogenic response of Leydig cells depends on previous exposure to gonadotropins. After exposure of rat Leydig cells to high doses of gonadotropins, the degree of stimulation of adenylyl cyclase is greatly diminished within several hours, and after 24 h the number of LH receptors is diminished (Saez 1989). These phenomena have been described as desensitization and receptor down regulation, respectively, and they have often been used to explain the decreased production of androgens that develops after initial stimulation. However, as mentioned earlier, Leydig cells are still active in the production of steroids other than androgens. Moreover, the regulation of steroidogenic activities is complex and not all changes in cellular activities are synchronized in time. For instance, in Leydig cells from mature rats isolated 10 days after injections of hCG (administered at day 0 and day 7), LH receptors were down regulated and adenylate cyclase desensitized. However, LH-dependent androgen production was increased (Calvo *et al.* 1984). Similarly, in the period of low receptor number and reduced cyclic AMP response, LH-dependent prostaglandin production in the rat testis is very high (Haour *et al.* 1979) and Leydig cells show hypertrophy (Hodgson and de Kretser 1984). High doses of hCG that cause desensitisation of steroid production stimulate proliferation of Leydig cells (Teerds *et al.* 1988). Thus rodent Leydig cells do not always show a diminished responsiveness after exposure to gonadotropins. Especially when LH is released in a pulsatile fashion it is difficult to predict how the cells respond. In mice Leydig cells do not show any sign of desensitisation after exposure to a chaotic pattern of LH pulses (Coquelin and Desjardins 1982), whereas in rats the response depends on the profile of LH administration (Hakola *et al.* 1998). The steroidogenic response also depends on the age of the animal. Foetal Leydig cells do not show the desensitisation phenomena as described for mature animals (Huhtaniemi 1996). On the other hand, in Leydig cells from aged animals the initial signal transduction after LH stimulation appears to be diminished. These cells can produce normal amounts of androgens when stimulated at the level of cAMP (Chen *et al.* 2002). Long-term suppression of steroid production ("steroidogenic hibernation") may reduce this age-related decline in sensitivity to steroid production (Chen and Zirkin 1999).

Leydig cells can therefore adapt their activities to changes in the environment such as exposure to hCG. Depending on the species, developmental stage, the function of interest and the time interval after exposure to the gonadotropin, this adaptive process includes inhibitory changes (desensitisation), but also stimulatory actions.

1.4 Regulation of androgen synthesis by factors other than LH

1.4.1 Locally produced factors

Leydig cells in the testis are surrounded by other cells belonging either to the seminiferous tubules, such as Sertoli cells, or by cells in the interstitial tissue, such as macrophages. Many observations indicate that these neighbouring cells can potentially influence the function of Leydig cells in a paracrine fashion. FSH stimulates development of Leydig cells, probably via Sertoli cell products. Disturbances in the spermatogenic epithelium also affect Leydig cells. Moreover, conditioned media from Sertoli cells or seminiferous tubules can modify the steroidogenic activities of Leydig cells, suggesting the presence of many stimulatory and inhibitory components (reviewed by Sharpe 1993; Saez 1989 and 1994; Gnessi *et al.* 1997). Although the existence of paracrine regulating systems can be inferred from these data, in the literature there is almost no consistency in the various reports. In most studies the results appear to depend chiefly on the species used, the techniques applied for the isolation of cells or secretion products, cell culture conditions, etc. Even when one batch of secreted Sertoli cell products was used to regulate steroid production in one standardised Leydig cell preparation, it was found that the short-term effects of the Sertoli cell products were stimulatory, whereas the long-term effects were strongly inhibitory (van Haren *et al.* 1995). These long-term inhibitory effects of Sertoli cell products in vitro are in sharp contrast with the long-term stimulatory effects that Sertoli cells exert on Leydig cells in vivo. In a recent study using knock out mice, it was again shown that Sertoli cells are important, but the data also illustrated that the constitutive activity of the receptor is sufficient to stimulate this process. The presence of active FSH seems not to be required (Baker *et al.* 2003). In a limited number of studies specific (recombinant) growth factors have been used, but this has not resolved the existing confusion. Another question is whether specific products, which are active when added to isolated Leydig cells in a chemically defined medium, are also effective in vivo when they act together with many other local products. in vitro experiments have already demonstrated that the activity of a certain compound can be inhibitory or stimulatory, depending on previous exposure or permanent exposure to other signal molecules. The action of growth factors is thus context-dependent (Sporn and Roberts 1988) or in other words, it can be a part of a cellular signalling language with individual growth factors functioning as

the letters of the alphabet. In a similar fashion, as letters in the alphabet can form words with a particular meaning, a certain combination of growth factors may give a (more) useful message to the cell than the isolated growth factors. It will not be difficult to understand that this cellular language can become complex when many signal molecules are used.

There is now agreement that individual local secretion products can only be considered as potential paracrine factors if four criteria are fulfilled: 1) the molecule should regulate at least one biological activity of the target cell; 2) the molecule must be secreted in adequate quantities to guarantee a physiological response; 3) regulation of secretion of the molecule must be possible; 4) changes in the local concentration of the molecule should influence the properties of the target cell in vivo. Since it is almost impossible to fulfil all these criteria for one particular compound, this section on the physiological relevance of local regulation could be very short. However, since local regulatory systems are in general very important for specific cell function and because so much research effort has been made to understand these systems, a brief summary of the past and the present of paracrine regulation will be given before some conclusions are made.

The appreciation of local regulation and the shift from endocrine research to paracrine/autocrine research started approx. 25 years ago when it was shown that FSH could stimulate Leydig cells in hypophysectomized rats (Odell *et al.* 1973). A second wave occurred when LHRH or LHRH-like molecules became available and when direct effects on Leydig cells could be shown (Hsueh and Jones 1981). This period of initial great excitement was followed by a period of disappointment when it was not possible to show significant secretion of endogenous "LHRH-like" compounds by testicular cells. In the following years results became available from many studies using growth factors which are known to be produced in the testis such as IGF-1, TGFβ, EGF/TGFα, FGF, PDGF, inhibin/activin, interleukin, TNFα, etc. (reviewed by Saez 1994 and Gnessi *et al.* 1997). In more than hundreds of publications many inhibitory and stimulatory effects of these compounds could be shown on steroidogenesis in vitro. Again, much was speculated about the biological relevance of these findings for the regulation of Leydig cells in the testis. However, when the results of all these investigations are taken together, it is still not clear how important these molecules, alone or together, are for the regulation of Leydig cells in vivo. When using information obtained from in vitro experiments, to explain Leydig cell functioning *in situ*, two typical aspects of Leydig cells in vivo should not be forgotten. Firstly, Leydig cells in vivo are surrounded by an interstitial fluid that will average out specific paracrine influences of neighbouring cells. Secondly, the Leydig cells are a part of a closed feedback system with the brain and the pituitary as the sensor and regulator of LH secretion. Local testicular influences that result

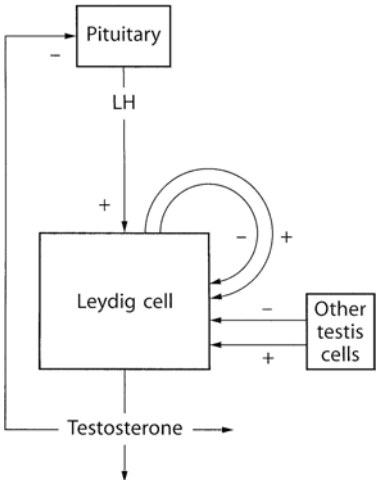


Fig. 1.5 Regulation of Leydig cell steroidogenesis by LH and locally produced factors.

in activation or inhibition of steroid production will therefore be compensated by alterations in LH secretion (see Figure 1.5).

The problems connected with understanding the physiological role of paracrine factors in general will be illustrated with the possible paracrine role of IGF-1 for Leydig cell steroidogenesis (for a review of the experimental findings related to this subject, see Lin 1996) because all the known properties make this molecule a most promising paracrine regulator. IGF-1 is produced locally in many tissues and in combination with the six different IGF binding proteins, the IFG/IGF-BP system has been proposed as a super-system for fine tuning of local hormone action. Since IGF-1, IGF binding proteins and specific proteases can be produced by the target cells themselves as well as by neighbouring cells, autocrine and paracrine regulatory systems can be integrated (Collett-Solberg and Cohen 1996). Many in vitro studies have shown that this IGF-1 system can also influence Leydig cells. IGF-1 can enhance the stimulatory effects of LH/hCG on Leydig cells through specific IGF-1 receptors and IGF-1 and also some IGF binding proteins can be produced by Leydig cells themselves. One could postulate therefore that part of the stimulatory action of LH on steroid production is mediated by an external and obligatory IGF-1 loop (production of IGF-1 by the Leydig cell and immediate stimulation of the IGF-1 receptors on the Leydig cell). Other testicular cells that can secrete compounds into the interstitial fluid can amplify or inhibit this external loop by either increasing IGF-1 concentration or by decreasing IGF-1 through binding to the specific binding proteins. Thus in theory the Leydig cell appears to be surrounded by a network of regulatory molecules with IGF-1 as co-stimulator and fine

tuner of LH and the binding proteins as fine-tuners of IGF-1 action. Many in vitro data support this model, but van Haren *et al.* 1992 could not show any effect of IGF-1 on the induction of cholesterol side chain cleavage P450 enzyme activity by LH in cultured Leydig cells. On the other hand, actions of IGF-II are obligatory for the FSH induction of aromatase in human follicles (Yan and Giudice 1999). The completely different effects of IGFs (no effect versus obligatory role) illustrate that in vitro investigations cannot answer the question on the physiological importance of the IGF system for induction of steroidogenic enzymes in gonadal cells. Transgenic animals with specific genes knocked out could shed new light on this problem. In this connection Baker *et al.* 1996 showed that mutant male mice with an inactive IGF-1 gene were reduced in size and were infertile. However, a close inspection showed that although the size of the testis was approx 40% of the normal and the total (not the free levels) peripheral testosterone levels were approx 20% of normal values, sperm cells of these mutant mice were able to fertilize wild type oocytes. Moreover, the infertility of the males was caused by the absence of mating behaviour. These results show that in the complete absence of the IGF-1 system indeed the normal growth of the animal is disturbed, but the data also show that the Leydig cells are still functional and that the testis, although reduced in size, can still produce active spermatozoa. It appears from this study as well as from many other studies using knock out animals, that the importance of a particular gene product (such as IGF-1) cannot be answered with a firm yes or no. It is very likely, as is the experience from many other studies with knock out animals for growth factors and regulating molecules, that the function of one defective gene can be compensated by other components of the external cellular regulatory network. It is becoming increasingly understood that physiological regulation of cell function is more than regulation of a simple linear process. Cell regulation involves many regulatory systems, and adaptive epigenetic networks and redundancy are now well known features of the cellular regulatory systems that operate (Strohman 1993). Although we know that a complex epigenetic regulatory network exists we are still far away from understanding this extensive signalling system (Dumont *et al.* 2001). Answering the question of the importance of the IGF-1 system for the regulation of steroid production in a normal organism is only possible when the amount of IGF-1 can be increased or decreased near the Leydig cells within the testis after normal development of the animal. This can be accomplished with animals in which genes in specific cell types can be manipulated conditionally (conditional knock out). Such experimental animals are now being investigated, but so far the focus has not been on Leydig cells.

Altogether, regulation of steroid production by LH not only involves complex interactions of many transcription factors for regulating gene expression in the nucleus and other regulatory pathways in the extra-nuclear compartment of the

cell, but a similar level of complexity of local regulating molecules exists outside the Leydig cell. Although this extra-cellular local regulatory system is complex, it seems to be very flexible and adaptive as shown by many gene knock out experiments. In contrast to the flexibility of local regulatory systems, LH receptor activation is always required for proper cell function. This has clearly been illustrated by the abnormalities caused by activating and inactivating mutations in the LH receptor gene, as discussed previously. LH thus appears to be high in the hierarchy of regulating molecules. This is in accordance with its role as an endocrine regulator. Another argument for a subordinate role of the paracrine system is that for regulation of steroidogenesis in testicular Leydig cells there are no reports that the local regulatory network can modify the properties of the Leydig cell to such an extent that maintenance of peripheral testosterone levels requires abnormally high or low levels of LH. An imbalance between LH and testosterone levels, however, can occur when genetic defects in steroidogenic enzymes or in the LH receptor cause insufficient production of testosterone. Under these conditions the feedback system tries to compensate for this deficiency with high levels of LH. In light of the many problems that still exist in our understanding of the paracrine control of rodent Leydig cells, the mechanisms involved in paracrine regulation of human Leydig cells remain totally obscure. On the other hand, when all the information discussed above is taken together, it is not difficult to conclude that the main regulator for the steroid production of the human Leydig cells is still LH. A recent clinical study on the effect of human recombinant FSH supports this view, because it was concluded that "Sertoli cell paracrine factors do not seem to play a major physiologic role in man when LH is active" (Young *et al.* 2000).

1.4.2 Other influences

Although LH appears to be the most important hormone, Leydig cells are also target cells for other hormones. In addition, the neural network must be considered. For a long time the importance of neuronal connections between the brain and the testis was neglected, but recent data show that the neural network can regulate the sensitivity of the Leydig cells towards LH (Csaba *et al.* 1998; Selvage and Rivier 2003). The relative importance of this neuronal network under normal physiological conditions is yet unknown, but since current research activities are more directed to the mechanisms of fine control, interactions between the neural and endocrine network must be the subject of future investigations.

Over the past years there has been a substantial number of publications on effects of thyroid hormones and glucocorticoids on Leydig cells. Thyroid hormone actions were mainly studied in connection with stimulation of Leydig cell activities, while most effects of glucocorticoid hormones were correlated with a decrease in androgen production.

Thyroid hormone accelerates the differentiation of Leydig cells (Ariyaratne *et al.* 2000) and also stimulates the StAR expression and steroid production in fully developed cells (Manna *et al.* 1999). Glucocorticoids inhibit steroidogenic enzymes and induce apoptosis in rat Leydig cells (Gao *et al.* 2002). The ultimate effects of glucocorticoids in vivo depend very much on local metabolism within the target cells. In this connection the activity of 11β-hydroxysteroid dehydrogenase as a local amplifier of glucocorticoid action is also of importance (Seckl and Walker 2001).

1.5 Transport of steroids

1.5.1 Trafficking inside cells

The formation of androgens from pregnenolone in the smooth endoplasmic reticulum of Leydig cells is the result of interactions between different membrane-bound enzymes with steroids as mobile elements. Within the smooth endoplasmic reticulum, to a great extent trafficking of the steroids is influenced by the affinities of the enzymes and binding properties of other membrane components. Apart from these binding entities, the movements of steroids in tissues appear to depend mostly on diffusion (reviewed by Mendel 1989). There are no reports that steroids are secreted like proteins that are released from vesicles after fusion with the cell membrane. Conjugated steroids, however, cannot easily pass cell membranes by diffusion and for this class of steroids specific transport systems are required (Mulder *et al.* 1973). Binding proteins for androgens or other steroids can play an important role in decreasing the concentration of unbound steroids outside the cell and so enhance the diffusion process, but there are no indications that such proteins are also important inside the cell. Another argument against an important role of specific transport systems for steroids in Leydig cells is the observation that secretion of pregnenolone that is normally very low, can become as high as secretion of testosterone when metabolism of pregnenolone to testosterone is inhibited (van Haren *et al.* 1989). Although diffusion is probably the main driving force for steroid trafficking within the cell, it has been shown that multiple-drug resistance proteins in the plasma membrane can increase the rate of transport of steroids over the plasma membrane (Ueda *et al.* 1992). If these transport proteins can also transport androgens and are localised in the Leydig cell, they could aid in the secretion of androgens. Alternatively these steroid transport proteins could alter the steady state concentrations of androgens in target cells. However, since the transport activities of these proteins have mainly been shown for corticosteroids and their derivatives (Gruol and Bourgeois 1997) the relevance of this transport system for androgens is unknown.

1.5.2 Trafficking between the testicular compartments

Steroids such as pregnenolone, progesterone and testosterone not only rapidly pass the Leydig cell membranes but they can also equilibrate rapidly between different testicular compartments (van Doorn *et al.* 1974). The secretion pattern in the testis is thus most likely determined by amounts that are produced inside the tissue, the permeability characteristics of the membranes and the binding proteins in various testicular fluids. The Leydig cells in the testis are surrounded by an interstitial fluid that is rich in plasma proteins and the cells are also in close contact with blood vessels. The preferential direction of secretion in the testis is mainly determined by the concentration gradient and flow rates of the various fluids. Since the blood flow is much higher than the flow of the interstitial fluid, most of the unconjugated steroids diffuse from the interstitial space to the blood and leave the testis via the venous blood (Maddocks and Sharpe 1989). The porcine testis is an exception that supports this view. In this species steroid sulphates, which cannot readily diffuse through the walls surrounding the interstitial space, were 13 to 35 fold more concentrated in interstitial fluid than in venous blood (Setchell *et al.* 1983). During the passage of venous blood through the pampiniform plexus the primary venous blood is diluted approximately 2 fold by incoming arterial blood. This occurs through anastomoses present in this network of interacting blood vessels (Noordhuizen-Stassen *et al.* 1985). The presence of relatively high levels of dihydrotestosterone in human spermatic venous blood has been taken as evidence that dihydrotestosterone is produced in the testis (Hammond *et al.* 1977). However, other studies have shown virtually no 5α-reductase in human testis (Miautani *et al.* 1977). Although these data are contradictory, dihydrotestosterone is most likely an epididymal steroid and it is conceivable that dihydrotestosterone produced in the epididymis is transported to testicular venous blood during the dilution process that occurs in the pampiniform plexus. Although dihydrotestosterone is not produced in the testis from testosterone, another derivative from testosterone, 17β-estradiol, is produced by Leydig cells. The testicular contribution to total estrogen production, however, is small (in the order of 20%) as compared to peripheral aromatisation. The local production of 17β-estradiol may be of great importance for regulating Leydig cell functions, for instance in the previously mentioned development of steroidogenic lesions.

1.5.3 Transport of androgens in the body

In the periphery steroids equilibrate rapidly between various organs and blood. This fact can be derived from identical levels of free testosterone in saliva and blood (Wang *et al.* 1981). The total concentrations of steroids in target tissues and body fluids is mainly dependent on the presence of binding proteins such as

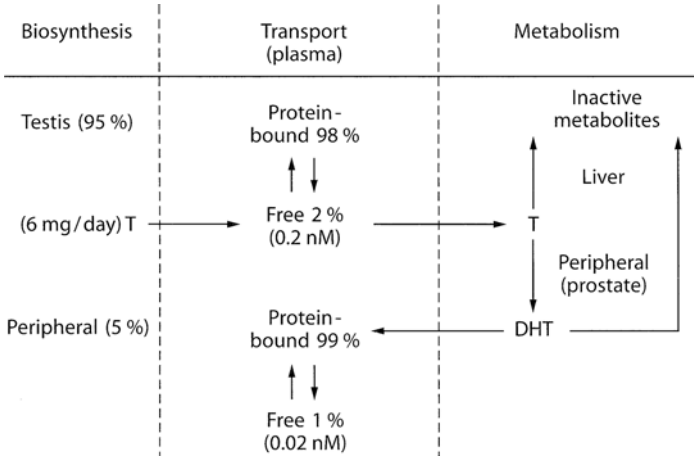


Fig. 1.6 Production, transport and metabolism of biologically active androgens.

sex hormone binding globulin (SHBG), and albumin (see Figure 1.6). Binding proteins in body fluids can act as a storage form for steroids that have a high rate of metabolism during passage of blood through the liver (Mendel 1989). In this way, extensive metabolism of active steroids can be inhibited. However, the presence of SHBG and albumin in body fluids is not essential for steroid homeostasis. This can be inferred from analbuminemic rats that possess neither SHBG nor albumin. In these rats, which are fertile, the total plasma concentration of testosterone is much closer to the free concentration in normal rats (Mendel *et al.* 1989). The free testosterone concentration is within the range of the affinity constant of the androgen receptor K_d 10^{-9}–10^{-10}M. Changes in the peripheral free testosterone concentration can therefore be directly sensed by the androgen receptor if there is equilibration between the exterior and interior of androgen target cells. It is not easy to envisage how steroids such as dihydrotestosterone and 17β-estradiol which can be present in picomolar concentrations (free) can be biologically active when the affinity constants for the receptors are in the (sub)nanomolar range. However, it has become clear that steroid receptors can also be (partly) activated, independent of steroids, by phosphorylation. It is not impossible that such "predisposed receptors" can be activated further by binding very small amounts of steroid (O'Malley *et al.* 1995). In some cases local production can furnish these active steroids, as for instance dihydrotestosterone in the prostate (Coffey 1988) and 17β-estradiol in the brain (Michael *et al.* 1986). If the capacity of such local production is sufficiently high, this could explain the apparent discrepancy between receptor affinity and the plasma availability. Alternatively, specific transport systems for steroids into or out of target cells are a possibility, as discussed previously for corticosteroids and their derivatives. Although such transport systems have not been shown to affect the

distribution of natural androgens, this may not hold true for synthetic androgenic compounds.

1.6 Metabolism of testosterone

The steady state level of biologically active steroids in the body as a whole is determined by the rate of synthesis and the rate of degradation. To maintain a steady state concentration of active steroids in a target cell a similar balance between the supply and removal must be maintained. The supply side of the balance is determined by the rate of inward transport of active steroid, sometimes in combination with activation through metabolism of the precursor. Similarly, factors that control removal are the rate of outward transport and the rate of degradation. A network of different factors contributes to the control of the level of a particular steroid in the target cell. Outside the target cell: flow rate of biological fluid (blood or lymph), release from binding proteins, transport through membranes, connective tissue, cell layers, in which sometimes inactivation of steroid can occur during transport. Inside the target cell: local activation or inactivation reactions and outward transport. Alterations in the rate of degradation of androgens induced by disease, ageing, treatment with drugs etc. are therefore as important as changes in the rate of the testicular synthesis. As an example, it could be possible that altered androgen metabolism during ageing may be responsible for a local hyperandrogenic state in the prostate leading to benign prostatic hyperplasia (Ishimaru *et al.* 1977).

There are several possibilities for the metabolism of testosterone (see Figure 1.7). Aromatization or reduction of the $\Delta 4$ bond of testosterone give rise to 17β-estradiol and 5α-dihydrotestosterone, respectively. These steroids have completely different biological activities since they interact with discrete receptors in the cell. Actions of testosterone on target tissues are therefore significantly modulated by metabolic reactions. When a target cell is estrogen-dependent, the aromatase activity in target cells and the supply of androgen substrate are of major importance for determining the rate of synthesis of estrogens. In humans the aromatase cytochrome P450 enzyme (p450arom or CYP19) is encoded by a single gene. This gene is expressed in many tissues including the placenta, ovary, testis, fat tissue, liver, brain, hair, follicles and the brain. A very few cases of complete aromatase deficiency due to a gene defect have been noted (Morishima *et al.* 1995; Bulun 1996). The activity of 17βHSD, especially the type 2 isoform that favours oxidative reactions, determines how much of the active oestradiol is converted to the biological inactive estron (Andersson and Moghrabi 1997). The importance of estrogens in males is reviewed by De Ronde *et al.* (2003).

For proper action of androgens it is sometimes necessary to convert testosterone into 5α-dihydrotestosterone before it can fully activate the androgen receptor. Two

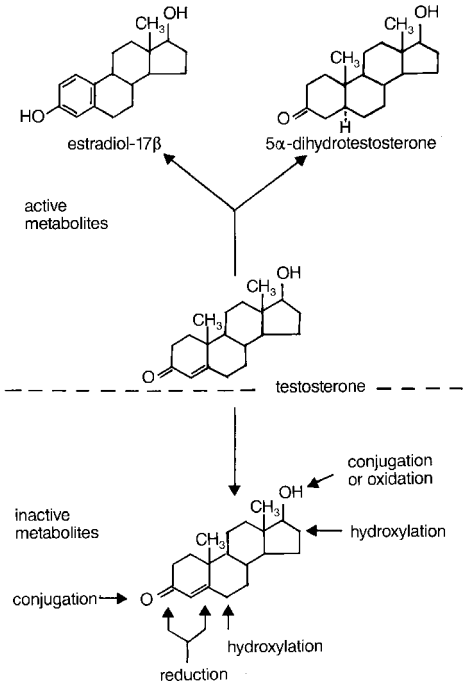


Fig. 1.7 Various possibilities for metabolism of testosterone.

isoforms of 5α-reductase exist and isoform 2 is most important because deficiencies of reductase type 2 are correlated with abnormal clinical manifestations (Wilson *et al.* 1993). To establish a critical steady state concentration of DHT, not only the activity of the 5α reductase must be high enough, but also the metabolism of DHT must be low. In the prostate of the dog the activity of the reductive 3α/3β- steroid dehydrogenase activities are low, and this favours the formation of DHT. The low rate of metabolism through the 3α/3β- dehydrogenase pathway may be the consequence of a low expression of one or both of these enzymes in the prostate, but it may also be possible that within one cell there is a balance between oxidative and reductive actions of two different iso-enzymes. Support for this hypothesis is a report showing that rat and human prostate contain an oxidative 3α-hydroxysteroid dehydrogenase that can convert 5α-androstane-diol back to dihydrotestosterone (Biswas and Russell 1997). This could explain why 5α-androstane-3α, 17β-diol is a more potent androgen for maintaining epididymis function than dihydrotestosterone or testosterone (Lubicz-Nawrocki 1973). Prostate tissue also contains the type 2, 17β hydroxysteroid dehydrogenase that is primarily oxidative in nature. This enzyme does not metabolise DHT but it does convert testosterone in androstenedione, especially when 5α reductase is inhibited (George 1997). In muscle there is a high

activity of 3αHSD and a low 5α-reductase (Luke and Coffey 1994). This combination of enzymes seems to operate to optimise the amount of testosterone for testosterone-dependent receptor stimulation in this cell type. In other target cells such as the skin and the hair follicle, the level of DHT, as the most active ligand, can depend on the supply of testosterone and conversion to DHT on one hand, balanced by the catabolism of DHT via reducing 3α/3β- steroid dehydrogenases and glucuronidation on the other hand (Rittmaster 1994). Oxidizing activities of 3α-steroid dehydrogenases may, however, offset this inactivation of DHT (Penning 1997). Thus the pattern of active and inactive androgen metabolites depends on a network of steroid-metabolising enzyme activities. Owing to these local conversions, the peripheral plasma concentrations of androgens are only a rough indicator for their biological activities. It has already been known for many years how androgen action in certain tissues can be amplified by enzymes that favour DHT formation. Much less is known about the regulation of these enzymes under physiological conditions. For instance, how does ageing affect the true levels of active androgens within the target cells? It is known that in the kidney, cortisol can be completely inactivated by oxidative actions of 11β-hydroxysteroid dehydrogenase in cell layers that surround the target cells for aldosterone (White *et al.* 1997). Thus a "metabolic shield" protects the receptors for mineralocorticoids in the target cells from unwanted actions of glucocorticoids. The reverse is also possible; local amplification of glucocorticoid action can occur by reducing inactive cortisone to cortisol. This can occur in liver, fat cells and in the brain and has enormous implications for the slow development of diseases such as diabetes type 2 (reviewed by Seckl and Walker 2001). Altogether these new observations have stimulated investigators to study further the details of corticosteroid metabolism. Although a "metabolic shield" for protecting cells from actions of androgens has not been shown, the physiological implications of interacting androgen metabolising enzymes requires more attention. Over the past years we have learned a lot from knock out studies or from over-expression of enzyme activities in tissues and cells. Now it is time to study the detailed interactions between the natural enzyme activities under different physiological conditions, for instance during ageing.

Although the balance of specific "activating" and "inactivating" steroid conversions is of great importance for the manipulation of the androgen response of the target cells, they are less important for the overall degradation and clearance of androgens. The pathway for degradation of androgens in various tissues is determined by the profile of enzymes involved in the inactivation process. Enzymes that are active in degrading androgens are 5α- and 5β-steroid reductases, 17β-hydroxysteroid dehydrogenase and 3α- and 3β-hydroxysteroid dehydrogenases. In addition to these enzymes that convert existing functional groups, androgens can also be hydroxylated at the 6, 7, 15 or 16 positions (Träger 1977). Most of these

androgen metabolites are intrinsically inactive. However, some steroids such as 5α-androstanediol can be "reconverted" to dihydrotestosterone and these steroids may therefore be considered as potentially active androgens. The androgenic effects will depend on the degree of metabolism. The 5β-androgenic metabolites are a special group of compounds that stimulate the production of heme in bone marrow and liver (Besa and Bullock 1981). These biological effects are not mediated by the classical androgen receptor. Thus steroid metabolites that cannot bind to nuclear steroid receptors can still express biological activity (see also next section). Metabolism or even catabolism should therefore not always be considered as an inactivating pathway, preparing a steroid for excretion. Androsterone (3α-hydroxy-5α-androstane-17-one) and etiocholanolone (3α-hydroxy-5β-androstane-17-one) are the most abundant urinary androgen metabolites. Some androgen metabolites are excreted as free steroids, whereas others are conjugated. These conjugated steroids carry a charged group such as a sulphate or a glucuronide group on the 3- or 17-position. Dehydroepiandrosterone-sulphate is a well-known example of a conjugated steroid which is produced by the adrenal cortex and that is present in the circulation at micromolar levels without a clear physiological function. In adult men, glucuronides of the 5β-androstane compounds are most abundant. The majority of the catabolic reactions take place in the liver but the prostate and the skin also contribute significantly to the metabolism of androgens. All the steroid-metabolising enzymes together constitute a network for transforming androgens into secretion products that finally leave the body via the urine or the skin. The flux through this network is great because the overall halflife of testosterone in men is only 12 minutes. It is clear that to maintain of a constant level of testosterone in the body, this breakdown must be balanced by a continuous supply from the testis.

1.7 Non-genomic effects of androgens

Most androgenic hormone action is thought to go through direct activation of DNA transcription via high affinity interactions with the androgen receptor. Information on the physiology and pathophysiology of these receptor actions will be given in the next chapters. In this section, complementary non-genomic effects of androgens will be discussed shortly.

In recent years, a variety of rapid "non-genomic" effects of sex steroids has been documented for these "nuclear-oriented" ligands (reviewed by Simoncini and Genazzani 2003). Androgens can also activate transcription-independent signalling pathways (Heinlein and Chang 2002). Rapid effects of androgens have been shown on calcium fluxes (Guo *et al.* 2002) and on intracellular phosphorylation cascades such as the Map-kinase pathway (Castoria *et al.*, 2003). Membrane effects

of androgens have also been implicated in functional responses such as rapid secretion of the prostate specific antigen (PSA) by prostatic cells (Papakonstanti *et al.* 2003) and the secretion of GnRH by pituitary cells (Shakil *et al.* 2002). In NIH 3T3 cells DNA synthesis is triggered after association between the androgen receptor and the membrane components has occurred under the influence of nanomolar concentrations of androgens. It appears that in these cells the very low density of androgen receptors is not sufficient to stimulate gene transcription (Castoria *et al.* 2003). Androgen-stimulated gene transcription only occurs when the intracellular receptor concentration is elevated. The membranous effects of low concentrations of "nuclear oriented" receptors could represent a more general mode for steroid action in general. More investigations in this direction are required.

Not all the membrane effects of androgens (and other sex steroids) are mediated by the classical receptor. There are several good indications that other steroid-binding proteins localised in the plasma membrane are essential for signal transduction, but for many years the structure of these proteins could not be elucidated and therefore it was not popular to study this subject. Recently an alternative receptor for membrane effects of progestins has been cloned (Zhu *et al.* 2003a). The protein has seven transmembrane domains and has similarities with G protein-coupled receptors. Hybridisation analyses have revealed that many mRNAs are present in a variety of human tissues (Zhu *et al.* 2003b). Although a similar protein has not been identified for androgens, it is known that humans can smell very small amounts of androstenone (16 ene-5α-androsten-3-one) as a volatile compound. Since only a very few isomers (but not testosterone) can be detected by the olfactory system, it is very likely that the smell is triggered by specific membrane receptors for androstenone in the olfactory sensory neurons (Snyder *et al.* 1988). It is known that all olfactory receptors are classical G protein-coupled proteins and since the alternative membrane receptor for progestins is homologous with G coupled-receptors, it is not unlikely that alternative androgen receptors in the olfactory system have a similar structure.

Recently, effects of testosterone on calcium mobility through cell membranes of T cells were reported (Benten *et al.* 1997). Since T cells do not possess the classical androgen receptors, this biological response also indicates the involvement of unconventional plasma membrane receptors for the expression of these androgen effects. Another example of the involvement of alternative androgen receptors can be found in eels. In eels nanomolar concentrations of 11-ketotestosterone, for which no nuclear receptor has been found, are essential for maintaining spermatogenesis in vitro. Under these conditions high concentrations of testosterone or dihydrotestosterone were inactive (Miura *et al.* 1991).

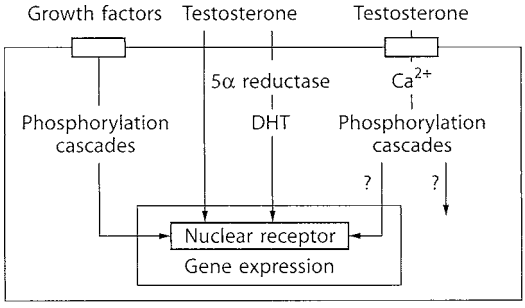


Fig. 1.8 Genomic and non-genomic actions of testosterone.

The dependence of spermatogenesis on high levels of testosterone can not be explained by properties of the classical nuclear receptor. Since the levels of testosterone required for maintaining normal spermatogenesis are much higher than the saturation level of the high-affinity androgen receptor, an alternative sensing system with a lower affinity has been postulated to operate (Rommerts 1988 and 1992) and later identified (Lyng *et al.* 2000). In this connection it is striking to note that the alternative membrane receptor for progestins mentioned earlier also has a 10 fold lower affinity than the classical progesterone receptor.

So far the non-genomic effects of steroids have received much less attention than the genomic effects. Increasing evidence collected in the last 5 years strongly suggests that the nucleus may not be the prime target for steroid actions. More and more we discover that biological systems in general are fine-regulated by networks of molecules and each day new signalling pathways and connections between these pathways are revealed. The importance of external regulatory networks for the outcome of steroid hormone action in the nucleus was stressed by O'Malley *et al.* (1995) when he proposed that membrane transduction pathways, activated by growth factors that interact with the nuclear receptor via intracellular phosphorylation cascades may "set the nuclear receptor thermostat" for proper responses to steroids. In a similar fashion the signal transduction pathways activated by the membrane effects of steroids could also influence its genomic actions, indicating that nuclear and membrane effects of steroids are probably more closely linked than previously thought (see Fig. 1.8).

1.8 Key messages

- Steroidogenesis is the cleavage of the carbon chain of cholesterol inside the mitochondria with formation of the biologically inactive steroid pregnenolone as endproduct. Specific enzymes in the endoplasmic reticulum of the Leydig cells form a metabolic network to catalyse the transformation of pregnenolone into biologically active testosterone.

- LH regulates the transport of cholesterol to the inside of the mitochondria (short-term regulation of steroidogenesis) as well as the profile and activities of the pregnenolone-metabolising enzymes (long-term regulation of steroidogenesis).
- The physiological role of specific paracrine factors for regulation of Leydig cell steroidogenesis is less clear than the role of LH.
- For extracellular and intracellular transport of cholesterol, specific transport systems are required. In contrast, steroids diffuse through tissues without specific transport systems and, as a result, all cells in the body "see" roughly the same concentration of unbound testosterone.
- In target cells transformations of testosterone into more active ligands can take place when the rate of inactivation is low.
- The response of target cells depends on the occupancy of the receptors that in turn depends on the intracellular concentration of unbound active androgens.
- In addition to genomic effects of androgens through the nuclear receptor, androgens can also stimulate non-genomic effects through interactions with receptors in the cell membrane.
- Disturbances in the biosynthesis of androgens through enzyme defects or in the actions of androgen are often the cause of abnormal sexual differentiation.

1.9 REFERENCES

Andersson S, Moghrabi N (1997) Physiology and molecular genetics of 17β- hydroxysteroid dehydrogenases. Steroids 62:143–147

Ariyaratne HBS, Mills N, Mason JI, Mendis-Handagama SMLC (2000) Effects of thyroid hormone on leydig cell regeneration in the adult rat following ethane dimethane sulphonate treatment. Biol Reprod 63:1115–1123

Ascoli M, Fanelli F, Segaloff DL (2002) The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev 23:141–174

Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR, Efstratiadis A (1996) Effects of an IGF-1 gene null mutation on mouse reproduction. Molec Endocr 10:903–918

Baker PJ, Pakarinen P, Hutaniemi IT, Abel MH, Charlton HM, Kumar TR, Shaughnessy PJ (2003). Failure of normal Leydig cell development in follicle-stimulating hormone (FSH) receptor-deficient mice, but not FSHbeta-deficient mice: role for constitutive FSH receptor activity. Endocrinology 144:138–145

Baulieu EE (1997) Neurosteroids: of the nervous system, by the nervous system, for the nervous system. In: Rec Prog Horm Res 52: pp 1–32

Benten WPM, Lieberherr, M, Sekeris CE, Wunderlich F (1997) Testosterone induces Ca^{2+} influx via non-genomic surface receptors in activated T cells. FEBS Letters 407:211–214

Berridge MJ, Galione A (1988) Cytosolic calcium oscillators. Fed Proc Am Soc Exp Biol 2: 3074–3082

Besa CE, Bullock LP (1981) The role of the androgen receptor in erythropoiesis. Endocrinology 109:1983–1989

Biswas MG, Russell DW (1997) Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate. J Biol Chem 272:15959–15966

Brinkmann AO, Leemborg FG, Rommerts FFG, van der Molen HJ (1982) Translocation of the testicular oestradiol receptor is not an obligatory step in the gonadotropin-induced inhibition of C17–20-lyase. Endocrinology 110:1834–1836

Bulun SE (1996) Aromatase deficiency in women and men: would you have predicted the phenotypes? J Clin Endocr Metab 81:867–871

Calvo JC, Radicella JP, Pignataro OP, Charreau EH (1984) Effect of a second injection of human chorionic gonadotrophin on the desensitized Leydig cells. Mol Cell Endocr 34:31–38

Castoria G, Lombardi M, Barone MV, Bilancio A, Domenico MD, Bottero D, Vitale F, Migliacchio A, Aurricchio F (2003) Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblast by a nontranscriptional receptor action. J Cell Biol 161:547–556

Chemes HE (1996) Leydig cell development in humans. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig cell. Cache River Press, Vienna, Il pp 176–202

Chen HL, Zirkin BR (1999) Long term suppression of Leydig cell steroidogenesis prevents Leydig cell aging. Proc Natl Acad Sci USA 96:14877–14881

Chen HL, Hardy MP, Zirkin BR (2002) Age related decreases in Leydig cell testosterone production are not restored by exposure to LH in vitro. Endocrinology 143:1637–1642

Clark BJ, Soo SC, Caron KM, Ikeda Y, Parker KL, Stocco DM (1995) Hormonal and developmental regulation of the steroidogenic acute regulatory (StAR) protein. Mol Endocrinol 9:1346–1355

Coffey DS (1988) Androgen action and the sex accessory tissues. In: Knobil E, Neill (eds) The physiology of reproduction. J. Raven Press, New York, pp 1081–1119

Collett-Solberg PF, Cohen P (1996) The role of the insulin-like growth factor binding proteins and the IGFBP proteases in modulation IGF action. Endocrinol Metab Clin North America 25:591–614

Combarnous Y, Guillou F, Martinat N (1986) Functional states of the luteinizing hormone/choriogonadotropin-receptor complex in rat Leydig cells. J Biol Chem 261:6868–71

Comfort A (1971) Likelihood of human pheromones. Nature 230:432–433

Cooke BA (1996) Transduction of the luteinizing hormone signal within the Leydig cell. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig cell. Cache River Press, Vienna, Il, pp 352–366

Coquelin A, Desjardins C (1982) Luteinizing hormone and testosterone secretion in young and old male mice. Am J Physiol 243:E257-E263

Cowley JJ, Brooksbank BW (1991) Human exposure to putative pheromones and changes in aspects of social behaviour. J Steroid Biochem Mol Biol 39:647–59

Csaba Z, Csernus V, Gerendai I (1998) Intratesticular serotonin affects steroidogenesis in the rat testis. J Neuroendocrinol 10:371–376

De Ronde W, Pols HAP, Johannes PTM, van Leeuwen PTM, de Jong FH (2003) The importance of oestrogens in males. Clin Endocr 58:529–542

Dumont JE, Pecasse F, Maenhaut C (2001) Crosstalk and specificity in signaling, are we crosstalking ourselves into general confusion? Cell Signalling 13:457–463

Ewing LL, Zirkin B (1983) Leydig cell structure and steroidogenic function. Rec Progr Horm Res 39:599–635

Forest MG (1989) Physiological changes in circulating androgens Pediatr Adolesc Endocrinol 19:104–129

Freeman DA, Rommerts FFG (1996) Regulation of Leydig cell cholesterol transport. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig cell. Cache River Press, Vienna, Il pp 232–239

Gao HB, Tong MH, Hu YQ, Guo QS, Ge R, Hardy MP (2002) Glucocorticoid induces apoptosis in rat Leydig cells. Endocrinology 143:130–138

Geissler WM, Davis DL, Wu l, Bradshaw KD, Patel S, Mendonca BB, Elliston KO, Wilson JD, Russell DW, Andersson S (1994) Male pseudohermaphroditism caused by mutations of testicular 17ß-hydroxysteroid dehydrogenase 3. Nature Genet 7:4–39

George FW (1997) Androgen metabolism in the prostate of the finasteride treated adult rat: a possible explanation for the differential action of testosterone and 5α-dihydrotestosterone during development of the male urogenital tract. Endocrinology 138:871–877

Gnessi L, Fabri A, Spera G (1997) Gonadal peptides as mediators of development and functional control of the testis: An integrated system with hormones and local environment. Endocr Rev 18:541–609

Gower DB, Cooke GM (1983) Regulation of steroid-transforming enzymes by endogenous steroids. J Steroid Biochem 19:1527–1556

Gruol DJ, Bourgeois S (1997) Chemosensitizing steroids: glucocorticoid receptor agonists capable of inhibiting P-glycoprotein function. Cancer Res 54:720–727

Guo Z, Benten WP, Krucken J, Wunderlich F (2002) Nongenomic testosterone calcium signaling. Genotropic actions in androgen receptor-free macrophages. J Biol Chem 277:29600–29607

Hakola K, Pierroz DD, Aebi A, Vuagnat BAM, Aubert ML, Huhtaniemi I (1998) Dose and time relationships of intravenously injected rat recombinant luteinizing hormone and testicular testosterone secretion in the male rat. Biol Reprod 59:338–343

Hall, PF (1988). Testicular steroid synthesis: organization and regulation. In: Knobil E, Neill J (eds) The physiology of reproduction, Raven Press, New York pp 975–998

Hall PF (1991) Cytochrome P450 C21scc: one enzyme with two actions: hydroxylase and lyase. J Steroid Biochem Mol Biol 40:527–532

Hammond GL, Ruokonen A, Kontturi M, Koskela E, Vihko R (1977) The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. J Clin Endocrinol Metab 45:16–24

Haour F, Kovanetzova B, Dray F, Saez JM (1979) hCG induced prostaglandin E2 and F2a release in adult rat testis: role in Leydig cell desensitization to hCG. Life Sci, 24:2151–2158

Hasegawa T, Zhao LP, Caron KM, Majdic G, Suzuki T, Shizawa S, Parker KL (2000) developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol Endocrinol 14:1462–1471

Heinlein CA, Chang C (2002) The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 16:2181–2187

Hodgson YM, de Kretser DM (1984) Acute responses of Leydig cells to hCG: evidence for early hypertrophy of Leydig cells. Mol Cell Endocr 35:75–82

Huhtaniemi, I. (1996) Ontogeny of the luteinizing hormone action in the male. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig cell. Cache River Press, Vienna, Il, pp 365–382

Huhtaniemi I, Bolton NJ, Martikainen H, Vihko R (1983) Comparison of serum steroid responses to a single injection of hCG in man and rat. J Steroid Biochem 19:1147–1151

Hunzicker-Dunn M, Gurevich VV, Casanova JE, Mukherjee S (2002) ARF6: a newly appreciated player in G protein-coupled receptor desensitization. FEBS letters 521:3–8

Ishimaru T, Pages L, Horton R (1977) Altered metabolism of androgens in elderly men with benign prostatic hyperplasia. J Clin Endocrinol Metab 45:695–701

King SR, Manna, PR, Ishii T, Syapin PJ, Ginsberg SD, Wilson K, Walsh LP, Parker KL, Stocco DM, Smith, RG, Lamb DJ (2002) An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain. J Neurosci 22: 10613–10620

Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, Belanger A (1997) The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology. Steroids 62:148–158

Lachane Y, Luu-The V, Labrie C, Simard C, Dumont M, de Launou Y, Guerin S, Leblanc G, Labrie F (1990) Chracterization of the structure-actvity relationship of rat types I and II 3β hydroxysteroid dehydrogenase/ 5- 4 -isomerase gene and its expression in mammalian cells. J Biol Chem 265:20469–20475

Laurich VM, Trbovich AM, O'Neill FH, Houk CP, Sluss PM, Payne AH, Donahoe PK, Teixeira J (2002) Mullerian inhibiting substance blocks the protein kinase A-induced expression of cytochrome p450 17alpha-hydroxylase/C(17–20) lyase mRNA in a mouse Leydig cell line independent of cAMP responsive element binding protein phosphorylation. Endocrinology 143:3351–3360

Lin T (1996) Insulin-like growth factor-I regulation of the Leydig cell In The Leydig cell, (ed) Payne AH, Hardy MP, Russell LD Cache River Press, Vienna, Il, pp 478–491

Loosfelt H, Misrahi M, Atger M, Salesse R, Thi MTVHL, Jolivet A, Guiochon-Mantel A, Sar S, Jallal B, Garnier J, Milgrom E (1989) Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain. Science 245:525–528

Lubicz-Nawrocki CM (1973) The effect of metabolites of testosterone on the viability of hamster epididymal spermatozoa. J Endocrinol 58:193–198

Luke MC, Coffey DS (1994) The male sex accessory tissues: structure, androgen action and physiology. In The physiology of reproduction, Knobil E, Neill JD (eds), Raven Press, New York, pp 1435–1480

Luu-The V, Takahashi Y, de Lanoit M, Dumont M, Lachane Y, Labrie F (1991) Evidence for distinct dehydrogenase and isomerase sites within a single 3β-hydroxysteroid dehydrogenase 5-ene-4-ene isomerase protein. Biochemistry 30:8861–8865

Lyng FM, Jones GR, Rommerts FFG (2000) Rapid androgen actions on calcium signaling in rat Sertoli cells and two human prostatic cell lines, similar biphasic responses between 1 picomolar and 100 nanomolar concentrations. Biol Reprod 63:736–747

Maddocks S, Sharpe RM (1989) Dynamics of testosterone secretion by the rat testis: implications for measurement of the intratesticular levels of testosterone. J Endocr 122:323–329

Manna PR, Tena-Sempere M, Hutaniemi IT (1999) Molecular mechanisms of thyroid hormone-stimulated steroidogenesis in mouse leydig tumor cells-involvement of the steroidogenic acute regulatory (StAR) protein. J Biol Chem 274:5909–5918

McFarland KC, Sprengel R, Phillips HS, Koher M, Rosemblit N, Nikolics K, Segaloff DL, Seeburg PH (1989) Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science 245:494–499

Mendel CM (1989) The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 10:232–274

Mendel CM, Murai JT, Siiteri PK, Monroe SE, Inove M (1989) Conservation of free but not total or non-sex-hormone binding-globulin-bound testosterone in serum from nagase analbuminemic rats. Endocrinology 124:3128–3130

Monno S, Ogawa H, Date T, Fujioka WL, Millar WL, Kobayashi M (1993) Mutation of histidine -373 to leucine in cytochrome-P450c17 causes 17α hydroxylase deficiency. J Biol Chem 268:25811–25817

Michael RP, Bonsall RW, Rees HD (1986) Accumulation of 3H testosterone and 3H estradiol in the brain of the female primate: evidence for the aromatization hypothesis. Endocrinology 118:1935–1944

Miller WL (1988) Molecular biology of steroid hormone synthesis. Endocr Rev 9:295–318

Miura T, Yamauchi K, Takahashi H, Nagahama Y (1991) Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). Proc Natl Acad Sci USA 88:5774–5778

Miautani S, Tsujimura T, Akashi S, Matsumoto K (1977) Lack of metabolism of progesterone, testosterone and pregnenolone to 5α-products in monkey and human testes compared with rodent testes. J Clin Endocrinol Metab 44:1023–1031

Mooradian AD, Morley JE, Korenman SG (1987) Biological actions of androgens. Endocr Rev 8:1–27

Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of oestrogens. J Clin Endocr Metab 80:3689–3698

Mulder E, Lamers-Stahlhofen GJM, van der Molen HJ (1973) Interaction between steroids and membranes. Uptake of steroids and steroid sulphates by resealed erythrocyte ghosts. J Steroid Biochem 4:369–379

Noordhuizen-Stassen EW, Charbon GA, de Jong FH, Wensing CJG (1985) Functional arteriovenous anastomoses between the testicular artery and the pampiniform plexus in the spermatic cord of rams. J Reprod Fert 75:193–201

Odell WD, Swerdloff RS, Jacobs HS, Hescox MA (1973) FSH induction of sensitivity to LH; one cause of sexual maturation in the male rat. Endocrinology 92:160–165

O'Malley BW, Schrader WT, Mani S, Smith C, Weigel NL, Conneely OM, Clark JH (1995) An alternative ligand-independent pathway for activation of steroid receptors. Rec Prog Horm Res 50:333–353

Pandey AV, Mellon SH, Miller WL (2003) Protein phosphatase 2A and phosphoprotein SET regulate androgen production by P450c17. J Biol Chem 278:2837–2844

Papadopoulos V. (1993) Peripheral-type benzo-diazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. Endocr Rev 14:22–240

Papakonstanti EA, Kampa M, Castanas E, Stournaras C (2003) A rapid nongenomic, signalling pathway regulates the actin reorganization induced by activation of membrane testosterone receptors. Mol Endocrinol 17:870–881

Parker KL, Schimmer BP (1997) Steroidogenic factor 1: A key determinant of endocrine development and function. Endocr Rev 18:361–377

Payne AH, Quinn PG, Rani CSS (1985) Regulation of microsomal cytochrome P-450 enzymes and testosterone production in Leydig cells. Rec Progr Horm Res 41:153–185

Payne AH, Hardy MP, Russell LD (eds) (1996) The Leydig cell. Cache River Press, Vienna, IL

Payne AH, O'Shaughnessy P (1996) Structure,function,and regulation of steroidogenic enzymes in the Leydig cell. In: The Leydig cell. Payne AH, Hardy MP, Russell LD (eds), Cache River Press, Vienna, IL. pp 260–285

Penning TM (1997) Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Reviews 18:281–305

Rao RM, Jo Y, Leers-Sucheta S, Bose HS, Miller W, Azhar S, Stocco DM (2003) Differential regulation of steroid hormonebiosynthesis in R2C and MA-10 Leydig tumor cells: Role of SR-B1- mediated selective cholesteryl ester transport. Biol Reprod 68:114–121

Rice DA, Mouw AR, Bogerd AM, Parker KL (1991) A shared promoter element regulates the expression of three steroidogenic enzymes. Mol Endocrinol 5:1552–1556

Richards JS (2001) New signaling pathways for hormones and cyclic adenosine 3′, 5′-monophosphate action in endocrine cells. Mol Endocrinol 15:209–218

Rittmaster RS (1994) Finasteride. New Eng J Med 330:120–125

Rhéaume E, Sanchez F, Mebarki F, Gagnon E, Carel JC, Chaussain JL, Morel Y, Labrie F, Simard JN (1995) Identification and characterization of the G15D mutation found in a male patient with 3ß-hydroxysteroid dehydrogenase (3β-HSD) deficiency – alteration of the putative NAD-binding domain of type-II 3β-HSD. Biochem 34:2893–2990

Rommerts FFG (1988) How much androgen is required for maintenance of spermatogenesis? J Endocr 116:7–9

Rommerts FFG (1992) Cell surface actions of steroids: A complementary mechanism for regulation of spermatogenesis? In: Spermatogenesis, fertilization and contraception (Molecular, cellular and endocrine events in male reproduction). Nieschlag E, Habenicht UF (eds), Schering Foundation Workshop 4, Springer Verlag, Berlin, pp 1–19

Rommerts FFG, Brinkmann AO (1981) Modulation of steroidogenic activities in testis Leydig cells. Mol Cell Endocr 21:15–28

Rommerts FFG, Cooke BA (1988) The mechanisms of action of luteinizing hormone. II. Transducing systems and biological effects. In: Hormones and their actions, part II (ed) Cooke BA, King RJB, van der Molen HJ. Elsevier Science Publishers BV (Biomedical Division), Amsterdam, pp 163–180

Rommerts FFG, van der Molen HJ (1989) Testicular steroidogenesis. In: The testis. Burger H, de Kretser D (eds), 2nd edition, Raven Press, New York, pp 303–328

Saez JM (1989) Endocrine and paracrine regulation of testicular functions. Pediatr Adolesc Endocrinol 19:37–55

Saez JM (1994) Leydig cells: endocrine, paracrine and autocrine regulation. Endocr Rev 16: 574–626

Schlatt S, Meinhardt A, Nieschlag E (1997) Paracrine regulation of cellular interactions in the testis: factors in search of a function. Eur J Endocr 137:107–117

Seckl SR, Walker BR (2001) 11α-hydroxysteroid dehydrogenase type 1-a tissue specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376

Selvage DJ, Rivier C (2003) Importance of the paraventricular nucleus of the hypothalamus as a component of a neural pathway between the brain and the testes that modulates testosterone secretion independently of the pituitary. Endocrinology 144:594–598

Setchel BP, Laurie MS, Flint APF, Heap RB (1983) Transport of free and conjugated steroids from the boar testis in lymph, venous blood and rete testis fluid. J Endocr 96:127–136

Shakil T, Ehsanul Hoque AN, Husain M, Belsham DD (2002) Differential regulation of gonadotropin-releasing hormone secretion and gene expression by androgen: membrane versus nuclear receptor activation. Mol Endocrinol 16:2592–2602

Sharpe RM (1993) Experimental evidence for Sertoli-germ cell and Sertoli- Leydig cell interactions. In: The Sertoli cell. Russell LD, Griswold MD (eds), Cache River Press, Vienna, Il. pp. 391–418

Simoncini T, Genazzani A (2003) Non-genomic actions of sex steroids. Eur J Endocrinol 148: 281–292

Simpson BJB, Wu FCW, Sharpe RM (1987) Isolation of human Leydig cells which are highly responsive to human chorionic gonadotropin. J Clin Endocrinol Metab 65:415–422

Smals AGH, Pieters GFFM, Lozekoot DC, Benraad TJ, Kloppenborg PWC (1980) Dissociated responses of plasma testosterone and 17-hydroxyprogesterone to single or repeated human chorionic gonadotropin administration in normal men. J Clin Endocrinol Metab 50: 190–193

Snyder SH, Sklar PB, Pevsner J (1988) Molecular mechanisms of olfaction. J Biol Chem 263:13971–13974

Sporn MB, Roberts AB (1988) Peptide growth factors are multifunctional. Nature 132: 217–219

Stocco DM, Clark BJ (1996) Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 17:221–244

Stocco DM (2001) Tracking the role of a star in the sky of the new millennium. Mol Endocrinol 15:1245–1254

Stocco DM (2002) Clinical disorders associated with abnormal cholesterol transport: mutations in the steroidogenic acute regulatory protein. Mol Cell Endocrinol 191:19–25

Strohman RC (1993) Ancient genomes, wise bodies, unhealthy people: limits of genetic thinking in biology and medicine. Perspec Biol Med 37:112–145

Teerds KJ, de Rooij DG, Rommerts FFG, Wensing CJG (1988) The regulation of the proliferation and differentiation of Leydig cell precursors after EDS administration of daily hCG treatment. J Androl 9:343–351

Themmen APN, Huhtaniemi IT (2000) Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev 21:551–583

Träger L (1977) Steroidhormone: Biosynthese, Stoffwechsel, Wirkung, Springer Verlag, Berlin, pp 164–197

Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R (1992) Human P-glycoprotein transports cortisol, aldosterone and dexamethasone but not progesterone. J Biol Chem 267:24248–24252

van den Akker EL, Koper JW, Boehmer AL, Themmen AP, Verhoef-Post M, Timmerman MA, Otten BJ, Drop SL, de Jong FH (2002) Differential inhibition of 17alpha-hydroxylase and 17,20-lyase activities by three novel missense CYP17 mutations identified in patients with P450c17 deficiency. J Clin Endocrinol Metab 87:5714–21

van Doorn LG, de Bruijn HWA, Galjaard H, van der Molen HJ (1974) Intercellular transport of steroids in the infused rabbit testis. Biol Reprod 10:47–53

van Haren L, Cailleau J, Rommerts FFG (1989) Measurement of steroidogenesis in rodent Leydig cells: a comparison between pregnenolone and testosterone production. Mol Cell Endocr 65:157–164

van Haren L, Flinterman JF, Orly J, Rommerts FFG (1992) Luteinizing hormone induction of the cholesterol side-chain cleavage enzyme in cultured immature rat Leydig cells: no role for insulin-like growth factor-I? Molec Cell Endocr 87:57–67

van Haren L, Flinterman JF, Rommerts FFG (1995) Inhibition of luteinizing hormone-dependent induction of cholesterol side-chain cleavage enzyme in immature rat Leydig cells by Sertoli cell products. Eur J Endocrinol 132:627–634

van Loenen HJ, Flinterman JF, Rommerts FFG (1994) High affinity FSH receptor binding is a slowly nonreversible process that appears not to be important for rapid receptor activation. Endocrine 2:1031–1035

van Noort M, Rommerts FFG, van Amerongen A, Wirtz KWA (1988) Intracellular redistribution of SCP2 in Leydig cells after hormonal stimulation may contribute to increased pregnenolone production. Biochem Biophys Res Commun 154:60–65

van Straten NCR, Schoonus-Gerritsma GG, van Someren RG, Draaijer J, Adang AEP, Timmers CM, Hanssen RGJM, van Boeckel CAA (2002) The first orally low molecular weight agonists for the LH receptor: thienopyr(im)idines with therapeutic potential for ovulation induction. Chem Bio Chem 10:1023–1026

Wang C, Plymate E, Nieschlag E, Paulsen CA (1981) Salivary testosterone in men. Further evidence of a direct correlation with free serum testosterone. J Clin Endocrinol Metab 53:1021–1024

Wang X, Walsh LP, Reinhart AJ, Stocco DM (2000) The role of arachidonic acid in steroidogenesis and steroidogenic acute regulatory (StAR) gene and protein expression. J Biol Chem 275:20204–20209

Weusten JJAM, Smals AGH, Hofman JA, Kloppenborg PWC, Benraad ThJ (1987a) The sex pheromone precursor androsta-5,16 dien-3α ol is a major early metabolite in in vitro pregnenolone metabolism in human testicular homogenates. J Clin Endocrinol Metab 65:753–756

White PC, Mune T, Agarwal AR (1997) 11ß-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. Endocr Rev 18:135–156

Wiebe JP (1997) Nongenomic actions of steroids on gonadotropin release. Rec Prog Horm Res 52:71–102

Wilson JD (1988) Androgen abuse by athletes. Endocr Rev 9:181–199

Wilson JD, Griffin JE, Russell DW (1993) Steroid 5α-reductase 2 deficiency. Endocr Rev 14:577–593

Yan W, Giudice LC (1999) Insulin-like growth factor II mediates the steroidogenic and growth promoting actions of follicle stimulating hormone on human ovarian preantral follicles cultured in vitro. J Clin Endocr Metab 84:1479–1482

Young J, Couzinet B, Chanson P, Brailly S, Loumay E, Schaison G (2000) Effects of human recombinant luteinizing hormone and follicle stimulating hormone in patients with acquired hypogonadotropic hypogonadism: study of Sertoli and Leydig cell secretions and interactions. J Clin Endocrnol Metab 85:3239–3244

Zhang LH, Rodriguez H, Ohno S, Miller WL (1995) Serine phosphorylation of human P450c17 increases 17,20-lyase activity: Implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci USA 92:10619–10623

Zhu Y, Rice CD, Pang Y, Pace M, Thomas P (2003a) Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc Natl Acad Sci USA 100:2231–2236

Zhu Y, Bond J, Thomas P (2003b) Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc Natl Acad Sci USA 100:2237–2242

Zuber MX, Simpson ER, Waterman MR (1986) Expression of bovine 17α-hydroxylase cytochrome P450cDNA in non-steroidogenic (COS1) cells. Science 234:1258–1260

2

The androgen receptor: molecular biology

H. Klocker, J. Gromoll and A.C.B. Cato

Contents

2.1 Introduction

Androgens are key regulators of male sexual differentiation and development of a normal male phenotype. In the adult they are required for maintenance and function of male genital organs and spermatogenesis. In addition, they are involved in a large number of physiological processes such as stimulating muscle and hair growth, bone development, erythropoiesis as well as controlling male psychosocial behavior. The two main androgens in the human body are testosterone and dihydrotestosterone, the 5α-reduced derivative of testosterone. Each of them has its distinct role and target tissues. During development, testosterone produced in the testis triggers the development of the Wolffian duct structures which results in the development of the seminal vesicles, vas deferens and urethra, whereas dihydrotestosterone, synthesized in the periphery through the action of the enzyme 5α-reductase, is crucial for development of external genitalia and the prostate from the urogenital sinus.

At the cellular level, androgen action is mediated by a high affinity receptor, the androgen receptor (AR) that functions as a ligand-activated transcription factor. This receptor is a member of the large superfamily of ligand-inducible transcription factors that share the structural and functional organization in three domains (transactivation, DNA-binding and ligand-binding domains). Binding of androgens induces a cascade of activation steps that finally result in a transcriptionally active AR capable of regulating the transcription of genes by binding to target sequences in the chromatin, termed androgen response elements.

The gene encoding the AR is located on the long arm of the X-chromosome close to the centromere region. The structural organization into 8 exons is essentially identical to those of the genes encoding other steroid receptors. Due to the sex chromosomal inheritance trait of the AR gene, AR malfunction affects males whereas females transmit genetic alterations to the next generation. Three diseases associated with AR defects are: 1) Male pseudohermaphroditism due to AR abnormalities causing complete or partial androgen insensitivity of target tissues in genetic and gonadal males. This is the most frequent cause of male sex ambiguity, and depending on the severity of the defect, the clinical symptoms span a wide spectrum from a female phenotype to patients with partial disorders and phenotypically normal males with infertility. 2) Escape from hormone ablation therapy in prostatic carcinoma is associated with a number of AR alterations that contribute to the development of hormone-insensitive tumor cells. 3) Spinal and bulbar muscular atrophy, a disease associated with a pathological AR function that is characterized by late onset, progressive weakening of skeletal muscles and impairment of motoneuron function.

2.2 The androgen receptor gene

2.2.1 Genomic localization

The AR gene is located on the long arm of the X chromosome close to the centromere spanning the chromosomal region Xq11.2 to Xq12 and is over 180 kb (http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=367). Prior to the cloning of the AR cDNA and physical mapping, its localization on the X chromosome had already been recognized from genetic studies. In 1975, Meyer *et al.*, who studied the genetics and inheritance of male pseudohermaphroditism, postulated that the dihydrotestosterone receptor gene must be on the X chromosome (Meyer *et al.* 1975). The same group assigned the gene to a position between Xq11 and Xq13 a few years later, by cell fusion complementation experiments with an androgen binding deficient mouse kidney cell and human cells containing different X – autosomal translocations expressing only segments of the X chromosome (Migeon *et al.* 1981). This locus was confirmed by a restriction fragment length polymorphism (RFLP) study (Wieacker *et al.* 1987).

The location on a sex chromosome implies that males possess only one allele whereas females have two. As a result, mutant AR genes are inherited in an X-linked fashion affecting men, whereas women carry and transmit the gene to the next generation without themselves being affected.

The AR cDNA was the last of the steroid receptors to be cloned. Using the high degree of homology of steroid receptor DNA-binding domains to screen cDNA libraries for similar sequences, several groups independently isolated the AR cDNA from different sources and determined its sequence at the end of the eighties (Chang *et al.* 1988a; 1988b; Lubahn *et al.* 1988a; 1988b; Trapman *et al.* 1988). Using a cDNA probe Brown *et al.* (1989) then localized the AR gene to Xq11-q12 by analysis of somatic cell hybrid panels segregating portions of the X chromosome.

2.2.2 Structure of the androgen receptor gene and its mRNA

The AR gene consists of 8 exons (Fig. 2.1) (Lubahn *et al.* 1989); it shares this gene structure with the other steroid receptors which form a subfamily of the nuclear receptors. Besides the steroid receptors this superfamily also includes the retinoid and thyroid hormone receptors, vitamin D receptors, peroxisome proliferator-activated receptors and a number of orphan receptors (Hager 2000; Owen and Zelent 2000; Whitfield *et al.* 1999). All the members have a unique molecular structure comprising a COOH-terminal ligand-binding domain, a central DNA-binding domain that is built up of two zinc finger motifs, a hinge region, and an NH_2- terminal transactivation domain (Fig. 2.1) (Fuller 1991; Glass *et al.* 1997; Tilley *et al.* 1989). In the AR gene the large exon 1 (2731 bp, 1616 bp coding) encodes the transactivation domain; the two zinc fingers of the DNA-binding domain are

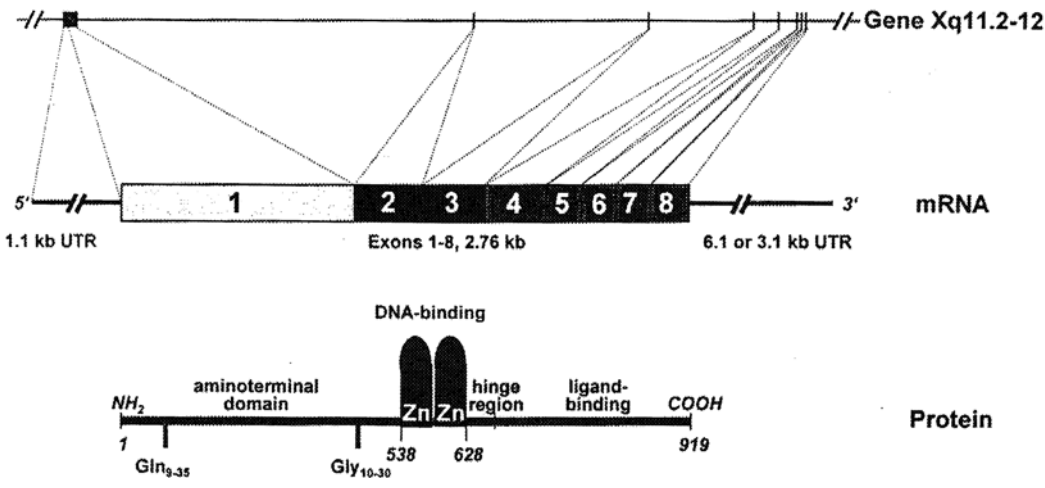


Fig. 2.1 Androgen receptor gene and protein
The domain structure of the androgen receptor comprises a COOH-terminal ligand-binding domain, a central DNA-binding domain made up of two zinc finger motifs, a hinge region, and an NH_2-terminal transactivation domain. The AR protein is 919 amino acids long. Due to two polymorphic regions, a polyglutamine and a polyglycine stretch, respectively, in the NH_2-terminal domain, the individual AR protein size varies to some extent. The AR gene is located on the X chromosome close to the centromere in bands 11.2–12 and comprises 8 exons. The large exon 1 (2731 bp, 1616 bp coding) encodes the transactivation domain. The two zinc fingers of the DNA-binding domain are encoded by exons 2 and 3 (153 and 117 bp), respectively. A small part of exon 3 and the first part of exon 4 (288 bp) contain the information for the hinge region that includes a nuclear translocation signal. The rest of exon 4 together with exons 5 to 8 (145, 131, 158, 157 bp) encode the ligand-binding domain. Two major mRNA species with 10 and 7 kb, respectively, are transcribed from the AR gene. They differ in the length of the 3′ untranslated region.

encoded by exons 2 and 3 (153 and 117 bp), respectively. A small part of exon 3 and the first part of exon 4 (288 bp) contain the information for the hinge region that includes a nuclear translocation signal. The rest of exon 4 together with exons 5 to 8 (145, 131, 158, 157 bp) encode the ligand-binding domain (Jenster *et al.* 1992; Simental *et al.* 1992).

The promoter of the AR gene lacks TATA and CAAT boxes typical for most eucaryotic promoters. The major start site of transcription is 1.1 kb upstream of the initiator ATG triplet and a second transcription initiation site is at +13 (Faber *et al.* 1991). The promoter is characterized by a short GC-box at −59 to −31 and a homopurine stretch of alternating adenosine and guanosine residues from −117 to −60 (Tilley *et al.* 1990a; Faber *et al.* 1993). Within the GC-box is a binding site for the transcription factor Sp1. Using footprint analysis and reporter gene

assays, Faber *et al.* found out that initiation from the second transcription start site is directed by the GC-box, whereas the first initiation site is dependent upon sequences between −5 and +57 (Faber *et al.* 1991). Upstream of the transcription initiation site between −380 and −530 is a cAMP response element (CRE) that confers cAMP induction of AR transcription (Mizokami *et al.* 1994). The region +109 to +129 forms a stem-loop secondary structure and was shown in reporter gene assays to play an essential role in the induction of AR translation (Mizokami and Chang 1994). Androgens induce downregulation of AR mRNA (Quarmby *et al.* 1990). Since classical androgen-responsive elements were not found in the AR promoter, this regulation seems to be indirect through interaction with other transcription factors or via binding to sequences similar to androgen-response element half sites.

The major AR mRNA species is 10 kb in size. In addition, a less abundant mRNA of approximately 7 kb is present in human prostate and different androgen responsive tissues (Burgess and Handa 1993; Hirai *et al.* 1994; Lubahn *et al.* 1988a). In a LNCaP prostate cancer cell line derived from a lymph node metastasis, an extra low abundant mRNA of 4.7 kb has been described (Trapman *et al.* 1988). In the major mRNA sequence the open reading frame of approximately 2.7 kb is flanked by a 1.1 kb 5' untranslated region and a very large (6.8 kb) 3' untranslated region (Faber *et al.* 1991). The 3' untranslated region is shortened in the smaller mRNA species. Two poly-adenylation signals are located at the end of the mRNA, 221 bp apart, a ATTAAA and a CATAAA box (Faber *et al.* 1991).

The cDNA encodes a protein of 919 amino acids with a molecular weight of 98.999 kD (Lubahn *et al.* 1988a). Due to two trinucleotid-repeat polymorphisms in the NH_2-terminal region the individual size of the human AR can vary to some extent. A polymorphic CAG repeat encodes a polyglutamine stretch and a polymorphic GGC repeat a polyglycine stretch (La Spada *et al.* 1991; Lumbroso *et al.* 1997; Chang *et al.* 2002; Chen *et al.* 2002). Comparison with the amino acid sequence of previously cloned steroid hormone receptors showed a high degree of sequence conservation with the progesterone, glucocorticoid, and mineralocorticoid receptors with highest homology in the DNA-binding domain and a small region within the hydrophobic ligand-binding domain (Chang *et al.* 1988b). These AR domains also show the highest evolutionary conservation. In the rat the amino acid sequence of the DNA-binding and hormone binding domains are identical to the human protein with an overall homology of 85% (Lubahn *et al.* 1988a).

2.2.3 Evolution of the androgen receptor

The AR is an evolutionary well-conserved protein. Amino acid homology between the human and mouse, rat and frog ARs, respectively, are 88, 85 and 81% (He *et al.* 1990). It is assumed that the nuclear receptors evolved from a common ancestor by

gene duplications (Amero *et al.* 1992). The rate of amino acid replacement during evolution was very different for the different AR domains. Replacement rates per amino acids position per billion years were calculated for the human and *Xenopus laevis* receptors to range from 0.04 for the DNA-binding domain or 0.19 for the ligand-binding domain to 1.25 for the N-terminal domain with an overall rate of 0.69 (Thornton and Kelley 1998). Comparative analysis of all available AR sequences and other steroid receptor sequences allowed the identification of residues that are strictly conserved and specific for AR receptors (Thornton and Kelley 1998). These are clustered in specific regions, e.g. the regions immediately prior to and after the DNA-binding domain, the DNA-binding domain itself and a few regions in the ligand-binding domain (Thornton and Kelley 1998).

The structural organization of the AR gene was studied in detail in the human, the mouse and the rat. In these three species there is no essential difference (Baarends *et al.* 1990; Tilley *et al.* 1990a; Faber *et al.* 1993; Lindzey *et al.* 1993; Grossmann *et al.* 1994a; 1994b; Kumar *et al.* 1994; Mizokami *et al.* 1994; Grossmann and Tindall 1995).

2.3 Functional domains of the androgen receptor

2.3.1 Amino terminal domain

Steroid receptors contain activation functions 1 and 2 (AF1 and AF2) at their NH_2- and COOH- terminal regions. In contrast to other steroid hormone receptors that contain a strong transactivation function at their COOH-terminus, the COOH-terminal AF2 domain in AR has a weak transactivation potential. Most of the transactivation function of the AR is carried out by the NH_2-terminal domain of the receptor (Jenster *et al.* 1995; Poukka *et al.* 2000a). The mechanism of transcriptional activation by the NH_2-terminus is not quite understood but it is thought that the NH_2-terminus may represent a surface for recruitment of coregulators. Consistent with this idea, several coactivators including SRC-1, GRIP-1 and CBP have been shown to interact with the NH_2-terminus (Ikonen *et al.* 1997; Alen *et al.* 1999; Bevan *et al.* 1999; Ma *et al.* 1999). While most of the entire NH_2-terminus (amino acids 1–494) is required for full activity of the full length receptor, a core that contributes to 50% of its activity is located between residues 101 and 360 and this region has been termed $\tau 1$. However, in the absence of the HBD, a different region termed $\tau 5$ (residues 370 to 494) mediates transactivation (Jenster *et al.* 1995) (Fig. 2.2). The AF-1 at the NH_2-terminus has also been separated into AF1a (amino acids 154–167) and AF-1b (amino acids 259–459) both of which are required for full transactivational activity (Chamberlain *et al.* 1996). NH_2-terminal residues of the AR (142–485) have been shown to activate a minimal promoter construct with the transcription factor TFIIF and the TATA binding protein (TBP), suggesting

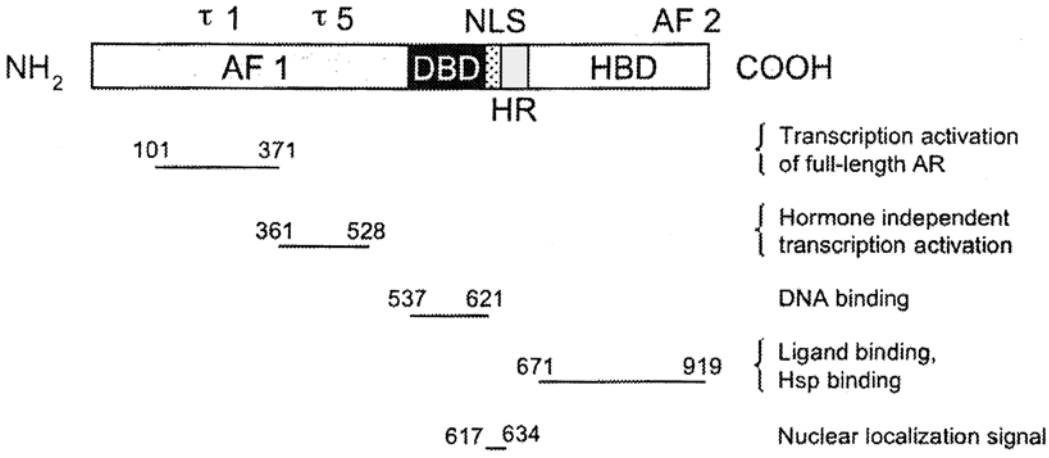


Fig. 2.2 Functional domain structure of the human androgen receptor
Schematic diagram of the AR showing the N-terminal AF1 activation domain with two independent transactivation functions, τ1 and τ5, DNA binding domain (DBD) in the middle of the molecule, hinge region (HR) and hormone binding domain (HBD) with a weak hormone-dependent transactivation function at the COOH-terminus of the receptor. The signal responsible for nuclear import is located at the junction of the DBD and hinge region.

a direct contact of the NH_2-terminus with the general transcriptional machinery (McEwan and Gustafsson 1997).

The amino terminus of the AR contains a CAG repeat stretch. Sequence analysis of the AR in a variety of species have shown that the amino-terminal CAG repeat increases exponentially with decreasing evolutionary distance from the human (Choong and Wilson 1998). For example, the number of CAG triplets is about 22 in humans, while in the prosimian lemur, a more distant primate species in evolutionary terms, it remains at 4 (Choong and Wilson 1998). The expansion of triplet motifs in the AR and their polymorphic nature in great apes which is not present in lower species indicate that differences in cellular factors or genetic changes could have led to an instable AR. The remaining amino acids residues in the AR extending from 1–53 and 360–429 including a polyproline stretch are completely conserved among primates. The high conservation of these other sequences might play an important role in the NH_2-/COOH- interactions which are so crucial for transactivation by the androgen receptor.

2.3.2 DNA binding domain

The DNA binding domain (DBD) is located in the central core of the receptor and consists of DNA binding zinc fingers which are two outloops of protein sequences,

each held in place by four conserved cysteine residues that coordinate with a zinc ion (Freedman *et al.* 1988). This motif is well conserved for members of the steroid receptor family such that information on the structure of this domain of the AR can be inferred from analysis of the equivalent domain of the other steroid receptors. The crystal structures of the glucocorticoid and estrogen receptor zinc fingers bound to their response elements have been solved (Luisi *et al.* 1991; Schwabe *et al.* 1993). Sequence specific DNA interactions occur through an α-helix which lies in the major groove of the DNA. The most important amino acids in this regard are the same for both the androgen and glucocorticoid receptors. As other domains of the AR, the DBD also serves as an interface for binding other factors. A 60 kDa polypeptide that shares N-terminal homology to a calcium binding protein, calreticulin, has been shown to bind the sequence KXFFKR in the DBD of the AR to inhibit DNA binding and transactivation by the receptor (Dedhar *et al.* 1994). Calreticulin is therefore a regulator of AR action and recent studies show that it is a receptor for nuclear export of proteins (Holaska *et al.* 2001). Thus the DBD of the AR contains a sequence for nuclear export, but how this functions in the signaling pathway of the receptor is not clear.

2.3.3 Nuclear localization and hinge region

A signal responsible for nuclear import is encoded by amino acid residues 608–625 (**Rk**cyeagmtlga**RK**l**KK**l) in the hinge region and is functionally similar to the bipartite nucleoplasmin nuclear localization signal (NLS). Mutational analysis showed that both basic parts of this nucleoplasmin-like sequence (shown in bold letters) contribute to the nuclear targeting of the AR (Jenster *et al.* 1993). It is possible that these portions of the AR are hidden but become exposed to mediate nuclear transport of the receptor following hormone binding.

In addition to providing the NLS for transport into the nucleus, the hinge region of the AR is also a surface for interaction with a number of proteins that modulate the transcriptional activity of the receptor. Among them are filamin A, a 280-kDa actin-binding protein (Loy *et al.* 2003), Ubc9, a homologue of the class E2 ubiquitin-conjugating enzymes (Poukka *et al.* 1999) as well as a 75 kDa protein termed p21-activating kinase 6 (PAK6) (Lee *et al.* 2002). All these proteins bind to the hinge region of the AR to negatively regulate the transactivating function of the receptor. Negative regulation of the AR function is not a universal feature of all the proteins interacting with the NLS. The RING finger protein SNURF that binds to a region overlapping the bipartite NLS and the DNA binding domain does not inhibit transactivation by the receptor. On the contrary it enhances transactivation by the receptor and facilitates AR import to nuclei while retarding its export on hormone withdrawal (Moilanen *et al.* 1998; Poukka *et al.* 2000b). Interestingly, some point mutations in the zinc finger region of the AR observed in patients with partial

androgen insensitivity syndrome or male breast cancer impair the interaction of AR with SNURF and render the AR refractory to the transcription-activating effects of SNURF (Poukka *et al.* 2000a).

2.3.4 Ligand binding domain

The main function of the ligand-binding domain (LBD) is to bind ligand and it is helped in this task by molecular chaperones. Genetic and biochemical studies have shown that the chaperones Hsp90 and Hsp70 participate in the activation process of the receptor. They maintain the apoAR (AR in the absence of hormone) in a high affinity ligand-binding conformation which is important for efficient response to hormone (Caplan *et al.* 1995; Fang *et al.* 1996).

To understand the specific recognition of ligands by the human AR, homology models of the ligand-binding domain were constructed based on the crystal structure of the progesterone receptor ligand binding domain. Several mutants in residues potentially involved in the specific recognition of ligands in the hAR were constructed and tested for their ability to bind agonists (Poujol *et al.* 2000). The homology model AR was refined using unrestrained multiple molecular dynamics simulations in explicit solvent (Marhefka *et al.* 2001). These models together with the recent crystal structure of the AR, show that the HBD of the AR is similar in structure to the HBD of other nuclear receptors. It is composed of 12 helices and a small β-sheet arranged in an α-helical sandwich. Depending on the nature of the ligand, agonists or antagonists, the carboxy-terminal helix H12 is found in either one of two orientations. In the agonists-bound conformation, helix H12 serves as a lid to close the ligand-binding pocket, whereas in the antagonist-bound conformation, helix H12 is positioned in a different orientation, thus opening the entrance of the ligand-binding pocket (Matias *et al.* 2000). The structure of the LBD of the wild-type AR and the T877A mutant found in the LNCaP prostate cancer cells that provide the receptor with a broad steroid specificity have been refined at 2.0Å resolution (Sack *et al.* 2001). The crystal structure of the mutant AR (L701H and T877A) reported to bind cortisol/cortisone with high affinity has however been determined at 1.95Å (Matias *et al.* 2002). These structural studies provide mechanistic explanation why non-androgenic ligands do function as agonists when bound to the AR.

2.3.5 Amino- and carboxyl-terminal interaction

A unique property of the AR in the regulation of gene expression is its ability to provide intra-domain interaction and communication between the amino- and the carboxyl-terminal domains of the receptor (Langley *et al.* 1995; 1998; He *et al.* 1999). This interaction is mediated through a FXXLF and WXXLF motifs at amino acids 23–27 and 429–439 in the NH_2-terminus of the receptor with distinct regions

in the HBD of the AR (He *et al.* 2000). Interaction between NH_2- and COOH-termini of the AR was shown to be necessary for the testosterone-induced AR stabilization and an antiparallel arrangement of AR monomers in the AR dimer (Berrevoets *et al.* 1998; Ikonen *et al.* 1997; Langley *et al.* 1995; 1998). The WXXLF motif has a significant but more minor role than the FXXLF motif in mediating the NH_2-/COOH- interaction (He *et al.* 2000). The activity of the more relevant FXXLF motif is further modulated by its flanking sequences (Steketee *et al.* 2002).

Recently it was demonstrated that interaction of the NH_2- and COOH-termini of the AR is essential for the recruitment of coactivator proteins SRC1, TIF2 and CBP (Saitoh *et al.* 2002). The importance of the NH_2-/COOH- terminal interaction was demonstrated in a mutational analysis of the AF2 domain of the AR, which showed that disruption of the functional interaction between NH_2- and COOH-termini of the AR is linked to androgen insensitivity syndrome (Thompson *et al.* 2001). Intriguingly promoter-specific differences exist in the requirements of the NH_2/COOH- interaction of the AR. While agonist-dependent transactivation of prostate-specific antigen (PSA) and probasin enhancer/promoter regions require the NH_2-/COOH- interaction, the sex-linked protein gene and mouse mammary tumor virus long terminal repeat do not (He *et al.* 2002). At present there are no evident predictive features that differentiate these enhancer/promoter androgen response elements in terms of their sensitivity to the NH_2-/COOH- interaction.

The NH_2- and COOH-terminal interaction is in itself a regulated process. While the coactivators such as SRC-1 (Ikonen *et al.* 1997; He *et al.* 1999) TIF2 (Berrevoets *et al.* 1998), CREB-binding protein (Ikonen *et al.* 1997) and c-Jun (Bubulya *et al.* 2001) positively mediate this interaction, other proteins such as the tumor suppressor protein p53 exert a negative effect on transactivation of the AR by disrupting its NH_2-/COOH- terminal interaction. Consistent with this, p53 is able to block DNA binding by the AR (Shenk *et al.* 2001). The negative effect of p53 is however blocked by overexpression of c-Jun, demonstrating antagonistic activities of these two proteins (Shenk *et al.* 2001). A p53 mutation found in metastatic prostate cancer severely disrupts the negative effect of p53 on the AR which would suggest that the inability of p53 mutants to downregulate the activity of the ARE may contribute to the metastatic phenotype (Shenk *et al.* 2001)

2.4 Molecular mechanisms of androgen receptor action

2.4.1 Chaperones and co-chaperones in androgen receptor action

In the absence of ligand, the AR exists in an inactive complex with molecular chaperones (Hsp90, Hsp70, Hsp56 and immunophilins). These proteins help in maintaining the correct conformation of the receptor necessary for efficient ligand binding (Pratt and Toft 1997).

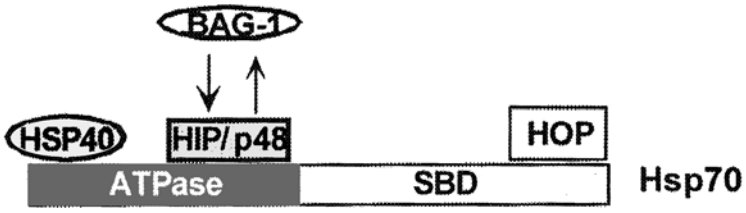


Fig. 2.3 Hsp70 and its co-chaperones
Schematic diagram of the Hsp70 showing the NH_2-terminal ATPase domain of the protein and the COOH-terminal substrate binding domain (SBD) responsible for interaction with unfolded proteins. Co-chaperones Hsp40, Hip and BAG-1 interact with the ATPase domain of Hsp70. Hop binds to the COOH-terminus of Hsp70 and serves as a bridging factor for interaction with Hsp90.

The minimal assembly of molecular chaperones necessary for efficient folding of nuclear receptors are Hsp90, Hsp70, p23 and co-chaperones Hsp40 and Hop (Dittmar *et al.* 1998; Kosano *et al.* 1998). Hsp70 is thought to be the first to bind the receptor (Morishima *et al.* 2000) and its function is positively regulated by the co-chaperones Hsp40 (Dittmar *et al.* 1998). Hop, binds to both Hsp90 and Hsp70 and functions as an adaptor protein, which brings a dimer of Hsp90 to the complex (Dittmar and Pratt 1997; Chen and Smith, 1998; Johnson *et al.* 1998). The function of p23 stays unclear, though this chaperone has been shown to be important for hormone binding by the glucocorticoid and progesterone receptors, where p23 stabilizes the aporeceptor complex at a late step of the receptor folding (Dittmar and Pratt 1997; Kosano *et al.* 1998).

The chaperone activity of Hsp70 is regulated by two other co-chaperones Hip and Bag-1 (Fig. 2.3). Hip (Höhfeld *et al.* 1995) positively regulates Hsp70 function, while Bag-1 competes with Hip for binding to Hsp70 ATPase domain and is a negative regulator of Hsp70 (Höhfeld and Jentsch, 1997; Takayama *et al.* 1997; Bimston *et al.* 1998). In addition Bag-1 indirectly interferes with the binding of Hop to Hsp70 (Gebauer *et al.* 1998). Bag-1, therefore, together with other chaperones and co-chaperones may be involved at different steps of protein folding.

Bag-1 is a family of proteins encoded by the same mRNA through the use of alternative translation-initiation sites. The largest isoform (Bag-1L) is translated from a noncanonical CUG codon followed by an in-frame downstream AUG start sites giving rise to Bag-1M, Bag-1S and p29 (Takayama *et al.* 1998; Yang *et al.* 1998). Bag-1L contains an NLS that allows this protein to be localized in the nucleus while the other Bag-1 proteins are mainly cytoplasmic.

The Bag-1 proteins are characterized by a strong binding to Hsp70 molecular chaperone through their carboxyl-terminal sequences. They act as nucleotide exchange factors of Hsp70 and are negative regulators of the refolding activity of

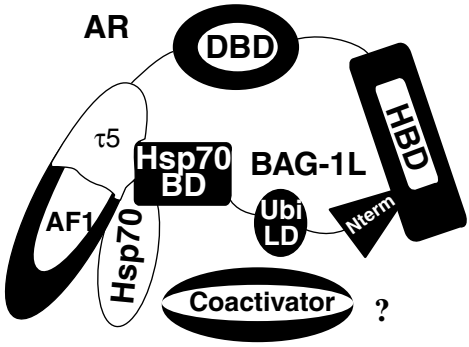


Fig. 2.4 A schematic representation of the structure of the AR-Bag-1L-Hsp70
Indicated are the domains of the AR consisting of the NH_2-terminal transactivation function AF1, the DNA binding domain (DBD) and the hormone binding domain (HBD). The site for the transactivation function $\tau 5$ is depicted in AF1. Presented in the figure are the interactions of the NH_2 and COOH-terminal domains of Bag-1L with the HBD of the AR and the ATPase domain of Hsp70 as well as the $\tau 5$ of the AR. This complex is required for the recruitment of coactivators but the exact binding sites of these molecules to the complex have not been determined.

this chaperone (Bimston *et al.* 1998; Höhfeld and Jentsch 1997; Takayama *et al.* 1997).

Bag-1L enhances the transactivation function of the AR (Froesch *et al.* 1998). It does so by using its Hsp70 binding domain to interact with the $\tau 5$ domain at the NH_2-terminus of the AR. In addition it interacts with the HBD of the AR through its N-terminal sequence (Shatkina *et al.* 2003). Thus it may function as a bridging factor to bring the NH_2- and COOH-terminal regions of the receptor together (Fig. 2.4). The AR, Hsp70 and Bag-1L are recruited to the androgen response element on the prostate specific antigen (PSA) gene (Shatkina *et al.* 2003). However, the molecular details as to how Hsp70 and Bag-1L enhance the transactivation function of the AR are still under investigation.

In addition to Bag-1L, the molecular chaperone Cdc37 specifically interacts with the HBD of the AR and plays a role in the transactivation function of the receptor in a manner that is not compatible with hormone binding. It appears that it functions at a late stage in receptor activation and may be related to the conversion of the ligand bound but inactive receptor into an active state but its exact mode of action is not clear. (Fliss *et al.* 1997; Rao *et al.* 2001).

2.4.2 Androgen response elements

Upon hormone binding, the AR is translocated from the cytoplasm into the nucleus where it uses its DNA binding domain to interact as a homodimer to specific

DNA sequences termed androgen response elements (AREs). These elements are generally located at the promoter or enhancer regions of AR target genes such as probasin (Rennie *et al.* 1993), prostate binding protein (PBP) (Claessens *et al.* 1989; 1993), glandular kallikrein-2 (hKLK2) (Murtha *et al.* 1993), prostate specific antigen (PSA) (Riegman *et al.* 1991) and many others (for review see Chang *et al.* 1995).

The consensus DNA-binding site for the AR is made up of two imperfect palindromic 6-base-pair (bp) elements (inverted repeats) separated by a 3-bp spacer: 5'-GG(A/T)ACAnnnTGTTCT-3' (Roche *et al.* 1992). Such a binding site can also be recognized by the progesterone, glucocorticoid and mineralocorticoid receptors. Besides this conventional ARE, specific sequences bound by the AR have been identified in a random sequence selection assay and in androgen-regulated genes (Adler *et al.* 1993; Claessens *et al.* 1996; Rennie *et al.* 1993; Rundlett and Miesfeld 1995; Verrijdt *et al.* 1999; 2002; Zhou *et al.* 1999). These motifs are partial direct repeats of the canonical 5'-TGTTCT-3' hexamer. AR binds this direct repeat possibly by dimerizing on the DNA in a "head-to-tail" conformation, while the consensus ARE receptor is bound in the dimer conformation "head-to-head" (Verrijdt *et al.* 2003). Binding to DNA is followed by interaction of the receptor with components of the basal transcription machinery such as TFIIH (Lee *et al.* 2000), TFIIF (Reid *et al.* 2002), TBP (McEwan and Gustafsson 1997), sequence specific transcription factors (Ning and Robins 1999), and different cofactor proteins. This leads to up- or downregulation of transcription of the target genes (Quigley *et al.* 1995; Tsai and O'Malley 1994).

In addition to the activation of gene expression, the AR is known to repress the expression of a number of genes (Léger *et al.* 1987; Persson *et al.* 1990) and signaling by β-catenin/TCF (Chesire and Issac 2002) but the mechanisms used are less well understood. A number of studies have produced the following results to describe negative regulation of gene expression by the AR.

Deletion and protein binding studies have shown that the NH_2-terminus of the AR is required for the inhibition of TGF-β signaling through binding of the AR to Smad3. This inhibits the interaction of Smad3 to Smad-binding element in TGF-β regulated promoters (Chipuk *et al.* 2002). NH_2-terminal region of the AR has also been reported to bind to Ets-related transcription factors in the inhibition of matrix metalloproteinase gene expression (Schneikert *et al.* 1996). Furthermore NH_2-terminal and COOH-terminal regions of the AR have been shown to form a complex with the pro-apoptotic protein forkhead and rhabdomyosarcoma (FKHR) to block the binding of this factor to its DNA response elements. This suppresses FKHR's transcriptional activity and its ability to regulate Fas ligand expression and to induce apoptosis and cell cycle arrest of prostate cancer cells (Li *et al.* 2003). Thus, although a number of data exist on how the AR downregulates gene expression, so far, no one particular region in the receptor has been identified for the repression.

The AR seems to use all its regulatory domains in protein-protein interactions to either inhibit the DNA binding activity or the transactivation function of a number of transcription factors without necessarily binding to DNA.

2.4.3 Co-activators and co-repressors

Activation of transcription by AR is regulated by a number of cellular proteins that interact with the receptor. The best characterized are the p160 SRC (steroid receptor co-activator) family members: SRC-1/NCoA-1 (Alen *et al.* 1999; Bevan *et al.* 1999), SRC2/GRIP1 (glucocorticoid receptor-interacting protein 1) /TIF2 (transcription intermediary factor 2) (Berrevoets *et al.* 1998; Kotaja *et al.* 2002a; Shang *et al.* 2002), and SRC3/ACTR (activator of the thyroid and retinoic receptor) / AIB1 (amplified in breast cancer)/pCIP (p300/CBP/co-integrator-associated protein)/RAC3 (receptor-associated co-activator 3) /TRAM 1(thyroid hormone receptor activator molecule 1) (Anzick *et al.* 1997; Chen *et al.* 1997; Li *et al.* 1997; Torchia *et al.* 1997), CREB-binding protein (CBP/p300) (Smith *et al.* 1996; Aarnisalo *et al.* 1998; Fronsdal *et al.* 1998), and P/CAF (CBP/p300 associated factor) (Reutens *et al.* 2001). AR recruits p160 proteins, CBP/p300 and P/CAF to the promoter of target genes (Berrevoets *et al.* 1998; Shang *et al.* 2002). These proteins possess histone acetyltransferase activity and acetylate both histones and steroid receptors, including the AR (Glass and Rosenfeld 2000; Fu *et al.* 2000; McKenna and O'Malley 2002). Acetylation of histone tails results in relaxation of chromatin packaging and thereby facilitates gene transcription. Acetylation of AR on the other hand has been shown to be essential for the transcription activation function of the receptor (Fu *et al.* 2000).

Other proteins, reported to modulate transactivation function of AR through ill-defined pathways are ARA70 (Yeh and Chang 1996), androgen receptor trapped clone 27 (ART-27) (Markus *et al.* 2002), FHL2 (Müller *et al.* 2000), ARA54 (Kang *et al.* 1999), Tip60 (Brady *et al.* 1999), Ubc9 (Poukka *et al.* 1999), ARIP3 (AR-interacting protein 3) (Kotaja *et al.* 2002b), cyclin E (Yamamoto *et al.* 2000), TRAP220, TRAP170 and TRAP100 subunits of the thyroid hormone receptor-associated protein (TRAP)-Mediator complex (Wang *et al.* 2002), histone methyltransferase CARM1 and β-Catenin (Koh *et al.*, 2002; Yang *et al.*, 2002; Truica *et al.*, 2000), the breast cancer susceptibility gene 1 (BRCA1) (Park *et al.* 2000; Yeh *et al.* 2000). Some of these factors, for example ARIP3 and ARA54 act synergistically with the p160 family members, most likely as bridging molecules for the AR and proteins possessing HAT activity (Kang *et al.* 1999; Kotaja *et al.* 2002b). Nonetheless, for most co-activator proteins, their mechanism of action is not yet clear.

Interaction of the nuclear receptor with co-activators is mediated through the transcriptional activation domains in the receptors. This includes AF1 in the NH_2-terminal domain and AF2 in the HBD. Co-activators interact with the AF2

hydrophobic surface in the HBD through conserved amphipathic alpha helical LXXLL motifs, where L is leucine and X is any amino acid (Le Douarin *et al.* 1996; Heery *et al.* 1997). However, it was shown that SRC1 mutant with disrupted LXXLL motif, deficient in binding to the HBD of other nuclear receptor, is still capable of potentiating the transactivation by the AR (Bevan *et al.* 1999), indicating that regulation of the transactivation function of the AR by SRC1 must occur through another domain of the receptor. Recently p160 co-activators SRC1 and TIF2 have been shown to interact directly with the AF1 function in the AR (Berrevoets *et al.* 1998; Bevan *et al.* 1999). This interaction is mediated by the τ5 domain in the AF-1 of the AR. AF2, on the other hand, demonstrated a reduced ability to recruit p160 coactivators (He *et al.* 1999). Thus, in contrast to other nuclear receptors, recruitment of the co-activators by the AR occurs primarily through the N-terminal AF-1 transactivation domain.

In addition to coactivators, other groups of proteins exist that regulate the activity of the AR in a negative way. These include AP-1 (Murtha *et al.* 1997; Sato *et al.* 1997), NFκB (Palvimo *et al.* 1996), TR4 (testicular orphan receptor 4) (Lee *et al.* 1999), HBO1 (histone acetyl transferase binding to origin recognition complex 1) (Sharma *et al.* 2000) and AES (amino-terminal enhancer of split), a member of the highly conserved Groucho/TLE family of corepressors (Yu *et al.* 2001). The molecular mechanisms that underlie the negative regulation of AR action by these proteins have remained mostly elusive.

2.5 Cross-talk of androgen receptor and growth factor signaling pathways

2.5.1 Rapid non-transcriptional action of the androgen receptor

Apart from its action as transcriptional factor, the AR has a function that does not require transport of the receptor into the nucleus. This process is manifested within seconds to a few minutes, in contrast to the other genomic activity of the receptor that takes 30–60 min. Thus these effects are too rapid to be due to changes at the genomic level and are therefore termed non-genomic or rapid nontranscriptional action of androgens (Cato and Peterziel 1998; Cato *et al.* 2002).

These effects range from activation of mitogen-activated protein kinases (MAPKs), adenylyl cyclase, protein kinase C, to activation of heterotrimeric guanosine triphosphate-binding proteins. The effects are diverse and are sometimes inhibited by classical androgen antagonists but are sometimes resistant to these ligands. The effects can also be mediated by androgens linked to large protein moieties making them unable to traverse the plasma membrane. This has therefore led to the idea that these rapid actions of androgens may be mediated by a novel type of membrane-bound androgen receptors. The identity of these molecules remains elusive.

The rapid action of androgens can be classified into two groups: rapid effects of androgens in receptor-negative cells and rapid effects in cells that contain the classical AR. In the first class of response, androgens are reported to increase intracellular levels of calcium in cells that lack intracellular AR such as mouse macrophages and splenic T cells (Benten *et al.* 1997; 1999). This response is thought to be mediated by a novel type of membrane-bound AR. However, recent studies show that cells thought to lack AR such as NIH 3T3 because they do not show transactivaton function in response to androgens, nevertheless possess low levels of classical AR (identified by PCR), enough to mediate rapid action of androgens (Castoria *et al.* 2003).

Cells that contain classical AR such as LNCaP or osteoblasts (Lieberherr and Grosse 1994; Peterziel *et al.* 1999) mediate rapid action of androgens. This was demonstrated in experiments in which ERK-1 and ERK-2 were shown to be activated in receptor negative PC3 or COS-7 following transfection of the AR and androgen treatment (Peterziel *et al.* 1999; Migliaccio *et al.* 2000).

The most extensively analysed mechanism of the rapid action mediated by the AR occurs through the activation of c-Src. Rapid activation of ERK-1 and ERK-2 by the androgen dihydrotestosterone was blocked by the Src family tyrosine kinase inhibitor PP1 (Migliaccio *et al.* 2000). Furthermore embryonic fibroblasts derived from Src-/- mice when transfected with the AR failed to show a rapid activation of ERK-1 and ERK-2 in response to androgen, confirming the role of Src in the AR. At the mechanistic level, the AR is thought to activate Src by binding to the SH3 domain of this protein. This activation process is also aided by the α or β-isoforms of the estrogen receptor (ERα of ERβ). These receptors are thought to bind to the SH2 domain of c-Src to activate this kinase together with the AR. In cells expressing both the AR and ER, activation of ERK-1 and ERK-2 by DHT can be inhibited by antiestrogen and the rapid effect of estrogen can be inhibited by antiandrogen (Migliaccio *et al.* 2000). This effect of ER and AR can explain recent reports of cross talk between estrogen and androgen in rapid action of the AR and ER (Kousteni *et al.* 2001). The interaction of AR with the SH3 domain of c-Src to activate ERK-1 and ERK-2 was however not observed when both proteins were extensively purified (Boonyaratanakornkit *et al.* 2001). This argues in favour of a bridging protein(s) playing a role in this interaction. The AR is also shown to rapidly activate PI3-kinase (Peterziel *et al.* 1999). This effect is most probably brought about by the interaction of the AR with the p85 subunit of PI3-kinase (Simoncini *et al.* 2000; Castoria *et al.* 2003).

The major question asked in the rapid action of androgens is the physiological meaning of this function of the AR. Prostate cell growth and S-phase entry in the cell cycle are consequences of some of the rapid action of the AE as they are inhibited by the MEK-1 inhibitor PD98059 or the Src inhibitor PP1 (Migliaccio *et al.* 2000). Furthermore microinjection of dominant negative Src or MEK-1 constructs into

prostate cancer LNCaP cells inhibited androgen-stimulated BrdU incorporation into DNA (Migliaccio *et al.* 2000). One of the classical examples of a physiological function of the rapid action of steroids is the steroid-induced maturation of *Xenopus* oocytes. Progesterone has been considered the relevant steroid controlling maturation through a non-transcriptional action. However, it has recently been shown that androgens are equally potent activators of maturation relative to progesterone and that they are more abundant in serum and ovaries of chorionic growth hormone-stimulated frogs (Lutz *et al.* 2001). Progesterone is rapidly converted to the androgen androstenedione in isolated oocytes by the enzyme CYP17. RNA interference and oocyte maturation studies indicated that the androgen-induced maturation was mediated by the *Xenopus* AR in a transcription-independent fashion, perhaps by altering G protein-mediated signaling (Lutz *et al.* 2003). Interestingly, only testosterone and androstenedione were potent inducers of oocyte maturation and not dihydrotestosterone or the synthetic androgen R1881, indicating that the type of ligand could have an important effect in mediating the rapid action of androgen.

2.5.2 Ligand independent activation of the androgen receptor

In the absence of ligand, several molecules or signal transduction cascades have been shown to activate the transcriptional activity of the AR. For example the protein kinase C (PKC) activator 12-O-tetradecanoylphorbol-13 acetate enhanced AR activity 10–12-fold in the absence of androgen (Darne *et al.* 1998). In androgen depleted LNCaP prostate cells, stimulation of the transactivation by the protein kinase A activator forskolin was shown to require the N-terminal region of the receptor (Nazareth and Weigel 1996; Sadar 1999). Other signaling factors such as insulin-like growth factor I, epidermal growth factor and keratinocyte growth factor or the inhibitor of phosphotyrosine phosphatases vanadate are all reported to activate the AR (Culig *et al.* 1994; Ikonen *et al.* 1994; Reinikainen *et al.* 1996). In most of the cases, the regulatory pathway that leads to the activation of the AR is not known.

More mechanistic evidence on how peptide hormones or their receptors modulate the action of the AR comes from studies on the ligand independent activation of the AR by HER-2/Neu (Craft *et al.* 1999b; Wen *et al.* 2000). In this regulatory pathway HER-2/Neu activates Akt (protein kinase B) to promote prostate cancer cell survival and growth in the absence of androgen. Akt specifically binds to the AR and phosphorylates serine 213 and 791 of the AR (Wen *et al.* 2000). Exchange of these serine residues into alanine prevented phosphorylation by Akt (Wen *et al.* 2000). It is, however, not clear whether these mutated sites on the AR are the only parameters that mediate the activation of the AR by Akt. The effect of the overexpression of Akt of HER-2/Neu on the mutated sites was not analyzed (Fig. 2.5).

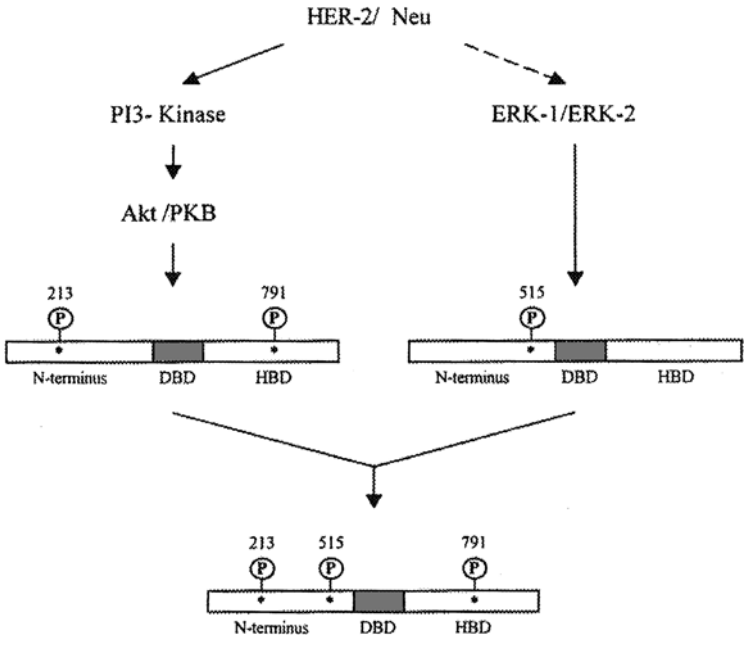


Fig. 2.5 Signaling pathway of HER-2/Neu in prostate cancer
A scheme showing two different pathways used by HER2/Neu to activate the androgen receptor. Also shown are the sites at the NH_2- and COOH-terminal regions in the AR that are phosphorylated as a result of the activation of the signaling pathways. These phosphorylated sites are responsible for enhancing the transactivation function of the receptor.

In an alternative pathway, HER2/Neu-induced activation of the AR is shown to function through activation of the ERK-1 and ERK-2 signaling pathway since this effect is sensitive to PD98059, the inhibitor of the MAP kinase cascade, and to the MAP kinase phosphatase MKP-1 (Yeh *et al.* 1999). Evidence for the involvement of ERK-1 and ERK-2 in the HER-1/Neu is the demonstration of a MAP kinase recognition site at amino acids 511–515 (Fig. 2.5). Mutation of the serine residue at this site into alanine abolished the HER-2/Neu-induced AR transactivation (Yeh *et al.* 1999). It is not only the MAP kinase signaling cascade that has been shown to activate the AR in the absence of ligand. The Janus kinase is reported to signal through the signal transducer and activator of transcription 3 (STAT 3) leading to activation of the AR in a mechanism that is still not clearly understood (Chen *et al.* 2000). It is, however, clear that activation of the AR by interleukin-6 (Hobisch *et al.* 1998) may occur via activation of JAK-2 and later activation of STAT 3 (Chen *et al.* 2000).

Bombesin/gastric-releasing peptide family of neuropeptides act as survival and migratory factors for androgen-independent prostate cancers. These neuropeptides exert their effect via the induction of the transactivation function of the AR but

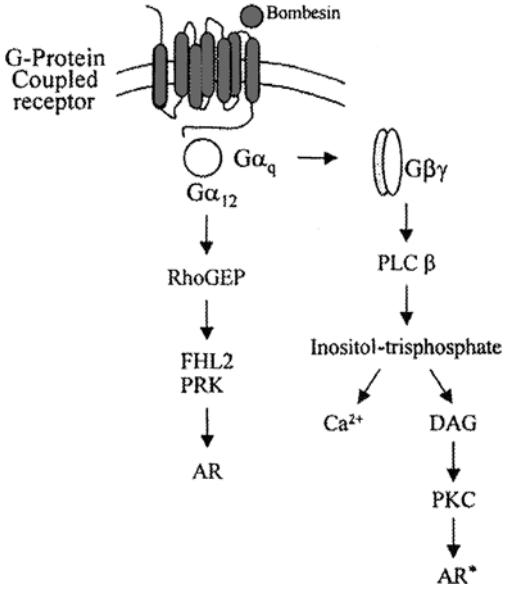


Fig. 2.6 Signal transduction pathway of bombesin to the androgen receptor
A schematic diagram illustrating how activation of G-protein coupled receptor can lead to the activation of the androgen receptor.

they do not directly bind the AR (Lee *et al.* 2001). Upon binding to their receptors, bombesin and neurotensin activate the AR through engagement of G proteins (Gαq or Gα12) and a cross-talk of G-protein tyrosine kinases. Both bombesin and neurotensin bind G-protein coupled receptors. The engagement of Gαq to the receptor liberates Gβγ which activates phospholipase β (PLCβ). PLCβ produces inositol trisphosphates which mobilizes Ca^{++} from internal stores and diacylglycerol which in turn activates PKC. PKC has been shown to activate the AR (Darne *et al.* 1998) (Fig. 2.6). A recent study has shown that bombesin also activates Gα12 which directly associates with RhoGEF, leading to the activation of the small G-protein Rho. Activation of Rho family of proteins enhances transactivation by the AR via activation of the LIM-only coactivator of the AR, FHL2 or the signal transduction pathway through the protein kinase C-related kinase (Müller *et al.* 2002; Metzger 2003).

2.6 Androgen receptor function in prostate cancer

2.6.1 Prostate development

The prostate is the prototype of a hormone-dependent organ. During embryogenesis dihydrotestosterone triggers its development from the urogenital sinus. In this process, the interaction of the stromal and the epithelial compartments of the

prostate gland are of crucial importance. The AR is first expressed in the stromal cells, which makes the cells responsive to dihydrotestosterone to stimulate proliferation and determine differentiation of the epithelial cells in a paracrine manner through the secretion of growth factors (Cunha 1984; 1992; Kratochwil 1986). Later, the AR is expressed in the epithelial cells and androgens can directly stimulate the growth of this cell type as well. The prostate finally grows to the normal size of about 20 cm^3 coupled with the rise of serum levels of androgen that occurs during puberty with a prostatic weight doubling time of 2.8 years (Coffey and Isaacs 1981).

Growth and function of the prostate are critically dependent on the presence of androgens and the function of the AR. The major androgen required for this process is DHT. Its concentration is about 10 times higher within the prostate than the concentration of testosterone (Lamb *et al.* 1992) and in case of an inability to produce DHT – for example due to a defect of the 5α-reductase enzyme – the prostate does not develop, even if testosterone levels are normal (Griffin and Wilson 1989; Thigpen *et al.* 1992). In addition to the normal level of DHT, AR function is critical. Mutations that impair ligand activation or receptor function as a transcription factor also result in lack of prostate development (McPhaul and Griffin 1999).

2.6.2 Androgen receptor function and prostate disease

The human prostate is a major site of disease, especially in elderly men. An increasing number is affected by prostate cancer and/or benign prostate hyperplasia (BPH). Established risk factors are the presence of androgens and age (Cook and Watson 1968). In addition, genetic factors and environmental influences such as high saturated fat, low fruit/vegetable diet and decreased sunlight exposure (vitamin D production) and life style also influence the risk for prostate disease (Stanford *et al.* 1999). In benign prostate hyperplasia, the presence of the androgen DHT that stimulates prostate growth through androgen receptor activation seems to be critical and reduction of DHT levels by inhibition of 5α-reductase is a successful therapy (Bartsch *et al.* 2000). In addition, alterations of the AR signaling, function and structure are associated with the progression of prostate cancer from a hormone-sensitive to a therapy-refractory state.

2.6.3 Androgen ablation therapy of prostate cancer

Androgen withdrawal induces programmed cell death (apoptosis) in prostate cells resulting in prostate tissue involution and, after some time, only a rudimentary prostate is left that is composed mainly of stromal cells (Kyprianou and Isaacs 1988; English *et al.* 1989). This process is reversible. Re-stimulation with androgens results in rapid proliferation and growth of the gland to its adult size. When rats are castrated one can observe massive induction of programmed cell death

starting about 24–48 hours later and continuing 7–10 days (Denmeade *et al.* 1996). Associated with androgen withdrawal is a rapid increase in expression of transforming growth factor-ß (TGF-ß), an inhibitor of prostate cell proliferation, and of testosterone-repressed message-2 (Kyprianou and Isaacs 1989). The latter encodes a glycoprotein also known as clusterin or sulphated glycoprotein-2 (SGP-2) that acts as a chaperone and has antiapoptotic properties in prostate tumor cells (Sensibar *et al.* 1995; Humphreys *et al.* 1999). Recent fine dissection of the events occurring after androgen withdrawal in a mouse model revealed that a hypoxia response seems to be induced and several cell types are involved (Shabsigh *et al.* 2001). Apoptosis is first induced in the endothelial cells of the blood vessels in the prostate, followed by the epithelial cells and finally the stromal cells (Buttyan *et al.* 2000).

Induction of programmed cell death in prostate cells by withdrawal of androgenic stimulation is the basis for the treatment of non-organ-confined prostate cancer. Although the methods of androgen withdrawal have changed over the years, the basic principle has remained the same since introduction of androgen ablation by Charles Huggins, who received the Nobel Prize for his pioneering work on prostate cancer treatment (Huggins and Hodges 1941; Huggins and Stevens, 1940). In the past, surgical castration (orchiectomy) or chemical castration by high-dose estrogen treatment were used, whereas nowadays the preferred choice of androgen ablation is treatment with gonadotropin-releasing hormone (Gn-RH) agonists that block LH production and testicular testosterone biosynthesis (Afrin and Ergul 2000; Auclerc *et al.* 2000; DiPaola *et al.* 2001). Sometimes GnRH analogs are combined with antiandrogens that block the androgen receptor (Kuil and Mulder 1994) to achieve complete androgen blockade (Crawford *et al.* 1999; Geller 1991; Labrie 1995; 1998). This combination eliminates testicular androgens and, in addition, inhibits activation of the androgen receptor by adrenal androgens (mainly dihydroepiandrosterone, its sulphate and androstenedione) that contribute about 10% of total androgen activity (Leewansangtong and Crawford 1998). Commonly used antiandrogens are the steroid derivative cyproterone acetate, and the nonsteroidal antiandrogens flutamide, nilutamide, and bicalutamide.

The major problem in treating advanced prostate cancer is that all these treatment methods are only effective as long as the tumors grow androgen-dependently or are at least androgen sensitive. Almost all tumors will progress to an androgen-independent, hormone-refractory state during treatment, thus rendering all common therapies ineffective. Treatment-resistance develops after about two years in the mean. However, there are significant individual differences depending on the biological nature of the tumors. It was postulated that androgen ablation would provide a selective pressure for androgen-independently growing cells that will survive androgen withdrawal by adaptation to the condition of androgen shortage. These cells are those responsible for tumor recurrence and formation of distant

metastasis. In the last few years increasing experimental evidence has accumulated showing that changes in androgen receptor signaling are crucial in this process (Culig *et al.* 2002; Eder *et al.* 2001).

2.6.4 Androgen receptor involvement in failure of androgen ablation therapy

Initially, it was believed that the failure of androgen ablation therapy is due to a loss of AR, the target of this therapy (Coffey and Isaacs 1981). This hypothesis was based on findings in the rat Dunning tumor system and human prostate cancer cell lines. In these models sensitivity towards androgen withdrawal correlates with the presence of AR protein (Diamond and Barrack 1984; Schuurmans *et al.* 1989; Tilley *et al.* 1990b). Cell lines that inactivate or lose the AR gene are not affected by ablation therapy and are more aggressive than their AR expressing counterparts (Isaacs *et al.* 1982). However, immunohistochemistry data unequivocally revealed that this model does not reflect the situation in the human cancer patient. Now it is generally accepted that the AR is not lost during prostate cancer progression from an androgen ablation-sensitive to an insensitive tumor state. Primary prostate tumors, as well as locally recurrent, therapy-resistant tumors (van der Kwast *et al.* 1991; Ruizeveld de Winter *et al.* 1994), lymph node metastasis (Hobisch *et al.* 1995a), and distant metastasis (Hobisch *et al.* 1995b) all express the androgen receptor and there is no correlation with the response to ablation therapy. The only difference that was described is a more heterogeneous expression pattern (Buchanan *et al.* 2001; Culig *et al.* 2003; De Winter *et al.* 1990; Miyamoto *et al.* 1993; Marcelli *et al.* 2000; Segawa *et al.* 2002).

To study the molecular events that occur during the transition from an androgen-dependent to an androgen-independent state, androgen ablation was simulated in a cell culture system using the prostate cancer cell line LNCaP. These cells, derived from a lymph node metastasis of a prostate cancer patient, express a high amount of AR and grow in an androgen-sensitive manner and thus represent early prostate cancer (Horoszewicz *et al.* 1983). They adapt to the condition of low androgen abundance during long-term culture in steroid-depleted medium and after some months, they change their phenotype to represent cells that are androgen-independent (Culig *et al.* 1999; Gao *et al.* 1999; Kokontis *et al.* 1994). They then became hypersensitive to very low concentrations of androgens and their growth-response curve is shifted to lower concentrations of androgens by at least one order of magnitude (Culig *et al.* 1999; Kokontis *et al.* 1994). When the duration of culture in steroid-depleted medium is extended, they even become inhibited by testosterone and stimulated by an antiandrogen (Culig *et al.*, 2000; Kokontis *et al.* 1998).

A change that becomes obvious in the long-term androgen ablated LNCaP cells is a 3–4 fold elevated androgen receptor level and enhanced androgen receptor

transcriptional activity as revealed by reporter gene assays (Culig *et al.* 1999). A similar antagonist to agonist switch in LNCaP cells was also found after long-term treatment of these cells with tumor necrosis factor alpha (TNFα) (Harada *et al.* 2001). The switch of an antiandrogen to an AR activator – in this case this is observed for the antiandrogen bicalutamide – is also characteristic for the situation in patients, where prostate tumors escape androgen ablation therapy.

A phenomenon termed "withdrawal syndrome" is observed with all antiandrogens used in therapy. This phenomenon describes the situation whereby cessation of the antiandrogen medication in patients who show a rise in serum PSA during AR blockade leads to an improvement of clinical symptoms and a decrease in PSA serum levels. This effect is observed in about one third of the cases (Caldiroli *et al.* 2001; Scher and Kolvenbag 1997; Wirth and Froschermaier 1997). Although this phenomenon is not completely understood, aberrant activation of the androgen receptor by the antiandrogen is the only plausible explanation (Hara *et al.* 2003; Moul *et al.* 1995).

The finding that antiandrogens can become AR agonists in tumors that escape therapy, together with the observed AR expression in the advanced tumor stages underscores the crucial importance of androgen receptor signaling in all stages of prostate cancer. There have been worldwide efforts in the last decade to shed light on the underlying molecular mechanisms by which the AR promotes growth and survival of hormone-refractory prostate tumors in the absence of androgens (Culig *et al.* 2001; Eder *et al.* 2001; Feldman and Feldman 2001; Grossmann *et al.* 2001). Four different escape scenarios have been established: first, amplification of the AR gene and AR overexpression; second, gain of function AR mutations; third, non-classical activation of the androgen receptor and fourth, changes in coactivators and corepressors that modulate AR transcriptional activity (Fig. 2.7).

Increase of receptor concentration in the tumor cells seems to be one way of circumventing the effects of androgen withdrawal. Fluorescence in situ hybridization studies identified AR gene amplification in about one third of tumors that escaped androgen ablation therapy (Bubendorf *et al.* 1999; Koivisto *et al.* 1996; Visakorpi *et al.* 1995). Amplification is strictly dependent on previous treatment and was never observed in untreated patients, indicating that the androgen ablation most likely selects for tumor cells with AR gene amplification. Analysis of AR expression at the mRNA level by RT-PCR revealed increased AR levels in tumors with gene amplification but also in tumors with non-amplified AR gene (Linja *et al.* 2001). On the whole, AR mRNA levels were six-fold higher in androgen-independent as compared to androgen-dependent tumors and benign prostate tissue (Linja *et al.* 2001). Most interestingly, AR gene amplification was associated with a favorable response to second line androgen ablation treatment, although there was no evidence of an advantage in survival (Palmberg *et al.* 2000).

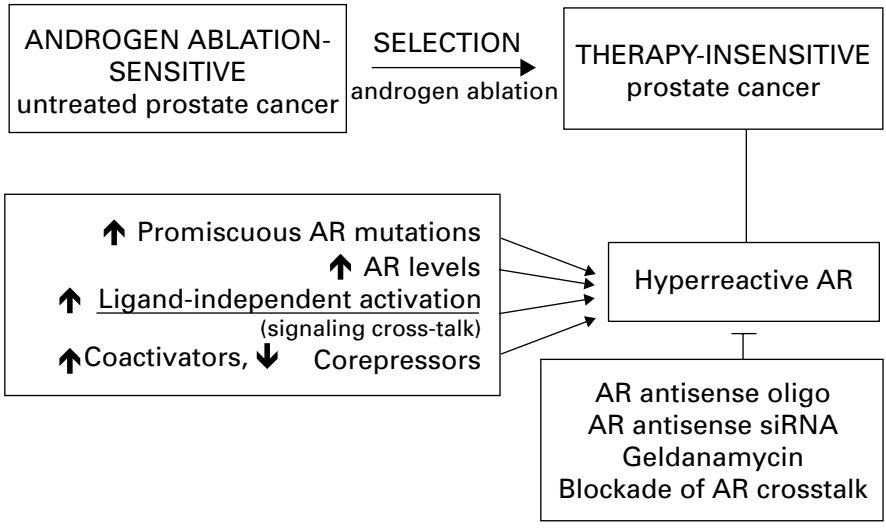


Fig. 2.7 Model of prostate cancer progression to androgen-ablation therapy resistance
The vast majority of prostate tumors is androgen-dependent and responds to androgen ablation therapy for a limited period of time. The selection pressure of androgen deprivation elicits the generation of a hyperreactive AR. The molecular changes involve mutation of the AR to generate promiscuous receptors that are activated by different steroid hormones and/or antiandrogens, overexpression of the AR protein, enhanced ligand-independent activation by cross-talk with other signaling pathways, and dysregulation of expression of AR coactivators and corepressors. New efforts to block the activity of the hyperreactive AR in therapy-resistant prostate cancer are focused on the use of antisense oligonucleotides or siRNA to knock down AR expression, the use of geldanamycin antibiotics that disturb AR interaction with heat shock protein Hsp 90 and thus trigger AR protein degradation, and, the blockade of signal tranduction pathways that activate the AR in a ligand-independent manner.

Another mechanism employed by prostate cancer cells to escape therapy is through mutation of the AR. Some eighty mutations have been found in prostate cancer specimens (Gottlieb *et al.* 2001). The vast majority are point mutations resulting in single amino acid exchanges, a few are mutations that introduce a premature stop codon or affect non-coding regions of the AR gene. With only few exceptions, AR mutations in prostate cancer are somatic (Buchanan *et al.* 2001; Culig *et al.* 2003; Marcelli *et al.* 2000). Most studies on prostate cancer revealed that AR mutations are rare in primary tumors from patients with localized prostate cancer and obviously do not account for prostate carcinogenesis. However, the frequency of AR mutations is much higher, probably up to 50%, in hormone-refractory recurrent tumors and distant metastases (Hyytinen *et al.* 2002; Suzuki *et al.* 1993; Taplin *et al.* 1995; 2003) indicating that AR mutations play a crucial role in progression to metastatic disease.

The predominate properties of mutant receptors found in prostate tumors are loss of androgen specificity and promiscuous activation by different steroids and antiandrogens (Table 2.1). This seems to improve the survival conditions of a tumor during androgen ablation therapy. Among the ligands that activate one or several mutant androgen receptors in prostate tumor cells are estradiol, progesterone, glucocorticoids, adrenal androgens, androgen metabolites and the antiandrogens cyproterone acetate, hydroxyflutamide, nilutinamide and bicalutamide (Hara *et al.* 2003; Suzuki *et al.* 1996; Veldscholte *et al.* 1992a; Wang *et al.* 2000).

Mutant androgen receptors are not only present in tumor tissue but also the majority of established AR expressing tumor cell lines harbor mutant ARs. In the LNCaP prostate cancer cells AR amino acid 877 is mutated (Thr→Ala) and in addition to androgens, this receptor is activated by estradiol, progesterone derivatives and the antiandrogens hydroxyflutamide and nilutamide (Montgomery *et al.* 1992; Veldscholte *et al.* 1990b; 1992a). These hormones stimulate LNCaP cell growth and promote the dissociation of heat-shock proteins from the receptor, which is normally observed in presence of androgens (Veldscholte *et al.* 1990a; 1990b; 1992b). The amino acid site mutated in LNCaP cells seems to be a hot spot for mutations in prostate cancers that escaped androgen ablation therapy (Gaddipati *et al.* 1994).

Besides mutations, another possible reason for androgen-independent prostate tumor growth may be ligand-independent activation of the AR. Cross-talk with growth factors (IGF, EGF, KGF, Her-2/Neu receptor) and interleukin-6 signaling, the protein kinase A and protein kinase B (Akt) pathways activate the AR in prostate cancer cells under the conditions of androgen ablation. These have been described in section 2.5.2.

The androgen receptor, like other steroid receptors, interacts with a number of coregulatory proteins – such as coactivators, corepressors and bridging proteins, which modulate its activity as a transcription factor (for details see section 2.4.3). Dysregulation of expression of such proteins that enhance or suppress AR transcriptional activity also seem to be involved in the escape of tumor cells from therapy.

2.6.5 Androgen receptor as a therapy target in hormone-resistant prostate cancer

Currently there is no efficient method available to treat patients who relapse during androgen ablation therapy and develop an androgen-independently growing tumor. Based on an improved understanding of AR signaling in therapy-refractory prostate cancer, novel therapies are being developed that target AR in advanced tumor cells.

Specific antisense AR oligonucleotides were identified that inhibit AR expression. Treatment of such prostate cancer cells resulted in reduced androgen receptor levels, growth inhibition and reduced PSA production in vitro and in vivo (Eder *et al.* 2000; 2001). Another approach is the use of derivatives of the antibiotic geldanamycin that

Table 2.1 Promiscuous mutant androgen receptors in prostate cancer

Amino acid position	Mutation	Description	Promiscuous Activation	References
670	Glu→Arg	• Primary tumor of untreated patient • Mutation in the AR hinge region	Progesterone Adrenal androgens Hydroxyflutamide	(Buchanan *et al.* 2001; Tilley *et al.* 1996)
715	Val→Met	• Primary tumor of hormone-refractory patient	Progesterone Adrenal androgens DHT metabolites Hydroxyflutamide	(Culig *et al.* 1993; Peterziel *et al.* 1995)
726	Arg→Leu	• Germ line mutation • Overrepresented in Finnish PCa patients	Estradiol	(Elo *et al.* 1995; Mononen *et al.* 2000)
730	Arg→Leu	• Primary tumor	DHT metabolites Hydroxyflutamide	(Newmark *et al.* 1992; Peterziel *et al.* 1995)
874	His→Tyr	• CWR-22 PCa xenograft derived from a bone metastasis	DHEA Estradiol Progesterone Hydroxyflutamide	(Bubley *et al.* 1996; Shao *et al.* 2003; Tan *et al.* 1996; Taplin *et al.* 1995)
877	Thr→Ser	• Primary tumor	Estradiol Progesterone Hydroxyflutamide	(Bubley *et al.* 1996; Taplin *et al.* 1995)
877	Thr→Ala	• LNCaP cell line derived from lymph node metastasis • Several tumor specimens • Mutation hot spot in hormone-refractory tumors	Estradiol Progesterone Hydroxyflutamide Pregnenolone	(Gaddipati *et al.* 1994; Grigoryev *et al.* 2000; Suzuki *et al.* 1996; Suzuki *et al.* 1993; Veldscholte *et al.* 1990a; Veldscholte *et al.* 1992a)
877 701	Thr→Ala Leu→His	• Double mutation • MDA PCa 2b cell line derived from tumor metastasis • Androgen activation decreased	Cortisol Corticosterone C17, C19 and C21 steroids	(Krishnan *et al.* 2002; Matias *et al.* 2002; Zhao *et al.* 1999; Zhao *et al.* 2000)

Of the about eighty androgen receptor gene mutations detected in prostate cancer specimens only some have been analyzed in terms of their functional consequences. Most of these mutations result in promiscuous androgen receptors that, in addition to androgens, are activated by other steroids and/or the antiandrogen hydroxyflutamide. The table lists AR mutations showing promiscuous activation. For a complete list of all AR mutations detected in prostate cancer, see the AR database web site (www.mcgill.ca/androgendb).

interfere with the function of heat shock protein Hsp90 and results in destabilization and degradation of proteins dependent on Hsp90, among them Her-2/Neu receptor and the androgen receptor (Morris and Scher 2000; Solit *et al.* 2002).

Growth factors stimulate cell proliferation and cell survival through activation of the MAP kinase cascade that in turn induces ligand-independent AR stimulation. Therefore, another strategy is the direct inhibition of cell membrane receptors. Burfeind and coworkers, for example, suggested that targeting the IGF-I receptor may be a potential treatment for prostate cancer (Burfeind *et al.* 1996). Membrane receptors, especially those of the EGF receptor family have also been targeted using specific monoclonal antibodies. The EGF receptor seems to be a central component in MAP kinase signaling in prostate tumor cells and provides a valuable target for a therapeutic strategy (Putz *et al.* 1999). EGF receptor blocking antibodies and inhibitors of EGF receptor kinase have already entered clinical trials (Ciardello and Tortora 1998; Herbst 2002; Trump *et al.* 2002). Craft *et al.* (1999a) reported on the effects of monoclonal antibodies blocking the HER-2/neu receptor, another member of the EGF receptor family. This antibody had considerable tumor-inhibiting effects and well-tolerated toxicity in prostate cancer patients. Finally, inhibition of prostate tumor cells was demonstrated by antisense depletion of cyclic AMP-dependent protein kinase (PKA) that also plays a role in ligand-independent activation of the AR (Nesterova and Cho-Chung 2000).

2.7 Pathogenicity of CAG repeat amplification in the androgen receptor

In a recent study, Cram *et al.* (2000) have investigated CAG repeat numbers in the AR of 92 infertile men and their inheritance in 99 female offspring conceived after intracytoplasmic sperm injection (ICSI). It turned out that a stable inheritance in the female offspring could be detected in more than 95 of the cases investigated. A CAG expansion or contraction of the paternal AR allele was observed only in 4 father-daughter pairs. All alterations were within the normal range of CAG repeat number and no phenotypic consequences were observed. The study indicates a frequency of changes to occur in the order of 5%. The range of repeat numbers for the AR to be stably transmitted is presumably between 15 and 28 CAG. CAG repeats beyond 28 might bear the risk of instability, possibly up to a disease-causing length (Zhang *et al.* 1994). Single sperm analysis was performed in a patient with spinobulbar muscular atrophy (SBMA) with a CAG repeat number of 47 in the AR (Zhang *et al.* 1995). The number of CAG repeats equaled the donor's somatic DNA in only 19% of the analyzed sperm, whereas 66% expansion and 15% contractions were observed. The average expansion was approximately 3 repeats ranging from 1 to 25 repeats. These data highlight the instability of CAG repeats, once a distinct threshold is reached.

CAG repeats shift size when inherited paternally, thus influences transmission. However, the molecular basis for the "parent-of-origin effect" association is not known. For Huntingtons' disease (HD) caused by another gene with a CAG repeat expansion, it has recently been shown that mice harboring a mutant HD gene transmit predominantly through the male germ line since the CAG repeat size of the mutant HD gene is different in male and female progeny from identical fathers. Males predominantly expand the repeat, whereas females predominantly contract the repeat (Kovtun *et al.* 2000). This indicates that CAG expansion is influenced by the gender of the embryo and that X- or Y-linked factors influence repair or replication of DNA in the embryo. Gender dependency in the embryo might offer an explanation why CAG repeats expansion from a premutation to a disease primarily occur through the paternal line.

2.7.1 Kennedy syndrome (spinobulbar muscular atrophy – SBMA)

A rare inherited neurodegenerative disease, Kennedy syndrome or spinobulbar muscular atrophy (SBMA), is characterized by progressive neuromuscular weakness resulting from a loss of motor neurons in the spinal cord and brain stem. The onset of this disease occurs in the third to fifth decades of life and is often preceded by muscular cramps on exertion, tremor of the hands and elevated muscle creatine kinase (Kennedy *et al.* 1968). The initial description of Kennedy syndrome also contains one case with gynaecomastia. Subsequent reports confirmed the presence of androgen insensitivity in men with SBMA, showing various degrees of gynaecomastia, testicular atrophy, disorders of spermatogenesis, elevated serum gonadotropins and diabetes mellitus (Arbizu *et al.* 1983; Shimada *et al.* 1995). An alteration in the AR was regarded as a pathophysiological sign for SBMA and the expansion of a CAG tract encoding a polyglutamine (polyQ) stretch within the N-terminal region of the receptor was subsequently recognized as the cause of the disorder by La Spada *et al.* (1991) and confirmed by other studies (Belsham *et al.* 1992; Brooks and Fischbeck 1995). There is an inverse correlation between the polyQ length and the age at onset, or the disease severity adjusted by the age at examination. (Mariotti *et al.* 2000; Lund *et al.* 2001). In addition, the likelihood of gynaecomastia increases with triplet length (MacLean *et al.* 1995). Also cognitive functions, especially spatial cognition, seem to be less effective in Kennedy patients (for review see Zitzmann and Nieschlag 2003).

Since men have only one AR allele, SBMA occurs predominantly in the male gender. Nevertheless, heterozygous female carriers with one normal and one expanded AR allele often present with cramps after muscular exertion and subclinical muscle weakness. In some cases, slight tongue atrophy and sporadic tongue fasciculations can be observed. Mild signs of chronic denervation may be revealed by neurophysiological studies in 50–60% of heterozygous females (Guidetti *et al.* 1996).

Such observations are obviously not dependent on X-chromosome inactivation patterns since random methylation occurs in these patients (Chen *et al.* 1999).

In patients with complete androgen insensitivity syndrome (CAIS), the chance for neuromuscular deficits or respective degeneration is not increased (Quigley *et al.* 1995). This suggests that neurological deficits in SBMA are not caused by a lack of androgen influence but rather that neurotoxic effects are associated with the pathologically elongated polyQ, which possibly causes irregular processing of the AR protein and accumulation of end products.

In molecular terms, the basal and ligand-induced transactivation function of the AR is inversely associated with the length of this CAG repeat chain (Beilin *et al.* 2000). Several investigators have also shown a reduction in transactivation activity with increased number of CAG repeats (Mhatre *et al.* 1993; Chamberlain *et al.* 1994). The modulatory effect on androgen-dependent gene transcription seems to be rather linear over a range from 0 to 200 CAG repeats in in vitro studies (Tut *et al.* 1997).

The NH_2-terminal domain of AR is the target of a number of interacting factors involved in the regulation of its transactivation. In a recent study Irvine *et al.* (2002) could show that although the binding site for the p160 coactivators at the N-terminus of the AR is downstream from the polyQ stretch, increased length of the polyQ up to 42 repeats inhibited both basal and coactivator-mediated AR transactivation activity. A similar result was also obtained with a nuclear G protein ras related protein termed ARA24 (Hsiao *et al.* 1999). Presumably increased polyQ length causes allosteric changes at the N-terminal domain of the AR that negatively influence interactions with coactivators and thus result in reduced transactivation potency.

2.7.2 Characteristic features of the androgen receptor in SBMA

A characteristic feature of the AR with polyQ stretch amplification and all other disorders caused by polyQ tract amplification is the generation of cytoplasmic and/or nuclear aggregates (Becker *et al.* 2000; Cowan *et al.* 2003; Darrington *et al.* 2002; Stenoien *et al.* 1999). These aggregates are thought to form the toxic principle that causes the SBMA disorder (Merry *et al.* 1998). They possibly arise from misfolding of the AR with polyQ stretch expansion and breakdown in proteolytic cleavage of the receptor. The aggregates sequester nuclear receptor coactivators (SRC-1), molecular chaperones and proteasomal proteins (Stenoien *et al.* 1999). The homeostatic disturbance associated with aggregate formation affects normal cellular function and may result in cell death. Claims have been made that it is actually the nuclear but not the cytoplasmic aggregates that contribute to the SBMA disorder (Li *et al.* 1998). Other voices of discontent have been raised against the aggregates in general as the toxic components in the SBMA disorder.

In a study by Simeoni *et al.* 2000, the aggregates did not occur in immortalized motoneuronal NSC34 cells expressing green fluorescence protein (GFP)-tagged AR with a polyQ stretch of 48 under basal conditions. The inclusions were, however, evident after activation of the receptor by testosterone. The kinetics of aggregate formation in the NSC34 cells differed from the rate of survival of the cells. Cell death occurred in the absence of testosterone when inclusions were not detectable. On the other hand, cell survival was increased by hormone addition, a treatment that induced formation of large intracellular aggregates.

In addition to the formation of aggregates, the polyQ tract in SBMA AR appears to enhance the production of C-terminally truncated fragment of the receptor. A 74 kDa fragment was particularly prominent in cells expressing the SBMA AR. From its size, it was deduced that it lacks the hormone binding but retains the DNA binding domain of the receptor. This fragment is suggested to be the toxic factor in the motor neuron disorder and it is proposed to function by initiating the transcription of specific genes (Abdullah *et al.* 1998; Butler *et al.* 1998). The generation of truncated proteins is not only limited to the AR with an expanded polyQ stretch but was observed in other proteins containing an expanded polyQ tract. It was suggested that the generation of the fragments was triggered by caspases and cleavage by this enzyme may represent a common step in the pathogenesis of the polyQ stretch neurodegenerative disorders (Ellerby *et al.* 1999; Wellington *et al.* 1998). The AR, in particular, is cleaved by caspase–3 family of proteases at Asp^{146} and this cleavage is increased during apoptosis. Mutation of this site blocks the ability of the SBMA AR to form perinuclear aggregates (Ellerby *et al.* 1999).

Another hypothesis for a possible mechanism for the SBMA disorder is the activation of mitogen activated protein kinase (MAP kinase) pathways by the AR with polyQ stretch amplification. Inhibitors of the ERK pathway reduced cell death induced by AR with a polyQ stretch of 112. Exchange of the serine residue at position 514 (the ERK phosphorylation site) to an alanine blocked AR-induced cell death and the caspase-3 derived cleavage products (LaFevre-Brent and Ellerby 2003).

2.7.3 Animal models for SBMA

In the past few years it has been particularly difficult to generate transgenic mouse models using heterologous as well as homologous promoters to drive the expression of the AR with an expanded polyQ stretch. The mice generated showed neither neurological symptoms nor other overt pathology (Bingham *et al.* 1995; LaSpada *et al.* 1998).

Recently Abel *et al.* (2001) generated transgenic mice in which a truncated AR was used encompassing a longer polyQ stretch. AR expression was driven by the use of different promoters such as the neurofilament light chain promoter and the prion protein promoter. The neurological phenotypes developed were dependent on the

promoters used and on the expression levels and pattern of the distinct promoters. When the expression of the mutated AR transgene was limited through the use of the neurofilament light chain promoter, the mice developed a phenotype confined to the motor system. There were, however, upper motor neuron manifestations in addition to lower motor neuron disease which is inconsistent with the clinical data of SBMA patients. Furthermore, none of the transgenic mice in the study showed motor loss or muscular atrophy (Abel *et al.* 2001). In another approach, Adachi *et al.* (2001) generated mice with an expanded CAG repeat stretch controlled by the AR promoter. These mice developed progressive neurologic phenotypes of muscular weakness and ataxia but not neuronal cell death, as reported in SBMA. A model closely resembling the human phenotype was established in transgenic mice having a 120 CAG repeat insertion in the AR under the control of a cytomegalovirus promoter. These mice displayed behavioral and motor dysfunction, progressive muscle weakness and atrophy with the loss of alpha motor neurons in the spinal cord. The male mice displayed a progressive reduction of sperm production consistent with testis defects reported in human patients (McManamny *et al.* 2002). These mice represent clinically relevant models of SBMA for the study of the pathogenesis of the disorder and for testing potential therapeutics.

In addition to mouse models, a *Drosophila* model of SBMA has also been established. This is based on the use of the *Drosophila melanogaster* Gal 4-UAS system to target expression of the mutant AR into distinct tissues. This system was used to target AR with different polyglutamine stretch amplifications into the photoreceptor neurons and accessory pigment cells in the developing eye disc under the control of the glass multimer reporter gene promoter. These transgenic flies showed a ligand-dependent depigmentation and degeneration of the *Drosophila* compound eye dependent on the size of the polyQ stretch amplification (Takeyama *et al.* 2002).

Both the mouse and *Drosophila* models of SBMA have shown that the development of the disorder is strictly dependent on androgens (Katsuno *et al.* 2002; Takeyama *et al.* 2002). In the mouse models, the phenotype was markedly pronounced in male transgenics and drastically reduced by castration (Katsuno *et al.* 2002; 2003). Treatment with leuprorelin, a GnRH agonist that reduces testosterone release from the testis, rescued motor function. Moreover, leuprorelin treatment reversed the behavioral and histopathological phenotype that are caused by the increase in serum testosterone level (Katsuno *et al.* 2003). It is thought that castration and the use of GnRH agonists exert their effect through nuclear exclusion of the AR. Intriguingly, the use of antiandrogens such as flutamide, hydroxyflutamide and bicalutamide produced the opposite effect in both mice and *Drosophila* models of SBMA. Rather than the expected antagonism of AR action, these ligands yielded no therapeutic effect but even aggravated the symptoms in some instances (Katsuno *et al.* 2003; Takeyama *et al.* 2002).

2.7.4 Mitigation of the SBMA phenotype

Cell culture and animal model experiments have produced useful information as to how best to mitigate the SBMA phenotype. As molecular chaperones recognize and renature misfolded proteins (aggregate), it was thought that they might reverse the polyQ toxicity. In cell culture experiments, overexpression of molecular chaperones reduced aggregate formation and suppressed apoptosis in neuronal cell models of SBMA (Bailey *et al.* 2002; Ishihara *et al.* 2003; Kobayashi *et al.* 2000). Overexpression of molecular chaperones has also been shown to restore the disrupted eye phenotype obtained in the *Drosophila* SBMA model (Chan *et al.* 2002; Takeyama *et al.* 2002). The molecular chaperones that possess this salvaging function are diverse. They range from hsp70 to hsp40 and hsp105α (Bailey *et al.* 2002; Chan *et al.* 2002; Ishihara *et al.* 2003; Kobayashi *et al.* 2000; Takeyama *et al.* 2002).

One established property of glutamine residues is their ability to act as amine acceptors in transglutaminase-catalysed reactions resulting in proteolytic resistant glutamyl-lysine cross links. Thus the N-terminal fragment of the AR may function as substrate for transglutaminases (Mandrusiak *et al.* 2003). Transglutaminase-mediated isopeptide bonds have been detected in brains of SBMA transgenic mice but not in controls, suggesting the involvement of transglutaminase-catalysed reactions in polyQ disease pathology. Consistent with this, the transglutaminase inhibitor, cystamine, has been shown to prevent aggregates caused by expanded polyQ stretch in the AR or ligand-dependent proteasome dysfunction associated with polyQ amplification (Becker *et al.* 2000; Mandrusiak *et al.* 2003).

Cell death induced by the polyQ amplification can also be mitigated by overexpression of full-length cAMP response element binding protein (CREB)-binding protein (CBP). CBP is one of the several histone acetyltransferases sequestered by polyQ inclusions (McCampbell *et al.* 2000). Thus histone acetylation is reduced in cells expressing amplified polyQ stretches. Reversal of this hypoacetylation by overexpression of CBP or treatment with histone deacetylase inhibitors reduce the cell loss (McCampbell *et al.* 2001).

2.8 Key messages

- The AR has a modular structure composed of three main domains: the NH_2-terminal domain necessary for transactivation, the centrally located DNA binding domain and the COOH-terminal domain required for hormone binding.
- The hormone binding domain exists in a complex with molecular chaperones and co-chaperones that are necessary for providing the receptor with the correct conformation for hormone binding. The molecular chaperones and co-chaperones also play a more active role in transactivation by the receptor by possibly providing the appropriate conformation for coactivators to bind to the receptor.

- The AR exerts a novel action that leads to the activation of several signal transduction cascades. This response is rapid, non-transcriptional and occurs within seconds to minutes, mimicking the action of growth factor receptors.
- In addition to androgens, the AR can also be activated in a ligand-independent manner by MAP kinases, cAMP and IL-6. These factors activate diverse signaling pathways and they all seem to trigger the function of the AR via site-specific phosphorylation events.
- Growth and maintenance of function of the prostate are critically dependent on AR function. Androgen withdrawal or AR inhibition results in induction of apoptosis in prostate epithelial cells and this forms the basis for endocrine therapy of prostate cancer.
- Prostate tumors escape from androgen ablation therapy by developing a hyperreative AR that is activated under the condition of androgen deprivation by means of mutations that generate promiscuous receptors, increased AR expression, enhanced ligand-independent activation or dysregulation of AR activity modulating proteins.
- Spinal and bulbar muscular atrophy (SBMA; Kennedy syndrome) is caused by a pathological amplification of the CAG repeat number and it is associated with the formation of cytoplasmic and/or nuclear aggregates. This disorder is ligand-dependent and requires transport of the mutated receptor into the nucleus.

2.9 REFERENCES

Aarnisalo P, Palvimo JJ, Jänne OA (1998) CREB-binding protein in androgen receptor-mediated signaling. Proc Nal Acad Sci USA 95:2122–2127

Abel A, Walcott J, Woods JA, Duda J, Merry, DE (2001) Expression of expanded repeat androgen receptor produces neurologic disease in transgenic mice. Hum Mol Genet 2:107–116

Abdullah AAR, Trifiro MA, Panet-Raymond V, Alvarado, C, de Tourreil S, Frankel D, Schipper HM, Pinsky L (1998) Spinobulbar muscular atrophy: polyglutamine-expanded androgen receptor is proteolytically resistant in vitro and processed abnormally in transfected cells. Hum Mol Genet 7:379–384

Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, Do J, Sang C, Kobayashi Y, Doyu M, Sobue G (2001) Transgenic mice with an expanded CAG repeat controlled by human AR promoter show polyglutamine nuclear inclusions and neuronal dysfunction without neuronal cell death. Hum Mol Genet 10:1039–1048

Adler AJ, Scheller A, Robins DM (1993) The stringency and magnitude of androgen-specific gene activation are combinatorial functions of receptor and nonreceptor binding site sequences. Mol Cell Biol 13:6326–6335

Alen P, Claessens F, Verhoeven G, Rombauts W, Peeters B (1999) The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 19:6085–6097

Afrin LB, Ergul SM (2000) Medical therapy of prostate cancer: 1999. J S C Med Assoc 96:77–84

Amero SA, Kretsinger RH, Moncrief ND, Yamamoto KR, Pearson WR (1992) The origin of nuclear receptor proteins: a single precursor distinct from other transcription factors. Mol Endocrinol 6:3–7

Anzick SL, Kononen J, Walker RL, Azorsa DO. Tanner MM, Guan XY. Sauter G, Kallioniemi OP, Trent JM, Meltzer PS (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968

Arbizu T, Santamaria J, Gomez JM, Quilez A, Serra JP (1983) A family with adult onset spinal and bulbar muscular atrophy, X-linked inheritance and associated testicular failure. J Neurol Sci 59:371–382.

Auclerc G, Antoine EC, Cajfinger F, Brunet-Pommeyrol A, Agazia C, Khayat D (2000) Management of advanced prostate cancer. Oncologist 5:36–44

Baarends WM, Themmen AP, Blok LJ, Mackenbach P, Brinkmann AO, Meijer D, Faber PW, Trapman J, Grootegoed JA (1990) The rat androgen receptor gene promoter. Mol Cell Endocrinol 74:75–84

Bailey CK, Andriola IFM, Kampinga HH, Merry DE (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. Hum Mol Genet 5:515–523

Bartsch G, Rittmaster RS, Klocker H (2000) Dihydrotestosterone and the concept of 5alpha-reductase inhibition in human benign prostatic hyperplasia. Eur Urol 37:367–380

Becker M, Martin E, Schneikert J, Krug HF, Cato ACB (2000) Cytoplasmic localisation and the choice of ligand determine aggregate formation by androgen receptor with amplified polyglutamine stretch. J Cell Biol 149:255–262

Beilin J, Ball EM, Favaloro JM, Zajac JD (2000) Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines. J Mol Endocrinol 25:85–96

Belsham DD, Yee WC, Greenberg CR, Wrogemann K (1992) Analysis of the CAG repeat region of the androgen receptor gene in a kindred with X-linked spinal and bulbar muscular atrophy. J Neurol Sci 112:133–138

Benten WPM, Lieberherr M, Sekeris CE, Wunderlich F (1997) Testosterone induuces Ca^{2+} influx via non-genomic surface receptors in activated T cells. FEBS Lett 407:211–214

Benten WPM, Lieberherr M, Stamm O, Wrehlke C, Guo Z, Wunderlich F (1999) Testosterone signaling through internalizable surface receptors in anndrogen receptor-free macrophages. Mol Biol Cell 10:3113–3123

Berrevoets CA, Doesburg P, Steketee K, Trapman J, Brinkmann AO (1998) Functional interactions of the AF-2 activation domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF2 (transcriptional intermediary factor 2). Mol Endocrinol 12:1172–1183

Bevan CL, Hoare S, Claessens F, Heery DM, Parker MG (1999) The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1. Mol Cell Biol 19:8383–8392

Bimston D, Song J, Winchester D, Takayama S, Reed JC, Morimoto RI (1998) BAG-1, a negative regulator of Hsp70 chaperone activity, uncouples nucleotide hydrolysis from substrate release. EMBO J 17:6871–6878.

Bingham PM, Scott MO, Wang S, McPhaul MJ, Wilson EM, Garbern JY, Merry DE, Fischbeck KH (1995) Stability of an expanded trinucleotide repeat in the androgen receptor gene in transgenic mice. Nat Genet 9:191–196

Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT, Edwards DP (2001) Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family of tyrosine kinases. Mol Cell 8:269–280

Brady ME, Ozanne DM, Gaughan L, Waite I, Cook S, Neal DE, Robson, CN (1999) Tip60 is a nuclear hormone receptor coactivator. J Biol Chem 274:17599–17604

Brooks BP, Fischbeck KH (1995) Spinal and bulbar muscular atrophy: a trinucleotide-repeat expansion neurodegenerative disease. Trends Neurosci 18:459–461

Brown CJ, Goss SJ, Lubahn DB, Joseph DR, Wilson EM, French FS, Willard HF (1989) Androgen receptor locus on the human X chromosome: regional localization to Xq11–12 and description of a DNA polymorphism. Am J Hum Genet 44: 264–269

Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G, Kallioniemi OP (1999) Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. Cancer Res 59:803–806

Bubley G, Fenton M, Fertig A (1996). The hormonal sensitivity of androgen receptor (AR) mutations derived from human tumors (Abstract) Cancer Res 37:1680A

Bubulya A, Chen SY, Fisher CJ, Zheng Z, Shen XQ, Shemshedini L (2001) c-Jun potentiates the functional interaction between the amino and carboxyl termini of the androgen receptor. J Biol Chem 276:44704–44711

Buchanan G, Greenberg NM, Scher HI, Harris JM, Marshall VR, Tilley WD (2001) Collocation of androgen receptor gene mutations in prostate cancer. Clin Cancer Res 7:1273–1281

Buchanan G, Yang M, Harris JM, Nahm HS, Han G, Moore N, Bentel JM, Matusik RJ, Horsfall DJ, Marshall VR *et al.* (2001) Mutations at the boundary of the hinge and ligand binding domain of the androgen receptor confer increased transactivation function. Mol Endocrinol 15: 46–56

Burfeind P, Chernicky CL, Rininsland F, Ilan J (1996) Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo. Proc Natl Acad Sci USA 93:7263–7268

Burgess LH, Handa RJ (1993) Hormonal regulation of androgen receptor mRNA in the brain and anterior pituitary gland of the male rat. Brain Res Mol Brain Res 19:31–38

Butler R, Leigh PN, McPhaul MJ, Gallo J-M (1998) Truncated forms of the androgen receptor are associated with polyglutamine expansion in X-linked spinal and bulbar atrophy. Hum Mol Genet 7:121–127

Buttyan R, Ghafar MA, Shabsigh A (2000) The effects of androgen deprivation on the prostate gland: cell death mediated by vascular regression. Curr Opin Urol 10:415–420

Caldiroli M, Cova V, Lovisolo JA, Reali L, Bono, AV (2001) Antiandrogen withdrawal in the treatment of hormone-relapsed prostate cancer: single institutional experience. Eur Urol 39 Suppl S2:6–10

Caplan AJ, Langley E, Wilson EM, Vidal J (1995) Hormone-dependent transactivation by the human androgen receptor is regulated by a dnaJ protein. J Biol Chem 270:5251–5257

Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domanico M, Bottero D, Vitale F, Migliaccio A, Auricchio F (2003) Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a nontranscriptional receptor action. J Cell Biol 161:547–556

Cato ACB, Peterziel H (1998) The androgen receptor as mediator of gene expression and signal transduction pathways. Trends Endocrinol Metab 9:150–154

Cato ACB, Nestl A, Mink S (2002) Rapid actions of steroid receptors in cellular signaling pathways. Science's STKE 138 re9

Chamberlain NL, Driver, ED, Miesfeld, RL (1994) The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181–3186

Chamberlain NL, Whitacre DC, Miesfeld RL (1996) Delineation of two distinct type 1 activation functions in the androgen receptor amino-terminal domain. J Biol Chem 271:26772–26778

Chan HYE, Warrick JW, Andriola I, Merry D, Bonini NM (2002) Genetic modulation of polyglutamine toxicity by protein conjugation pathways in Drosophila. Hum Mol Gen 11:2895–2904

Chang CS, Kokontis J, Liao ST (1988a) Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324–326

Chang CS, Kokontis J, Liao ST (1988b) Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 85:7211–7215

Chang C, Saltzman A, Yeh S, Young W, Keller E, Lee HJ, Wang C, Mizokami A (1995) Androgen receptor: an overview. Crit Rev Eukaryot Gene Expr 5:97–125

Chang BL, Zheng SL, Hawkins GA, Isaacs SD, Wiley KE, Turner A, Carpten JD, Bleecker ER, Walsh PC, Trent JM, *et al.* (2002) Polymorphic GGC repeats in the androgen receptor gene are associated with hereditary and sporadic prostate cancer risk. Hum Genet 110:122–129

Chen S, Smith DF (1998) Hop as an adaptor in the heat shock protein 70 (Hsp70) and hsp90 chaperone machinery. J Biol Chem 273:35194–35200

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569–580

Chen RS, Huang CC, Chu NS, Cheng CC, Wei YH (1999) Random X chromosome methylation patterns in the carriers with clinical phenotypic expressions of X-linked recessive bulbospinal neuronopathy. Acta Neurol Scand 1004:249–53

Chen T, Wang LH, Farrar, WL (2000) Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. Cancer Res. 60:2132–2135

Chen C, Lamharzi N, Weiss NS, Etzioni R, Dightman DA, Barnett M, DiTommaso D, Goodman G (2002) Androgen receptor polymorphisms and the incidence of prostate cancer. Cancer Epidemiol Biomarkers Prev 11:1033–1040

Chesire DR, Isaacs WB (2002) Ligand-dependent inhibition of b-catenin/TCF signaling by androgen receptor. Oncogene 21:8453–8469

Chipuk JE, Cornelius SC, Pultz NJ, Jorgensen JS, Bonham MJ, Kim S-J, Danielpour D (2002) The androgen receptor represses transforming growth-b signaling through interaction with Smad3. J Biol Chem 277:1240–1248

Choong CS, Wilson EM (1998) Trinucleotide repeats in the human androgen receptor: a molecular basis for disease. J Mol Endocrinol 21:235–257

Ciardello F, Tortora G (1998) Interactions between the epidermal growth factor receptor and type I protein kinase A: biological significance and therapeutic implications. Clin Cancer Res 4:821–828

Claessens F, Dirckx L, Delaey B, Decourt JL, Hemschoote K, Peeters B, Rombauts W (1989) The androgen-dependent rat prostatic binding protein: comparison of the sequences in the 5' part and upstream region of the C1 and C2 genes and analysis of their transcripts. J Mol Endocrinol 3:93–103.

Claessens F, Celis L, De Vos P, Heyns W, Verhoeven G, Peeters B, Rombauts W (1993) Functional androgen response elements in the genes coding for prostatic binding protein. Ann N Y Acad Sci 684:199–201

Claessens F, Alen P, Devos A, Peeters B, Verhoeven G, Rombauts W (1996) The androgen-specific probasin response element 2 interacts differentially with androgen and glucocorticoid receptors. J Biol Chem 271:19013–19016

Coffey DS, Isaacs JT (1981) Control of prostate growth. Urology 17:17–24

Cook GB, Watson FR (1968) A comparison by age of death rates due to prostate cancer alone. J Urol 100:669–671

Cowan KJ, Diamond MI, Welch WJ (2003) Polyglutamine protein aggregation and toxicity are linked to the cellular stress response. Hum Mol Genet 12:1377–1391

Craft N, Chhor C, Tran C, Belldegrun A, DeKernion J, Witte QN, Said J, Reiter RE, Sawyers CL (1999a) Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-independent tumors through a two-step process. Cancer Res 59:5030–5036

Craft N, Shostak Y, Carey M, Sawyers CL (1999b) A mechanism for hormone-independent prostate cancer through modification of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Medicine 5:280–285

Cram DS, Song B, McLachlan RI, Trounson AO (2000) CAG trinucleotide repeats in the androgen receptor gene of infertile men exhibit stable inheritance in female offspring conceived after ICSI. Mol Hum Reprod 6:861–866

Crawford ED, Rosenblum M, Ziada AM, Lange PH (1999) Hormone refractory prostate cancer. Urology 54:1–7

Culig Z, Hobisch A, Cronauer MV, Cato AC, Hittmair A, Radmayr C, Eberle J, Bartsch G, Klocker H (1993) Mutant androgen receptor detected in an advanced stage of prostatic carcinoma is activated by adrenal androgens and progesterone. Mol Endocrinol 7:1541–1550

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H (1994) Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor, keratinocyte growth factor, and epidermal growth factor. Cancer Res 54:5474–5478

Culig Z, Hobisch A, Hittmair A, Cronauer MV, Radmayr C, Zhang J, Bartsch G, Klocker H (1997) Synergistic activation of androgen receptor by androgen and luteinizing hormone-releasing hormone in prostatic carcinoma cells. Prostate 32:106–114

Culig Z, Hoffmann J, Erdel M, Eder IE, Hobisch A, Hittmair A, Bartsch G, Utermann G, Schneider MR, Parczyk K, Klocker H (1999) Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. Br J Cancer 81:242–251

Culig Z, Hobisch A, Bartsch G, Klocker H (2000) Expression and function of androgen receptor in carcinoma of the prostate. Microsc Res Tech 51:447–455

Culig Z, Klocker H, Bartsch G, Hobisch A (2001) Androgen receptor mutations in carcinoma of the prostate: significance for endocrine therapy. Am J Pharmacogenomics 1:241–249

Culig Z, Klocker H, Bartsch G, Hobisch A (2002) Androgen receptors in prostate cancer. Endocr Relat Cancer 9:155–170

Culig Z, Hobisch A, Erdel M, Bartsch G, Klocker H (2003) Studies on androgen receptor mutations and amplification in human prostate cancer. Methods Mol Med 81:267–275

Cunha GR (1984) Androgenic effects upon prostatic epithelium via tropic influences from stroma. Prog Clin Biol Res 145:81–102

Cunha GR, Alarid ET, Turner T, Donjacour AA, Boutin EL, Foster BA (1992) Normal and abnormal development of the male urogenital tract – role of androgens, mesenchymal-epithelial interactions, and growth factors. J Androl 13:465–475

Darne C, Veyssiere G, Jean C (1998) Phorbol ester causes ligand-independent activation of the androgen receptor. Eur J Biochem 256:541–549

Darrington RS, Butler R, Leigh PN, McPhaul MJ, Gallo J-M (2002) Ligand-dependent aggregation of polyglutamine-expanded androgen receptor in neuronal cells. NeuroReport 13:2117–2120

De Winter JA, Trapman J, Brinkmann AO, Boersma WJA, Mulder E, Schroeder FH, Claassen E, van der Kwast TH (1990) Androgen receptor heterogenity in human prostatic carcinomas visualized by immunohistochemistry. J Pathol 160:329–332

Dedhar S, Rennie PS, Shago M, Hagesteijn C-YL, Yang H, Filmus J, Hawley RG, Bruchovsky N, Cheng H, Matusik RJ, Giguère V (1994) Inhibition of nuclear hormone receptor activity by calreticulin. Nature 367:480–483

Denmeade SR, Lin XS, Isaacs JT (1996) Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. Prostate 28:251–265

Diamond DA, Barrack ER (1984) The relationship of androgen receptor levels to androgen responsiveness in the Dunning R3327 rat prostate tumor sublines. J Urol 132:821–827

DiPaola RS, Kumar P, Hait WN, Weiss RE (2001) State-of-the-art prostate cancer treatment and research. A report from the Cancer Institute of New Jersey. N J Med 98:23–33

Dittmar KD, Pratt WB (1997) Folding of the glucocorticoid receptor by the reconstituted Hsp90-based chaperone machinery. The initial hsp90.p60.hsp70-dependent step is sufficient for creating the steroid binding conformation. J Biol Chem 272:13047–13054

Dittmar KD, Banach M, Galigniana MD, Pratt WB (1998) The role of DnaJ-like proteins in glucocorticoid receptor.hsp90 heterocomplex assembly by the reconstituted hsp90.p60.hsp70 foldosome complex. J Biol Chem 273:7358–7366

Eder I, Culig Z, Ramoner R, Thurnher M, Putz T, Nessler-Menardi C, Tiefenthaler M, Bartsch, G, Klocker H (2000) Inhibition of LNCaP prostate cancer cells by means of androgen receptor antisense oligonucleotides. Cancer Gene Therapy 7:997–1007

Eder IE, Culig Z, Putz T, Nessler-Menardi C, Bartsch G, Klocker H (2001) Molecular biology of the androgen receptor: from molecular understanding to the clinic. Eur Urol 40:241–251

Ellerby LM, Hackam AS, Propp SS, Ellerby HM, Rabizadeh S, Cashman NR, Trifiro MA, Pinsky L, Wellington CL, Salvesen GS, Hayden MR, Bredesen DE (1999) Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. J Neurochem 72:185–195

Elo JP, Kvist L, Leinonen K, Isomaa V, Henttu P, Lukkarinen O, Vihko P (1995) Mutated human androgen receptor gene detected in a prostatic cancer patient is also activated by estradiol. J Clin Endocrinol Metab 80:3494–3500

English HF, Kyprianou N, Isaacs JT (1989) Relationship between DNA fragmentation and apoptosis in the programmed cell death in the rat prostate following castration. Prostate 15:233–250

Faber, PW, van Rooij HC, van der Korput HA, Baarends WM, Brinkmann AO, Grootegoed JA, Trapman J (1991) Characterization of the human androgen receptor transcription unit. J Biol Chem 266:10743–10749

Faber PW, van Rooij HC, Schipper HJ, Brinkmann AO, Trapman J (1993) Two different, overlapping pathways of transcription initiation are active on the TATA-less human androgen receptor promoter. The role of Sp1. J Biol Chem 268:9296–9301

Fang Y, Fliss AE, Robins DM, Caplan AJ (1996) Hsp90 regulates androgen receptor hormone binding affinity in vivo, J Biol Chem 271:28697–28702

Feldman BJ, Feldman D (2001) The development of androgen-independent prostate cancer. Nature Rev Cancer 1:34–45

Fliss AE, Fang Y, Boschelli F Caplan AJ (1997) Differential in vivo regulation of steroid hormone receptor activation by Cdc37p. Mol Biol Cell 8:2501–2509

Freedman LP, Luisi BF, Korszun ZR, Basavappa R, Sigler PB, Yamamoto KR (1988) The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. Nature 334:543–546

Froesch, BA, Takayama S, Reed JC (1998) BAG-1L protein enhances androgen receptor action. J Biol Chem 273:11660–11666

Fronsdal K, Engedal N, Slagsvold T, Saatcioglu F (1998) CREB binding protein is a coactivator for the androgen receptor and mediates cross-talk with AP-1. J Biol Chem 273: 31853–31859

Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati M-L, Pestell RG (2000) p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 275:20853–20860

Fuller PJ (1991) The steroid nuclear receptor superfamily: mechanisms of diversity. FASEB J 5:2243–2249

Gaddipati JP, McLeod DG, Heidenberg HB, Sesterhenn IA, Finger MJ, Moul JW, Srivastava S (1994) Frequent detection of codon 877 mutation in the androgen receptor gene in advanced prostate cancers. Cancer Res 54:2861–2864

Gao M, Ossowski L, Ferrari AC (1999) Activation of Rb and decline in androgen receptor precede retinoic acid-induced apoptosis in androgen-dependent LNCaP cells and their androgen-independent derivative. J Cell Physiol 179:336–346.

Gebauer M, Zeiner M, Gehring U (1998) Interference between proteins Hap46 and Hop/p60, which bind to different domains of the molecular chaperone hsp70/hsc70. Mol Cell Biol 18:6238–6244

Geller J (1991) Review of assessment of total androgen blockade as treatment of metastatic prostate cancer. J Endocrinol Invest 14:881–891

Glass CK, Rose DW, Rosenfeld MG (1997) Nuclear receptor coactivators. Curr Opin Cell Biol 9:222–232

Glass CK, Rosenfeld MG (2000) The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141

Gottlieb B, Beitel LK, Trifiro MA (2001) Variable expressivity and mutation databases: The androgen receptor gene mutations database. Hum Mutat 17:382–388

Griffin JE, Wilson JD (1989) The androgen resistance syndromes: 5a-reductase deficiency, testicular feminization, and related syndromes. 6th ed McGraw-Hill New York

Grigoryev DN, Long BJ, Njar VC, Brodie AH (2000) Pregnenolone stimulates LNCaP prostate cancer cell growth via the mutated androgen receptor. J Steroid Biochem Mol Biol 75:1–10

Grossmann ME, Tindall DJ (1995) The androgen receptor is transcriptionally suppressed by proteins that bind single-stranded DNA. J Biol Chem 270:10968–10975

Grossmann ME, Lindzey J, Blok L, Perry JE, Kumar MV, Tindall DJ (1994a) The mouse androgen receptor gene contains a second functional promoter which is regulated by dihydrotestosterone. Biochemistry 33:14594–14600

Grossmann ME, Lindzey J, Kumar MV, Tindall DJ (1994b) The mouse androgen receptor is suppressed by the 5'-untranslated region of the gene. Mol Endocrinol 8:448–455

Grossmann ME, Huang H, Tindall DJ (2001) Androgen receptor signaling in androgen-refractory prostate cancer. J Natl Cancer Inst 93:1687–1697

Guidetti D, Vescovini E, Motti L, Ghidoni E, Gemignani F, Marbini A, Patrosso MC, Ferlini A, Solime F (1996) X-linked bulbar and spinal muscular atrophy, or Kennedy disease: clinical, neurophysiological, neuropathological, neuropsychological and molecular study of a large family. J Neurol Sci 135:140–148

Hager GL (2000) Understanding nuclear receptor function: from DNA to chromatin to the interphase nucleus. Prog Nucleic Acid Res Mol Biol 66:279–305

Hara T, Miyazaki J, Araki H, Yamaoka M, Kanzaki N, Kusaka M, Miyamoto M (2003) Novel mutations of androgen receptor: a possible mechanism of bicalutamide withdrawal syndrome. Cancer Res 63:149–153

Harada S, Keller ET, Fujimoto N, Koshida K, Namiki M, Matsumoto T, Mizokami A (2001) Long-term exposure of tumor necrosis factor alpha causes hypersensitivity to androgen and anti-androgen withdrawal phenomenon in LNCaP prostate cancer cells. Prostate 46:319–326.

He WW, Fischer LM, Sun S, Bilhartz DL, Zhu XP, Young CY, Kelley DB, Tindall DJ (1990) Molecular cloning of androgen receptors from divergent species with a polymerase chain reaction technique: complete cDNA sequence of the mouse androgen receptor and isolation of androgen receptor cDNA probes from dog, guinea pig and clawed frog. Biochem Biophys Res Commun 171:697–704

He B, Kemppainen JA, Voegel JJ, Gronemeyer H, Wilson EM (1999) Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH_2-terminal domain. J Biol Chem 274:37219–37225

He B, Kemppainen JA Wilson EM (2000) FXXLF and WXXLF sequences mediate the NH_2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 275:22986–22994

He B, Lee LW, Minges JT, Wilson EM (2002) Dependence of selective gene activation on the androgen receptor NH2- and COOH-terminal interaction. J Biol Chem 277:25631–25639

Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733–736

Herbst RS (2002) ZD1839: targeting the epidermal growth factor receptor in cancer therapy. Expert Opin Investig Drugs 11:837–849

Hirai M, Hirata S, Osada T, Hagihara K, Kato J (1994) Androgen receptor mRNA in the rat ovary and uterus. J Steroid Biochem Mol Biol 49:1–7

Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A (1995a) Androgen receptor status of lymph node metastases from prostatic carcinoma. Prostate 29:129–136

Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A (1995b) Distant metastases from prostatic carcinoma express androgen receptor protein. Cancer Res 55:3068–3072

Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, Culig K (1998) Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. Cancer Res 58:4640–4645

Höhfeld J, Jentsch S (1997) GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. EMBO J 16:6209–6216

Höhfeld J, Minami Y, Hartl FU (1995) Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. Cell 83:589–598

Holaska JM, Black BE, Love DC, Hanover JA, Leszyk J, Paschal BM (2001) Calreticulin is a receptor for nuclear export. J Cell Biol 152:127–140

Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP (1983). LNCaP model of human prostatic carcinoma. Cancer Res 43:1809–1818

Hsiao PW, Lin DL, Nakao R, Chang C (1999) The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem 29:20229–20234

Huggins C, Hodges CV (1941) Studies on prostatic cancer: the effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293–297

Huggins C, Stevens R (1940) The effect of castration on benign hypertrophy of the prostate in men. J Urol 43:705–714

Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem 274:6875–6881

Hyytinen ER, Haapala K, Thompson J, Lappalainen I, Roiha M, Rantala I, Helin HJ, Janne OA, Vihinen M, Palvimo JJ, Koivisto PA (2002) Pattern of somatic androgen receptor gene mutations in patients with hormone-refractory prostate cancer. Lab Invest 82:1591–1598

Ikonen T, Palvimo JJ, Kallio PJ, Reinikainen P, Jänne, OA (1994) Stimulation of androgen-regulated transactivation by modulators of protein phosphorylation. Endocrinology 135:1359–1366

Ikonen T, Palvimo JJ, Jänne OA (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear coactivators. J Biol Chem 272:29821–29828

Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 9:267–74

Isaacs JT, Wake N, Coffey DS, Sandberg AA (1982) Genetic instability coupled to clonal selection as a mechanism for tumor progression in the Dunning R-3327 rat prostatic adenocarcinoma system. Cancer Res 42:2353–2371

Ishihara K, Yamagishi NY, Saito Y, Adachi H, Kobayashi Y, Sobue G, Ohtsuka K, Hatayama T (2003) Hsp105a suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. J Biol Chem 278:25143–25150

Jenster G, Trapman J, Brinkmann AO (1993) Nuclear import of the human androgen receptor. Biochem J 293:761–768

Jenster G, van der Korput JA, Trapman J, Brinkmann AO (1992) Functional domains of the human androgen receptor. J Steroid Biochem Mol Biol 41:671–675

Jenster G, van der Korput JA, Trapman J, Brinkmann AO (1995) Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J Biol Chem 270:7341–7346

Johnson BD, Schumacher RJ, Ross, ED, Toft DO (1998) Hop modulates Hsp70/Hsp90 interactions in protein folding. J Biol Chem 273:3679–3686.

Kang HY, Yeh S, Fujimoto N Chang C (1999) Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 274:8570–8576

Katsuno M, Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, Sang C, Kobayashi Y, Doyu M, Sobue G (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 35:843–854

Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, Inukai A, Sobue G (2003) Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med 6:768–773

Kennedy WR, Alter M, Sung JH (1968) Progressive proximal spinal and bulbar muscular atrophy of late onset. Neurology 18:671–680

Kobayashi Y, Kume A, Li M, Doyu M, Hata M, Ohtsuka K, Sobue G (2000) Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. J Biol Chem 275:8772–8778

Koh SS, Li H, Lee YH, Widelitz RB, Chuong CM, Stallcup MR (2002) Synergistic coactivator function by coactivator-associated arginine methyltransferase (CARM) 1 and beta-catenin with two different classes of DNA-binding transcriptional activators. J Biol Chem 277:26031–26035

Koivisto P, Visakorpi T, Kallioniemi OP (1996) Androgen receptor gene amplification: a novel molecular mechanism for endocrine therapy resistance in human prostate cancer. Scand J Clin Lab Invest Suppl 226:57–63

Kokontis J, Takakura K, Hay N, Liao S (1994) Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation. Cancer Res 54:1566–1573

Kokontis JM, Hay N, Liao S (1998) Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for $p27^{Kip1}$ in androgen-induced cell cycle arrest. Mol Endocrinol 12:941–953

Kosano H, Stensgard B, Charlesworth MC, McMahon N, Toft D (1998) The assembly of progesterone receptor-hsp90 complexes using purified proteins. J Biol Chem 273:32973–32979

Kotaja N, Karvonen U, Jänne OA, Palvimo JJ (2002a) The nuclear interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. J Biol Chem 277:30283–30288

Kotaja N, Vihinen M, Palvimo JJ Jänne OA (2002b) Androgen receptor-interacting protein 3 and other PIAS proteins cooperate with glucocorticoid receptor-interacting protein 1 in steroid receptor-dependent signaling. J Biol Chem 277:17781–17788

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC (2001) Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104:719–730

Kovtun IV, Therneau TM, McMurray CT (2000) Gender of the embryo contributes to CAG instability in transgenic mice containing a Huntington's disease gene. Hum Mol Genet 18:2767–2775

Kratochwil K (1986) The stroma and the control of cell growth. J Pathol 149:23–24

Krishnan AV, Zhao XY, Swami S, Brive L, Peehl DM, Ely KR, Feldman D (2002) A glucocorticoid-responsive mutant androgen receptor exhibits unique ligand specificity: therapeutic implications for androgen-independent prostate cancer. Endocrinology 143:1889–1900

Kuil CW, Mulder E (1994) Mechanism of antiandrogen action: conformational changes of the receptor. Mol Cell Endocrinol 102:R1–R5

Kumar MV, Jones EA, Grossmann ME, Blexrud MD, Tindall DJ (1994) Identification and characterization of a suppressor element in the 5'-flanking region of the mouse androgen receptor gene. Nucleic Acids Res 22:3693–3698

Kyprianou N, Isaacs JT (1988) Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 122:552–562

Kyprianou N, Isaacs JT (1989) Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Mol Endocrinol 3:1515–1522

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79

La Spada AR, Peterson KR, Meadows SA, McClain ME, Jeng G, Chmelar RS, Haugen HA, Chen K, Singer MJ, Moore D, Trask BJ, Fischbeck KH, Clegg CH, McKnight GS (1998) Androgen receptor YAC transgenic mice carrying CAG 45 alleles show trinucleotide repeat instability. Hum Mol Genet 7:959–967

Labrie F (1995) Endocrine therapy of prostate cancer: optimal form and timing. J Clin Endocrinol Metab 80:1066–1071

Labrie F (1998) Combined androgen blockade and treatment of localized prostate cancer: A real hope when approaching the year 2000. Endocr Rel Cancer 5:341–351

LaFevre-Bernt MA, Ellerby LM (2003) Kennedy's disease: phosphorylation of the polyQ-expanded form of androgen receptor regulates its cleavage by caspase-3 and enhances cell death. J Biol Chem 278:34918–34924

Lamb JC, English H, Levandoski PL, Rhodes GR, Johnson RK, Isaacs JT (1992) Prostatic involution in rats induced by a novel 5 alpha-reductase inhibitor, SK&F 105657: role for testosterone in the androgenic response. Endocrinology 130:685–694

Langley E, Zhou ZX, Wilson EM (1995) Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 270:29983–29990

Langley E, Kemppainen JA, Wilson EM (1998) Intermolecular NH2-/carboxyl-terminal interactions in androgen receptor dimerization revealed by mutations that cause androgen insensitivity. J Biol Chem 273:92–101

Le Douarin B, Nielsen AL, Garnier JM, Ichinose H, Jeanmougin F, Losson R, Chambon P (1996) A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors. EMBO J 15:6701–6715

Lee Y-F, Shyr C-R, Thin TH, Lin W-J, Chang C (1999) Convergence of two repressors through heterodimer formation of androgen receptor and testicular orphan receptor-4: a unique signaling pathway in the steroid receptor superfamily. Proc Natl Acad Sci USA 96:14724–14729

Lee DK, Duan HO, Chang C (2000) From androgen receptor to the general transcription factor TFIIH. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator. J Biol Chem 275:9308–9313

Lee L-F, Guan J, Qui Y, Kung, H-J (2001) Neuropeptide-induced androgen independence in prostate cancer cells: roles of nonreceptor tyrosine kinases Etk/Bmx, Src, and focal adhesion kinase. Mol Cell Biol 21:8385–8397

Lee SR, Ramos SM, Ko A, Masiello D, Swanson KD, Lu ML, Balk SP (2002) AR and ER interaction with a p21-activated kinase (PAK6). Mol Endocrinol 16:85–99

Leewansangtong S, Crawford ED (1998) Maximal androgen withdrawal for prostate cancer therapy: Current status and future potential. Endocr Rel Cancer 5:325–339

Léger JG, Montpetit ML, Tenniswood MP (1987) Characterization and cloning of androgen-repressed mRNAs from rat ventral prostate. Biochem Biophys Res Commun 147:196–203

Li H, Gomes PJ, Chen JD (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. Proc Natl Acad Sci USA 94:8479–8484

Li M, Nakagomi Y, Kobayashi Y, Merry DE, Tanaka F, Doyu M, Mitsuma T, Hashizume Y, Fischbeck KH, Sobue G (1998) Nonneural nuclear inclusions of androgen receptor protein in spinal and bulbar muscular atrophy. Am J Pathol 153:695–701

Li P, Lee H, Guo S, Unterman TG, Jenster G, Bai W (2003) AKT-independent protection of prostate cancer cells from apoptosis mediated through complex formation between the androgen receptor and FKHR. Mol Cell Biol 23:104–118

Lieberherr M, Grosse B (1994) Androgens increase intracellular calcium concenttration and inositol 1,4,5-trisphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. J Biol Chem 269:7217–7223

Lindzey J, Grossmann M, Kumar MV, Tindall DJ (1993) Regulation of the 5'-flanking region of the mouse androgen receptor gene by cAMP and androgen. Mol Endocrinol 7:1530–1540

Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T (2001) Amplification and overexpression of androgen receptor gene in hormone- refractory prostate cancer. Cancer Res 61:3550–3555

Loy CJ, Sim KS, Young EL (2003) Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. Proc Natl Acad Sci USA 100:4562–4567

Lubahn DB, Joseph DR, Sar M, Tan J, Higgs HN, Larson RE, French FS, Wilson EM (1988a) The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. Mol Endocrinol 2:1265–127

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM (1988b) Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240:327–330

Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM, French FS (1989) Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity [published erratum appears in Proc Natl Acad Sci USA 1990; 87:4411]. Proc Natl Acad Sci USA 86:9534–9538

Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Singer PB (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature 352:497–505

Lumbroso R, Beitel LK, Vasiliou DM, Trifiro MA, Pinsky L (1997) Codon-usage variants in the polymorphic (GGN)n trinucleotide repeat of the human androgen receptor gene. Hum Genet 101:43–46

Lund A, Udd B, Juvonen V, Andersen PM, Cederquist K, Davis M, Gellera C, Kolmel C, Ronnevi LO, Sperfeld AD, Sorensen SA, Tranebjaerg L, Van Maldergem L, Watanabe M, Weber M, Yeung L, Savontaus ML (2001) Multiple founder effects in spinal and bulbar muscular atrophy SBMA, Kennedy disease around the world. Eur J Hum Genet 96:431–436

Lutz LB, Cole LM, Gupta MK, Kwist KW, Auchus RJ, Hammes SR (2001) Evidence that androgens are the primary steroids produced by *Xenopus laevis* ovaries and may signal through the classical androgen receptor to promote oocyte maturation. Proc Natl Acad Sci USA 98:13728–13733

Lutz LB, Jamnongjit M, Yang W-H, Jahani D, Gill A, Hammes SR (2003) Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. Mol Endocrinol 17:1106–1116

Ma H, Hong H, Huang SM, Irvine RA, Webb P, Kushner PJ, Coetzee GA, Stallcup MR (1999) Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol Cell Biol 19:6164–6173

MacLean HE, Choi WT, Rekaris G, Warne GL, Zajac JD (1995) Abnormal androgen receptor binding affinity in subjects with Kennedy's disease spinal and bulbar muscular atrophy. J Clin Endocrinol Metab 802:508–516

Mandrusiak LM, Beitel LK, Wang X, Scanlon TC, Chevalier-Larsen E, Merry DE, Trifiro MA (2003) Transglutaminase potentiates proteasome dysfunction induced by polyglutamine-expanded androgen receptor. Hum Mol Genet 12:1497–1506

Marcelli M, Ittmann M, Mariani S, Sutherland R, Nigam R, Murthy L, Zhao Y, DiConcini D, Puxeddu E, Esen A, Eastham J, Weigel NL, Lamb DJ (2000) Androgen receptor mutations in prostate cancer. Cancer Res 60:944–949

Marhefka, CA, Moore II, BM, Bishop, TC, Kirkovsky, L, Mukherjee, A, Dalton, JT, Miller, DD (2001) Homology modeling using multiple molecular dynamics simulations and docking studies of the human androgen receptor ligand binding domain bound to testosterone and nonsteroidal ligands. J Med Chem 44:1729–1740

Mariotti C, Castellotti B, Pareyson D, Testa D, Eoli M, Antozzi C, Silani V, Marconi R, Tezzon F, Siciliano G, Marchini C, Gellera C, Donato SD (2000) Phenotypic manifestations associated with CAG-repeat expansion in the androgen receptor gene in male patients and heterozygous females: a clinical and molecular study of 30 families. Neuromuscul Disord 106:391–397

Markus SM, Taneja SS, Logan SK, Li W, Ha S, Hittelman AB, Rogatsky I, Garabedian MJ (2002) Identification and characterization of ART-27, a novel coactivator for the androgen receptor N terminus. Mol Biol Cell 13:670–682

Matias, PM, Carrondo, MA, Coelho, R, Thomaz, M, Zhao, X-Y, Wegg, A, Crusius, K, Egner, U, Donner, P (2002) Structural basis for the glucocorticoid response in a mutant human androgen receptor (AR^{ccr}) derived from an androgen-independent prostate cancer. J Med Chem 45:1439–1446

Matias, PM, Donner, P, Coelho, R, Thomaz, M, Peixoto, C, Macedo, S, Otto, N, Joschko, S, Scholz, P, Wegg, A, Bäsler, S, Schäfer, M, Egner, U, Carrondo, MA (2000) Structural evidence for ligand specificity in the binding domain of the human androgen receptor. Implications for pathogenic gene mutations. J Biol Chem 275:26164–26171

McCampbell A, Taye AA, Whitty L, Penney E, Steffan JS, Fischbeck KH (2001) Histone deacetylase inhibitors reduce polyglutamine toxicity. Proc Natl Acad Sci USA 98:15179–15184

McEwan IJ, Gustafsson J (1997) Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci USA 94:8485–8490

McKenna NJ, O'Malley BW (2002) Minireview: nuclear receptor coactivators–an update. Endocrinology143:2461–2465

McManamny P, Chy HS, Finkelstein DI, Craythorn RG, Crack PJ, Kola I, Cheema SS, Horne MK, Wreford NG, O'Bryan MK, deKretser DM, Morrison JR (2002) A mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 18: 2103–2111

McPhaul MJ, Griffin JE (1999). Male pseudohermaphroditism caused by mutations of the human androgen receptor. J Clin Endocrinol Metab 84:3435–3441

Merry DE, Kobayashi Y, Bailley CK, Taye AA, Fischbeck KH (1998) Cleavage, aggregation and toxicity of the expanded androgen receptor in spinal and bulbar muscular atrophy. Hum Mol Genet 7: 693–701

Metzger E, Muller JM, Ferrari S, Buettner R, Schule R (2003) A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. EMBO J 22:270–280.

Meyer WJ 3rd, Migeon BR, Migeon CJ (1975). Locus on human X chromosome for dihydrotestosterone receptor and androgen insensitivity. Proc Natl Acad Sci USA 72: 1469–1472

Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nature Genet 5:184–188

Migeon BR, Brown TR, Axelman J, Migeon CJ (1981) Studies of the locus for androgen receptor: localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse. Proc Natl Acad Sci USA 78:6339–6343

Migliaccio A, Castoria G, Di Domenico M, De Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zannini MS, Abbondanza C, Auricchio F (2000) Steroid-induced androgen receptor-oestradiol receptor b-Src complex triggers prostate cancer cell proliferation. EMBO J 19:5406–5417

Miyamoto KK, McSherry SA, Dent GA, Sar M, Wilson EM, French FS, Sharief Y, Mohler JL (1993) Immunohistochemistry of the androgen receptor in human benign and malignant prostate tissue. J Urol 149:1015–1019

Mizokami A, Chang C (1994) Induction of translation by the 5'-untranslated region of human androgen receptor mRNA. J Biol Chem 269:25655–25659

Mizokami A, Yeh SY, Chang C (1994) Identification of 3',5'-cyclic adenosine monophosphate response element and other cis-acting elements in the human androgen receptor gene promoter. Mol Endocrinol 8:77–88

Moilanen A-M, Poukka H, Karvonen U, Häkli M, Jänne OA, Palvimo JJ (1998) Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. Mol Cell Biol 18: 5128–5139

Mononen N, Syrjakoski K, Matikainen M, Tammela TL, Schleutker J, Kallioniemi OP, Trapman J, Koivisto PA (2000) Two percent of Finnish prostate cancer patients have a germ-line mutation in the hormone-binding domain of the androgen receptor gene. Cancer Res 60:6479–6481

Montgomery BT, Young CY, Bilhartz DL, Andrews PE, Prescott JL, Thompson NF, Tindall DJ (1992) Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. Prostate 21:63–73

Morishima Y, Murphy PJ, Li DP, Sanchez ER, Pratt WB (2000) Stepwise assembly of a glucocorticoid receptor.hsp90 heterocomplex resolves two sequential ATP-dependent events involving first hsp70 and then hsp90 in opening of the steroid binding pocket. J Biol Chem 275:18054–18060

Morris MJ, Scher HI (2000) Novel strategies and therapeutics for the treatment of prostate carcinoma. Cancer 89:1329–1348

Moul JW, Srivastava S, McLeod DG (1995) Molecular implications of the antiandrogen withdrawal syndrome. Semin Urol 13:157–163

Müller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schüle R (2000) FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 19:359–369

Müller JM, Metzger E, Greschik H, Bosserhoff A-K, Mercep L, Buettner R, Schüle R (2002) The transcriptional coactivator FHL2 transmits Rho signals from the cell membrane into the nucleus. EMBO J 21:736–748

Murtha P, Tindall DJ, Young CY (1993) Androgen induction of a human prostate-specific kallikrein, hKLK2: characterization of an androgen response element in the 5' promoter region of the gene. Biochemistry 32:6459–6464

Murtha PE, Zhu W, Zhang J, Zhang S, Young CY (1997) Effects of Ca^{++} mobilization on expression of androgen-regulated genes: interference with androgen receptor-mediated transactivation by AP-I proteins. Prostate 33:264–270

Nazareth L, Weigel NL (1996) Activation of the human androgen receptor through a protein kinase A signaling pathway. J Biol Chem 271:19900–19907

Nesterova M, Cho-Chung YS (2000) Oligonucleotide sequence-specific inhibition of gene expression, tumor growth inhibition, and modulation of cAMP signaling by an RNA-DNA hybrid antisense targeted to protein kinase A RIalpha subunit. Antisense Nucleic Acid Drug Dev 10:423–433

Newmark JR, Hardy DO, Tonb DC, Carter BS, Epstein JI, Isaacs WB, Brown TR, Barrack ER (1992) Androgen receptor gene mutations in human prostate cancer. Proc Natl Acad Sci USA 89:6319–6323

Ning YM, Robins DM (1999) AML3/CBFalpha1 is required for androgen-specific activation of the enhancer of the mouse sex-limited protein (Slp) gene. J Biol Chem 274:30624–30630

Owen GI, Zelent A (2000) Origins and evolutionary diversification of the nuclear receptor superfamily. Cell Mol Life Sci 57:809–827

Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T (2000) Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. J Urol 164:1992–1995

Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Janne OA (1996) Mutual transcriptional interference between RelA and androgen receptor. J Biol Chem 271:24151–24156

Park JJ, Irvine RA, Buchanan G, Koh SS, Park JM, Tilley WD, Stallcup MR, Press MF, Coetzee GA (2000) Breast cancer susceptibility gene 1 (BRCA1) is a coactivator of the androgen receptor. Cancer Res 60:5946–5949

Persson H, Lievre CA-L, Söder O, Villar MJ, Metsis M, Olson L, Ritzen M, Hökfelt T (1990) Expression of b-nerve growth factor receptor mRNA in sertoli cells downregulated by testosterone. Science 247:704–707

Peterziel H, Culig Z, Stober J, Hobisch A, Radmayr C, Bartsch G, Klocker H, Cato AC (1995) Mutant androgen receptors in prostatic tumors distinguish between amino-acid-sequence requirements for transactivation and ligand binding. Int J Cancer 63:544–550

Peterziel H, Mink S, Schonert A, Becker M, Klocker H, Cato ACB (1999) Rapid signalling by androgen receptor in prostate cancer cells. Oncogene 18:6322–6329

Poujol N, Wurtz J-M, Tahiri B, Lumbroso S, Nicolas J-C, Moras D, Sultan C (2000) Specific recognition of androgens by their nuclear receptor. A structure-function study. J Biol Chem 275:24022–24031

Poukka H, Aarnisalo P, Karvonen U, Palvimo JJ, Jänne, OA (1999) Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. J Biol Chem 274:19441–19446

Poukka H, Aarnisalo P, Santti H, Jänne OA, Palvimo JJ (2000a) Coregulator small nuclear RING finger protein (SNURF) enhances Sp1- and steroid-mediated transcription by different mechanisms. J Biol Chem 275:571–579

Poukka H, Karvonen U, Yoshikawa N, Tanaka H, Palvimo JJ, Jänne OA (2000b) The RING finger protein SNURP modulates nuclear trafficking of the androgen receptor. J Cell Sci 113:2991–3001

Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev:18, 306–360

Putz T, Culig Z, Eder IE, Nessler-Menardi C, Bartsch G, Grunicke H, Uberall F, Klocker H (1999) Epidermal growth factor (EGF) receptor blockade inhibits the action of EGF, insulin-like growth factor I, and a protein kinase A activator on the mitogen-activated protein kinase pathway in prostate cancer cell lines. Cancer Res 59:227–233

Quarmby VE, Yarbrough WG, Lubahn DB, French FS, Wilson EM (1990) Autologous down-regulation of androgen receptor messenger ribonucleic acid. Mol Endocrinol 4:22–28

Quigley CA, De Bellis A, Marschke KB, El-Awady MK, Wilson EM, French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271–321

Rao J, Lee P, Benzeno S, Cardozo C, Albertus J, Robins DM, Caplan AJ (2001) Functional interaction of human Cdc37 with the androgen receptor but not with the glucocorticoid receptor. J Biol Chem 276:5814–5820

Reid J, Murray I, Watt K, Betney R, McEwan IJ (2002) The androgen receptor interacts with multiple regions of the large subunit of general transcription factor TFIIF. J Biol Chem 277:41247–41253

Reinikainen P, Palvimo JJ, Jänne OA (1996) Effects of mitogens on androgen receptor-mediated transactivation. Endocrinology 137:4351–4357

Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, Cheng H, Snoek R, Hamel A, Bock ME, MacDonald BS, Nickel BE, Chang C, Liao S, Cattini PA, Matusik RJ (1993) Characterization of two cis-acting DNA elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 7:23–36

Reutens AT, Fu M, Wang C, Albanese C, McPhaul MJ, Sun Z, Balk SP, Jänne OA, Palvimo JJ, Pestell RG (2001) Cyclin D1 binds the androgen receptor and regulates hormone-dependent signaling in a p300/CBP-associated factor (P/CAF)-dependent manner. Mol Endocrinol 15:797–811

Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J (1991) The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930

Roche PJ, Hoare SA, Parker MG (1992) A consensus DNA-binding site for the androgen receptor. Mol Endocrinol 6:2229–2235

Ruizeveld de Winter JA, Janssen PJ, Sleddens HM, Verleun Mooijman MC, Trapman J, Brinkmann AO, Santerse AB, Schroder FH, van der Kwast TH (1994). Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am J Pathol 144:735–746

Rundlett SE, Miesfeld RL (1995) Quantitative differences in androgen and glucocorticoid receptor DNA binding properties contribute to receptor-selective transcriptional regulation. Mol Cell Endocrinol 109:1–10.

Sack JS, Kish KF, Wang C, Attar RM, Kiefer SE, An Y, Wu GY, Scheffler JE, Salvati ME, Krystek Jr SR, Weinmann R, Einspahr HM (2001) Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural agonist dihydrotestosterone. Proc Natl Acad Sci USA 98:4904–4909

Sadar MD (1999) Androgen-independent induction of prostate-specific antigen expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways. J Biol Chem 274:7777–7783

Saitoh M, Takayanagi R, Goto K, Fukamizu A, Tomura A, Yanase T, Nawata H (2002) The presence of both the amino- and carboxyl-terminal domains in the AR is essential for the completion of a transcriptionally active form with coactivators and intranuclear compartmentalization common to the steroid hormone receptors: a three-dimensional imaging study. Mol Endocrinol 16:694–706

Sato N, Sadar MD, Bruchovsky N, Saatcioglu F, Rennie PS, Sato S, Lange PH, Gleave ME (1997) Androgenic induction of prostate-specific antigen gene is repressed by protein-protein interaction between the androgen receptor and AP-1/c-Jun in the human prostate cancer cell line LNCaP. J Biol Chem 272:17485–17494

Scher HI, Kolvenbag GJ (1997) The antiandrogen withdrawal syndrome in relapsed prostate cancer. Eur Urol 31:3–7; discussion 24–27

Schneikert J, Peterziel H, Defossez P-A, Klocker H, De Launoit Y, Cato ACB (1996) Androgen receptor-Ets protein interaction is a novel mechanism for steroid hormone-mediated down-modulation of matrix metalloproteinase expression. J Biol Chem 271:23907–23913

Schuurmans AL, Bolt J, Mulder E (1989) Androgen receptor-mediated growth and epidermal growth factor receptor induction in the human prostate cell line LNCaP. Urol Int 44:71–76

Schwabe J, Chapman L, Finch JT, Rhodes D (1993) The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements. Cell 75:567–578

Segawa N, Nakamura M, Shan L, Utsunomiya H, Nakamura Y, Mori I, Katsuoka Y, Kakudo, K (2002) Expression and somatic mutation on androgen receptor gene in prostate cancer. Int J Urol 9:545–553

Sensibar JA, Sutkowski DM, Raffo A, Buttyan R, Griswold MD, Sylvester SR, Kozlowski JM, Lee C (1995) Prevention of cell death induced by tumor necrosis factor alpha in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin). Cancer Res 55:2431–2437

Shabsigh A, Ghafar MA, de la Taille A, Burchardt M, Kaplan SA, Anastasiadis AG, Buttyan, R (2001) Biomarker analysis demonstrates a hypoxic environment in the castrated rat ventral prostate gland. J Cell Biochem 81:437–444

Shang Y, Myers M, Brown M (2002) Formation of the androgen receptor transcription complex. Mol Cell 9:601–610

Shao TC, Li H, Eid W, Ittmann M, Unni E, Cunningham GR (2003) In vivo preservation of steroid specificity in CWR22 xenografts having a mutated androgen receptor. Prostate 57:1–7

Sharma M, Zarnegar M, Li X, Lim B, Sun Z (2000) Androgen receptor interacts with a novel MYST protein, HBO1. J Biol Chem 275:35200–35208

Shatkina L, Mink S, Rogatsch H, Klocker H, Langer G, Nestl A, Cato ACB (2003) The cochaperone Bag-1L enhances androgen receptor action via interaction with the NH2-terminal region of the receptor. Mol Cell Biol 20:7189–7197

Shenk JL, Fisher CJ, Chen S-Y, Zhou X-F, Tillman K, Shemshedini L (2001) p53 represses androgen-induced transactivation of prostate-specific antigen by disrupting hAR amino- to carboxyl-terminal interaction. J Biol Chem 276:38472–38479

Shimada N, Sobue G, Doyu M, Yamamoto K, Yasuda T, Mukai E, Kachi T, Mitsuma T (1995) X-linked recessive bulbospinal neuronopathy: clinical phenotypes and CAG repeat size in androgen receptor gene. Muscle Nerve 18:1378–1384

Simental JA, Sar M, Wilson EM (1992) Domain functions of the androgen receptor. J Steroid Biochem Mol Biol 43:37–41

Simeoni S, Mancini MA, Stenoien DL, Marcelli M, Weigel NL, Zanisi M, Martini L, Poletti A (2000) Motoneuronal cell death is not correlated with aggregate formation of androgen receptors containing an elongated polyglutamine tract. Hum Mol Genet 9:133–44

Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK (2000) Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 407:538–541

Smith CL, Onate SA, Tsai MJ, O'Malley BW (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. Proc Natl Acad Sci USA 93:8884–8888

Solit DB, Zheng FF, Drobnjak M, Munster PN, Higgins B, Verbel D, Heller G, Tong W, Cordon-Cardo C, Agus DB, *et al.* (2002) 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. Clin Cancer Res 8:986–993

Stanford JL, Damber JE, Fair WR, Sancho-Garnier H, Griffiths K, Gu F-L, Kiemeney LA (1999) Epidemiology of prostate Cancer. In Prostate Cancer – 2nd International Consultation on Prostate Cancer, Murphy GA, Khoury S, Partin AW, Denis L (eds) Paris, Health Publication Ltd. Paris pp 21–55

Steketee K, Berrevoets CA, Dubbink HJ, Doesburg P, Hersmus R, Brinkmann AO, Trapman J (2002) Amino acids 3–13 and amino acids in and flanking the ^{23}FxxLF^{27} motif modulate the nteraction between the N-terminal and ligand-binding domain of the androgen receptor. Eur J Biochem 269:5780–5791

Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, DeMartino GN, Marcelli M, Weigel NL, Mancini MA (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet 8:731–741

Suzuki H, Sato N, Watabe Y, Masai M, Seino S, Shimazaki J (1993) Androgen receptor gene mutations in human prostate cancer. J Steroid Biochem Mol Biol 46:759–765

Suzuki H, Akakura K, Komiya A, Aida S, Akimoto S, Shimazaki J (1996) Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to antiandrogen withdrawal syndrome. Prostate 29:153–158

Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, Morimoto RI. Reed JC (1997) BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J 16:4887–4896

Takayama S, Krajewski S, Krajewska M, Kitada S, Zapata JM, Kochel K, Knee D, Scudiero D, Tudor G, Miller GJ, Miyashita T, Yamada M, Reed JC (1998) Expression and location of Hsp70/Hsc-binding anti-apoptotic protein BAG-1 and its variants in normal tissues and tumor cell lines. Cancer Res 58:3116–3131

Takeyama K-I, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, Miura M, Tabata T, Kato S (2002) Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. Neuron 35:855–864

Tan J, Sharief J, Hamil KG, Gregory CW, Zang D-Y, Sar M, French FS, Pretlow TG (1996) Altered ligand specificity of a mutant androgen receptor (AR) in the androgen dependent human prostate cancer cenograft, CWR-22: Comparison with the LNCaP mutant AR. J Urol 155:340A (Abstract)

Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN, Balk SP (1995) Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 332, 1393–1398.

Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J, Stadler W, Hayes DF, Kantoff PW, Vogelzang NJ, Small EJ (2003) Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol 21:2673–2678

Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, Francke U, Wilson JD, Russell DW (1992) Molecular genetics of steroid 5alpha-reductase 2 deficiency. J Clin Invest 90:799–809

Thompson J, Saatcioglu F, Jänne OA, Palvimo JJ (2001) Disrupted amino- and carboxyl-terminal interactions of the androgen receptor are linked to androgen insensitivity. Mol Endocrinol 15:923–935

Thornton JW, Kelley DB (1998) Evolution of the androgen receptor: structure-function implications. Bioessays 20:860–869

Tilley WD, Marcelli M, Wilson JD, McPhaul, MJ (1989) Characterization and expression of a cDNA encoding the human androgen receptor. Proc Natl Acad Sci USA 86:327–331

Tilley WD, Marcelli M, McPhaul MJ (1990a) Expression of the human androgen receptor gene utilizes a common promoter in diverse human tissues and cell lines. J Biol Chem 265:13776–13781

Tilley WD, Wilson CM, Marcelli M, McPhaul, M. J. (1990b) Androgen receptor gene expression in human prostate carcinoma cell lines. Cancer Res 50:5382–5386.

Tilley WD, Buchanan G, Hickey TE, Bentel JM (1996) Mutations in the androgen receptor gene are associated with progression of human prostate cancer to androgen independence. Clin Cancer Res 2:277–285

Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK Rosenfeld MG (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature 387:677–684

Trapman J, Klaassen P, Kuiper GG, van der Korput JA, Faber PW, van Rooij HC, Geurts van Kessel A, Voorhorst MM, Mulder E, Brinkmann AO (1988) Cloning, structure and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Commun 153:241–248

Truica CI, Byers S, Gelmann EP (2000) Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. Cancer Res 60:4709–4713

Trump D, Wilding G, Small EJ, Soulie P, Gupta A (2002) A pilot trial of ZD1839 (Iressa), an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), in combination with docetaxel and estramustine in patients with hormone-refractory prostate cancer (HRPC). J Urol 167, Abstract at the Annual Congress of the American Urological Association

Tsai MJ, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 82:3777–3782

van der Kwast T, Schalken J, Ruizeveld de Winter J, van Vroonhoven C, Mulder E, Boersma W, Trapman J (1991) Androgen receptors in endocrine therapy-resistant human prostate cancer. Int J Cancer 48:189–193

Veldscholte J, Ris Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, Van Rooij HC, Trapman J, Brinkmann AO, Mulder E (1990a) A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. Biochem Biophys Res Commun 173:534–540

Veldscholte J, Voorhorst OM, Bolt DVJ, Van RH, Trapman J, Mulder E (1990b) Unusual specificity of the androgen receptor in the human prostate tumor cell line LNCaP: high affinity for progestagenic and estrogenic steroids. Biochim Biophys Acta 1052:187–194

Veldscholte J, Berrevoets CA, Ris Stalpers C, Kuiper GG, Jenster G, Trapman J, Brinkmann AO, Mulder E (1992a) The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to antiandrogens. J Steroid Biochem Mol Biol 41:665–669

Veltscholte J, Berrevoets CA, Zegers ND, van der Kvast TH, Grootegoed JA, Mulder E (1992b) Hormone-induced dissociation of the androgen receptor-heat-shock protein complex. Use of a new monoclonal antibody to distinguish transformed from nontransformed receptors. Biochemistry 31:7422–7430

Verrijdt G, Haelens A, Claessens F (2003) Selective DNA recognition by the androgen receptor as a mechanism for hormone-specific regulation of gene expression. Mol Genet Metab 78:175–185

Verrijdt G, Schoenmakers E, Alen P, Haelens A, Peeters B, Rombauts W, Claessens F (1999) Androgen specificity of a response unit upstream of the human secretory component gene is mediated by differential receptor binding to an essential androgen response element. Mol Endocrinol 13:1558–1570

Verrijdt G, Schauwaers K, Haelens A, Rombauts W, Claessens F (2002) Functional interplay between two response elements with distinct binding characteristics dictates androgen specificity of the mouse sex-limited protein enhancer. J Biol Chem 277:35191–35201

Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi O-P (1995) In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9:401–406

Wang C, Young WJ, Chang C (2000) Isolation and characterization of the androgen receptor mutants with divergent transcriptional activity in response to hydroxyflutamide. Endocrine 12:69–76

Wang Q, Sharma D, Ren Y, Fondell JD (2002) A coregulatory role for the TRAP-mediator complex in androgen receptor-mediated gene expression. J Biol Chem 277:42852–42858

Wellington CL, Ellerby LM, Hackam AS, Margolis RL, Trifiro MA, Singaraja R, McCutcheon K, Salvesen GS, Propp SS, Bromm M, Rowland KJ, Zhang T, Rasper D, Roy S, Thornberry N, Pinsky L, Kakizuka A, Ross CA, Nicholson DW, Bredesen DE, Hayden MR (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates trucated fragments containing the polyglutamine tract. J Biol Chem 273:9158–9167

Wen Y, Hu MC-T, Makino K, Spohn B, Bartholomeusz G, Yan D-H, Hung M-C (2000) HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. Cancer Res 60:6841–6845

Whitfield GK, Jurutka PW, Haussler CA, Haussler MR (1999) Steroid hormone receptors: evolution, ligands, and molecular basis of biologic function. J Cell Biochem Suppl 32–33:110–122

Wieacker P, Griffin JE, Wienker T, Lopez JM, Wilson JD, Breckwoldt M (1987) Linkage analysis with RFLPs in families with androgen resistance syndromes: evidence for close linkage between the androgen receptor locus and the DXS1 segment. Hum Genet 76:248–252

Wirth, MP, Froschermaier SE (1997) The antiandrogen withdrawal syndrome. Urol Res 25:S67–S71

Yang X, Chernenko G, Hao Y, Ding Z, Pater MM, Pater A, Tang SC (1998) Human BAG-1/RAP46 protein is generated as four isoforms by alternative translation initiation and overexpressed in cancer cells. Oncogene 17:981–989

Yang F, Li X, Sharma M, Sasaki CY, Longo DL, Lim B, Sun Z (2002) Linking beta-catenin to androgen-signaling pathway. J Biol Chem 277:11336–11344

Yamamoto A, Hashimoto Y, Kohri K, Ogata E, Kato S, Ikeda K, Nakanishi M (2000) Cyclin E as a coactivator of the androgen receptor. J Cell Biol 150:873–880

Yeh S, Chan C (1996) Cloning and characterization of a specific coactivator, ARA70, the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93:5517–5521

Yeh S, Lin H-K, Kang H-Y, Thin TH, Lin M-F, Chang C (1999) From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci USA 96:5458–5463

Yeh S, Hu Y-C, Rahman M, Lin H-K, Hsu C-L, Ting H-J, Kang H-Y, Chang C (2000) Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells. Proc Natl Acad Sci USA 97:11256–11261

Yu X, Li PP, Roeder RG, Wang Z (2001) Inhibition of androgen receptor-mediated transcription by amino-terminal enhancer of split. Mol Cell Biol 21:4614–4625

Zhang L, Leeflang EP, Yu J, Arnheim N (1994) Studying mutations by sperm typing: instability of CAG trinucleotide repeats in the human androgen receptor gene. Nature Genet 8:203

Zhang L, Fischbeck KH, Arnheim N (1995) CAG repeat length varaition in sperm from a patient with Kennedy's disease. Hum Mol Genet 4:303–305

Zhao XY, Boyle B, Krishnan AV, Navone NM, Peehl DM, Feldman D (1999) Two mutations identified in the androgen receptor of the new human prostate cancer cell line MDA PCa 2a. J Urol 162:2192–2199

Zhao XY, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM, Feldman D (2000) Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. Nat Med 6:703–706

Zhou Z, Corden JL, Brown TR (1997) Identification and characterization of a novel androgen response element composed of a direct repeat. J Biol Chem 272:8227–8235

Zitzmann M, Nieschlag E (2003) The CAG repeat polymorphism within the androgen receptor gene and maleness. Int J Androl 26:76–83

3

Androgen receptor: pathophysiology

O. Hiort and M. Zitzmann

Contents

3.1 Introduction

The final biological steps in the cellular cascade of normal male sexual differentiation are initiated by the molecular interaction of testosterone and dihydrotestosterone with the androgen receptor (AR) in androgen-responsive target tissues. As complete insensitivity to androgens leads to a female phenotype (Meschede *et al.*

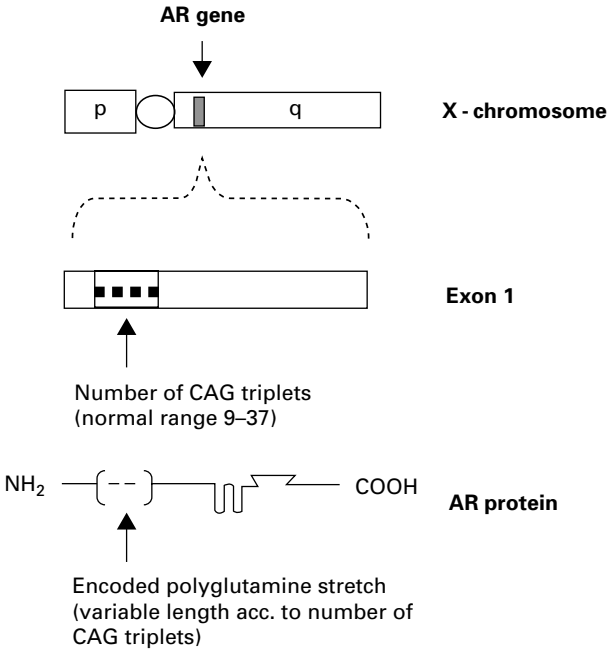


Fig. 3.1 Display of the X-chromosome with the androgen receptor (AR) gene. Exon 1 contains a variable number CAG repeats encoding a polyglutamine stretch of variable length in the receptor protein. The number of CAG repeats or length of polyglutamine residues is inversely associated with the transcriptional activity of androgen-dependent genes, hence androgen effects in target tissues.

2000; Quigley *et al.* 1995), maleness may be described as the sublimate of gender difference. Testosterone and its metabolite dihydrotestosterone (DHT) exert their effects on gene expression via the AR. A diverse range of clinical conditions starting with complete androgen insensitivity (CAIS) has been correlated with mutations in the AR (Hiort *et al.* 1996; Meschede *et al.* 2000; Quigley *et al.* 1995). Subtle modulations of the transcriptional activity induced by the AR have also been observed and frequently assigned to a polyglutamine stretch of variable length within the N-terminal domain of the receptor. This stretch is encoded by a variable number of CAG-triplets in exon 1 of the AR gene located on the X-chromosome (Fig. 3.1). First observations of pathologically elongated AR CAG repeats in patients with X-linked spinobulbar muscular atrophy (SBMA) showing marked hypoandrogenic traits (La Spada *et al.* 1991) were supplemented by partially conflicting findings of clinical significance also within the normal range of CAG repeat length. The modulatory effect on androgen-dependent gene transcription is linear and probably mediated by a differential affinity of coactivator proteins to the encoded polyglutamine stretch, such as ARA24 and p160 (Hsiao *et al.* 1999; Irvine *et al.* 2002). As

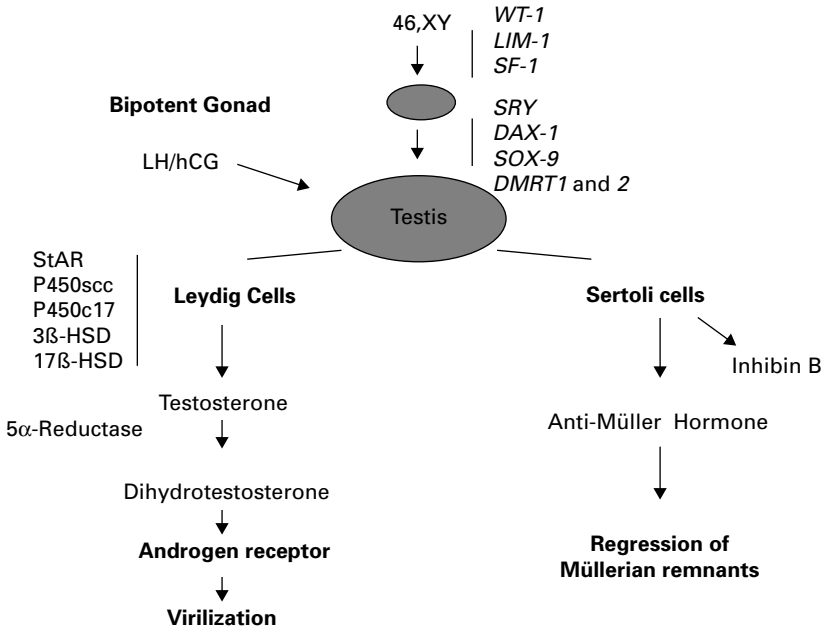


Fig. 3.2 Pathways of human sexual differentiation. The genetic cascade for testicular development first leads to initiation of a bipotent gonad before the testis is determined. Within the testis, the Leydig cells synthesize testosterone via five enzymatic steps from cholesterol which is secreted to react peripherally via the androgen receptor after being at least partially converted to dihydrotestosterone. The Sertoli cells synthesize anti-Mullerian hormone which is necessary for regression of Mullerian ducts.

these proteins are ubiquitously but nevertheless non-uniformly expressed, the modulatory effect of the CAG repeat polymorphism on AR target genes is most likely not only dependent on androgenic saturation and AR expression, but also varies from tissue to tissue. To date, an involvement of prostate cancer risk, spermatogenesis, bone density, hair growth, cardiovascular risk factors and psychological implications has been demonstrated.

3.2 Androgen action pathway

3.2.1 In foetal sexual differentiation

Normal male sexual development is dependent both on genetic events of gonadal development as well as on endocrine pathways initiated by hormones secreted from the testes (Fig. 3.2). Gonadal differentiation is initiated with the development of the bipotent gonad during early embryonal life (Hiort and Holterhus 2000). Several genes are known to be involved in this process leading to the creation of the undifferentiated gonad. Abnormalities in the Wilms tumour 1 (WT1) gene are associated

with failure of gonadal differentiation, nephropathy, development of Wilms tumours (Denys-Drash syndrome and Frasier syndrome), and in the WAGR syndrome which also involves anomalies of the eye (aniridia) and mental retardation.

Another gene involved in the development of the bipotential gonad and the kidneys is the recently cloned LIM1-gene. Homozygous deletions in this gene in mice lead to developmental failure of both gonads and kidneys. To date, no human mutations have been described in this gene, although a phenotype of renal and gonadal developmental defects in association with brain abnormalities might be anticipated. The role of the steroidogenic factor 1 (SF1) in the formation of the gonad is not yet clear. SF1 is the product of the FTZ1-F1-gene and is believed to be a nuclear orphan hormone receptor due to the presence of two zinc fingers and a ligand binding domain in its molecular structure. FTZ1-F1 mRNA is expressed in the urogenital ridge which forms both gonads and adrenals, and is also found in developing brain regions. Mice lacking SF1 fail to develop gonads, adrenals, and the hypothalamus. However, SF1 is probably also involved in other aspects of sexual development, as it regulates the expression of steroidogenic enzymes as well as the transcription of the anti-Müllerian hormone (AMH) (Ozisik *et al.* 2003).

Further progession of gonadal differentiation from the bipotential gonad is mediated through gonosomal and autosomal genes. It was long believed and has been proven that a specific testis-determining-factor (TDF) was essential for testicular development and that the encoding gene was located on the Y-chromosome. This gene, termed sex-determining-region of the Y-chromosome (SRY) is a single-exon gene which encodes a protein with a DNA-binding motif that acts as a transcription factor and in turn regulates the expression of other genes. Evidence was provided that SRY binds to the promoter of the AMH gene and also controls the expression of steroidogenic enzymes (Harley *et al.* 2003). Thus, SRY probably induces the expression of AMH to prevent the formation of Müllerian duct derivatives. Evidence that SRY is the TDF was presented when the mouse homologue SRY gene was introduced into the mouse germ line and genetic female offspring showed a normal male phenotype in these genetically engineered animals (Koopman *et al.* 1991). Furthermore, naturally occurring mutations of SRY have been described in humans (Hiort *et al.* 1995).

Autosomal genes which are structurally related to SRY genes have been described. These 'SRY-box-related' or SOX-genes are to some extent involved in testicular development. SOX 9 is connected with chondrogenesis and gonadal differentiation. This gene is transcribed especially following SRY-expression in male gonadal structures. Additionally, SOX 9 is an activator of the type II collagen gene which in turn is essential for formation of the extracellular matrix of cartilage (Harley *et al.* 2003). A gene which is involved in adrenal as well as in ovarian and testicular development is DAX 1. This gene is located on the X-chromosome and was termed:

Dosage-sensitive sex reversal locus – **A**drenal hypoplasia congenita – critical region on the **X**, gene 1. DAX 1 is expressed during ovarian development, but is silent during testis formation, implying a critial role in ovarian formation. Interestingly, DAX-1 is repressed by SRY during testicular development. However, if a duplication of the DAX-1 region on Xp21 is present in a 46,XY patient and, thus, the activity of its gene product is enhanced, testicular formation is impaired. In contrast, mutations in DAX-1 diminishing its activity lead to a lack of adrenal formation and also hypogonadal hypogonadism in congenital adrenal hypoplasia (Beuschlein *et al.* 2002). Further genes involved in testicular differentiation have been localized on chromosome 10 and on chromosome 9 (DMRT 1 and 2).

In early gestation, both the anlagen for the Wolffian and Müllerian ducts are present in the foetus regardless of the karyotype. If testicular formation is unhindered, the Sertoli cell will produce AMH. To exert the action of AMH, high concentrations of this hormone and active binding to a membrane receptor in the mesenchymal cells surrounding the Müllerian ducts are necessary. Therefore, reduced excretion of AMH due to lowered number of Sertoli cells is responsible for partial uterus formation disorders of sex determination. The AMH gene is under transcriptional control of several other proteins involved in sexual differentiation. SF-1 binds directly to the AMH gene promoter and activates its transcription in the Sertoli cells. A regulatory effect of SRY on AMH receptor expression has also been reported (Lim and Hawkins 1998).

Unhindered steroid hormone formation and action is necessary for the development of the external genitalia. Furthermore, defects in cholesterol synthesis may also lead to distinct phenotypes including deficiencies of genital development. The first steps of steroid biosynthesis are common pathways for glucocorticoids, mineralocorticoids, and sex steroids, while the formation of testosterone from androstenedione via 17ß-hydroxysteroid dehydrogenase type 3 is probably limited to testis (Hiort *et al.* 2000). In contrast, further conversion of testosterone to DHT is catalysed in the peripheral target tissues and not within the gonads. Androgen synthesis in the developing testes is controlled during early foetal life by human chorionic gonadotropin (hCG) and only later by the foetal luteinizing hormone (LH) itself.

Expression of the AR is present even prior to the onset of testicular androgen secretion. There is a marked similarity in distribution and intensity of AR staining in the external genitalia of male and female fetuses at 18 to 22 weeks gestation (Kalloo *et al.* 1993), a finding that explains the virilization of female fetuses when exposed to supranormal androgen concentrations as in congenital adrenal hyperplasia.

The major sites of action are the virilization of the male accessory glands and the male external genitalia. Testosterone may act differently in this process. Paracrine actions of high concentrations of testosterone result in differentiation of the Wolffian duct, thus forming the deferent ducts. Endocrine actions are caused

by testosterone which reaches its target tissues, e.g. the external male genitalia, via the blood stream. Depending on the anatomical region, testosterone can be further converted to dihydrotestosterone. Both testosterone and dihydrotestosterone enter the target cells and bind to the cytoplasmic AR. The AR belongs to the nuclear receptor superfamily and is a ligand activated transcription factor of androgen regulated genes (Hiort and Holterhus 2000). Binding of the ligand induces an activation cascade involving dissociation of the receptor from heat shock proteins, receptor phosphorylation, dimerization, translocation of the receptor into the nucleus, interaction with specific hormone responsive elements within the promoter region of androgen regulated genes and assembly of the basal transcription machinery finally resulting in specific gene transcription. Binding of the androgenic ligand to the AR is a highly specific event (Poujol *et al.* 2000). While earlier studies stressed the well-described fact that dihydrotestosterone is a much stronger ligand for the AR than testosterone (Deslypere *et al.* 1992), more recent data suggest that this concept needs qualitative extension. Hsiao *et al.* (2000) recently identified different androgen response elements which showed a differential response upon activating the AR through either testosterone or dihydrotestosterone. Moreover, recently it was demonstrated that structurally different androgens with different profiles of biological actions induced very different response patterns through the AR when using three structurally different androgen responsive promoters in co-transfection assays (Holterhus *et al.* 2002). The morphogenetic result of these specific actions of androgens is the irreversible virilization of the external male genitalia. This process is terminated in the 12th week of gestation. Hence, incomplete masculinization, e.g., incomplete closure of the midline (hypospadias) during the sensitive window between the 7th and the 12th week cannot be overcome by even high doses of androgens at later stages of development. This fact may seem trivial but it clearly indicates that the genomic programs provided by the androgen target tissues must have undergone comprehensive and definitive alterations in parallel to the ontogenetic process of external virilization.

3.2.2 In puberty and adulthood

Increasing androgenic steroid secretion from the adrenals is defined as adrenarche and precedes puberty. Adrenarche is associated with increased growth of pubic and axillary hair independent of gonadal androgen secretion. Adrenal androgens include mainly dehydroepiandrostendione, its sulfate, and androstendione, but also other adrenal steroids have androgenic potential. Adrenocorticotropic hormone (ACTH) is a potent stimulator of adrenal androgen secretion; however, its potency relative to cortisol secretion is much less. Also, substances other than ACTH may modulate adrenal androgen secretion. These include estrogens, prolactin, growth hormone, gonadotropins and lipotropin. None of these appear to be the usual physiological

modulator, although under some circumstances each may increase androgen production (Odell and Parker 1984). Adrenal androgen levels will continue to increase during adolescence until the third decade of life when a continuous and variable decrease will be prevalent.

Normal male puberty starts with the enlargement of testes and penis. Testicular volume increases from 1 to 2 cc prepubertally to 3 to 8 cc even before pubic hairs start to appear and reaches 20–30 cc in adulthood. In addition to changes in secondary hair and genital changes, increasing testosterone concentrations produce other changes in most tissues of the body. The larynx increases in size and the voice deepens. Also bone mass and muscle strength increase, a growth spurt occurs, the erythrocyte cell mass increases, the skin thickens, and hair growth on the trunk is enhanced, as well as androgenic hair recession may occur. Sex steroids, and specifically testosterone may alter behaviour, and central nervous effects include stimulation of sexual libido and aggressiveness.

Testosterone in conjunction with FSH is an essential endocrine factor for spermatogenesis in male mammals which acts directly on the germinal epithelium via the AR. During sex hormonal quiescence in prepuberty, germ cell proliferation is arrested until the juvenile phase. Testosterone alone can induce spermatogenesis if administered during this period (Marshall *et al.* 1984). However, quantitative maintenance of the spermatogenic process cannot be achieved by testosterone alone, but needs the supportive action of FSH (Weinbauer and Nieschlag 1990, see also Chapter 5).

3.3 Generalized androgen insensitivity in humans

Defective androgen action caused by cellular resistance to androgens causes the androgen insensitivity syndrome (AIS) (Hiort *et al.* 1996; Quigley *et al.* 1995). The end-organ resistance to androgens results in a wide clinical spectrum of defective virilization of the external genitalia in 46,XY individuals. Müllerian duct derivatives are usually completely absent because of the normal ability of the foetal testes to produce AMH. Since the AR gene has been cloned, it became obvious that inactivating mutations of the AR gene represent the major molecular genetic basis of AIS (Lubahn *et al.* 1988a). Due to the X-chromosomal recessive inheritance, healthy female carriers may typically be conductors (Lubahn *et al.* 1988b).

In the complete androgen insensitivity syndrome (CAIS), any in vivo androgen action is abolished due to complete inactivation of in vivo AR signalling. Therefore, these patients have normal female external genitalia with a short and blind-ending vagina. At puberty, CAIS patients acquire a normal female body shape and they show normal breast development. This is caused by increasing estradiol levels due to elevated testosterone biosynthesis during puberty and its conversion to estradiol

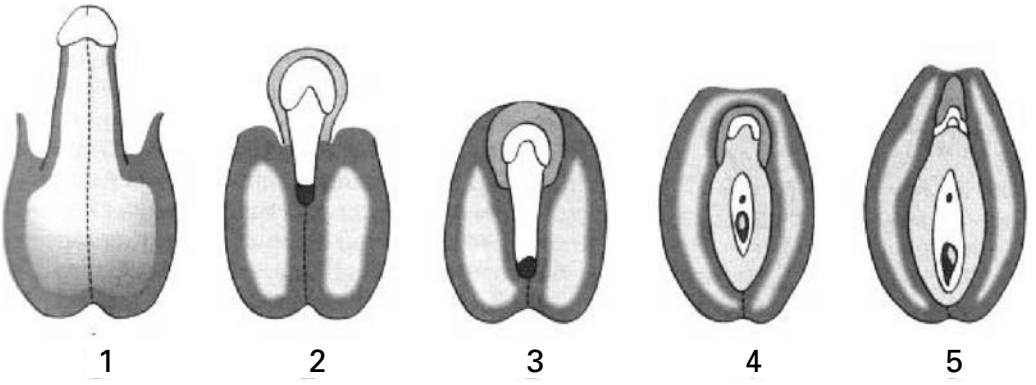


Fig. 3.3 Clinical grades of ambiguous genitalia. Virilization is diminished from grade 1 towards grade 5. According to Sinnecker *et al.* 1997.

by aromatization. Usually, no pubic or axillary hair is present (Hiort *et al.* 1996; Sinnecker *et al.* 1997).

Partial impairment of AR function is usually associated with partial androgen insensitivity syndrome (PAIS). The considerable variability in the degree of impaired AR activity accounts for a wide clinical spectrum of external undervirilization observed in PAIS. This may range from a female habitus with only a small degree of virilization as partial fusion of labioscrotal folds and minimal enlargement of the clitoris to patients with considerable degree of virilization with male habitus, gynecomastia, female pattern of secondary hair distribution, and genital malformations such as hypospadias (Hiort *et al.* 1993; 1996; Holterhus *et al.* 1997; 2000). Phenotype of external genitalia may be graded according to the scale of Sinnecker *et al.* (1997) (Fig. 3.3).

The minimal androgen insensitivity syndrome (MAIS) describes individuals with a normal male habitus without genital malformation with only a slight masculinization deficit such as high pitched voice and gynecomastia associated with sub- or infertility (Hiort *et al.* 1996; 2000).

Within families, the phenotype may vary considerably between MAIS and PAIS even with the same underlying molecular abnormality (Holterhus *et al.* 2000; Rodien *et al.* 1996).

3.3.1 Biochemical evidence for defective androgen receptor

3.3.1.1 Pituitary-testicular axis

In patients with AIS, assessment of the pituitary-gonadal axis is age-dependent. In the normal male, a rise of gonadotropins and testosterone is seen during the first months of life, usually starting after the first week postnatally with a decline after six months to prepubertally low levels (Forest *et al.* 1973). Recently, Bouvattier

described infants with CAIS and PAIS with regard to postnatal changes in testosterone and gonadotropins (Bouvattier *et al.* 2002). Interestingly, in CAIS testosterone as well as gonadotropin levels were lower than in normal 46,XY male infants in the second months of life. However, in patients with PAIS, while there were overall normal values for gonadotropins, the testosterone values in serum were markedly increased compared to CAIS and to the normal age range. This has also been described earlier (Hiort *et al.* 1993).

This observation of elevated testosterone may also be seen as a response to stimulation with human chorionic gonadotropin (hCG) in prepubertal children with PAIS after the first months of life. However, in other cases, mainly in CAIS, the testosterone response to hCG may be subnormal, falsely indicative of a testosterone biosynthesis defect (Ahmed *et al.* 1999; Hellwinkel *et al.* 1999). This makes diagnosis of androgen insensitivity in the hormonal quiescence of childhood very difficult.

After puberty, androgen insensitivity can be inferred from both elevated LH and testosterone levels. The elevation of LH and testosterone is most likely due to an impaired negative feedback control of the hypothalamic-pituitary-testicular axis in AIS (Aiman *et al.* 1979). It is discernible even in patients with the minimal form of AIS (Hiort *et al.* 2000); however, the androgen sensitivity index derived from the product of the values for LH and testosterone is not a specific parameter of AIS. In a recent study by Melo *et al.* (2003), the androgen sensitivity index was elevated in all postpubertal patients with AIS; however, the LH levels were higher in CAIS. The higher elevation of LH in patients with CAIS was attributed to the severity of the underlying molecular defect. These authors found the androgen sensitivity index a valuable parameter in postpubertal patients to distinguish AIS from other forms of intersex disorders.

3.3.1.2 SHBG androgen sensitivity test

Especially in infants and children with ambiguous genitalia, the diagnosis of AIS may be difficult due to the uninformative hormonal profile. Alternatively, ligand binding analysis in genital skin fibroblasts derived from a genital biopsy has been employed although this is a cumbersome, invasive and costly diagnostic approach to AIS (Hiort *et al.* 1993). In contrast, DNA analysis of the AR gene allows a definitive diagnosis (see below), but is also costly and time consuming, nor does it lead to a functional prognosis of future development in the tested infant. Therefore, a specific test of androgen sensitivity in vivo has been applied to children with AIS, based on the ability of the anabolic steroid stanozolol to induce a decline in serum sex hormone binding globulin (SHBG) (Sinnecker *et al.* 1989). Stanozolol is a non-virilizing anabolic steroid, inducing very promoter-specific effects via the AR in in vitro experiments (Holterhus *et al.* 2002). In a cohort of pre- and postpubertal patients with AIS, stanozolol given at a dose of 0.2 mg/kg per day on

three consecutive days led to a decline in serum SHBG levels between days 5 and 8 after start of the test, which was correlated to the phenotype (Sinnecker *et al.* 1997).

Thus, the SHBG-androgen sensitivity test is the only in vivo test known today to assess AR function in a given individual. This may be useful in the discussion of gender assignment and prognosis of future development. However, this test has two major drawbacks. Firstly, it is not sensitive during the first six months of life, the period when discriminative evaluation of a child with ambiguous genitalia is most needed. Secondly, the test is not sensitive in children bearing *de novo* or mosaic mutation of the AR gene as in these patients the normal AR function may be present in liver tissue and thus lead to false negative results (Hiort *et al.* 1998; Holterhus *et al.* 2001). Furthermore, stanozolol is not available as a prescriptive drug any more. However, the decline of SHBG has also been observed after stimulation of Leydig cells with hCG (Bertelloni *et al.* 1997) and may thus be employed as an alternative androgen sensitivity test.

3.3.2 Genetic aspects of the androgen receptor in human androgen insensitivity

The AR is a ligand-activated transcription factor of androgen-regulated genes. It is commonly assumed – though not experimentally proven to date – that a controlled temporal and spatial expression of androgen-regulated genes during early embryogenesis provokes a distinct spectrum of functional and structural alterations of the internal and external genitalia, ultimately resulting in the irreversible formation of the normal male phenotype (Holterhus *et al.* 2003).

The AR belongs to the intracellular family of structurally related steroid hormone receptors. Transcriptional regulation through the AR is a complex multistep process involving androgen binding, conformational changes of the AR protein, receptor phosphorylation, nuclear trafficking, DNA binding, cofactor interaction and finally transcription activation. It is now more than 15 years ago that the human AR gene was cloned by several groups and mapped to Xq11–12 (Chang *et al.* 1988; Lubahn *et al.* 1988a; 1988b; Tilley *et al.* 1989; Trapman *et al.* 1988). It spans approximately 90 kilobases (kb) and comprises 8 exons, named 1–8 or A–H. Transcription of the AR gene and subsequent splicing usually results in distinct AR-mRNA populations in genital fibroblasts. Translation of the mRNA into the AR protein usually leads to a product migrating at about 110 kDa in Western immunoblots comprising between 910 to 919 amino acids.

The AR shares its particular modular composition of three major functional domains with the other steroid hormone receptors. A large N-terminal domain precedes the DNA-binding domain, followed by the C-terminal ligand-binding domain. Additional functional subdomains could be identified by in vitro investigation of artificially truncated, deleted or point mutated ARs (for review see Quigley *et al.* 1995). Upon entering target cells, androgens interact very specifically

with the ligand-binding pocket of the AR. This initiates an activation cascade with conformational changes and nuclear translocation of the AR. Prior to receptor binding to target DNA, homodimerization of two AR proteins occurs in a ligand-dependent manner. This is mediated by distinct sequences within the second zinc finger of the DNA-binding domain as well as through specific structural N-C-terminal interactions. The AR-homodimer binds to hormone responsive elements (HRE) which usually consists of two palindromic (half-site) sequences within the promoter of androgen-regulated genes. Through chromatin remodelling, direct interaction with other transcription factors and specific coactivators and corepressors, a steroid-receptor specific modulation of the assembly of the preinitiation complex is achieved, resulting in specific activation or repression of target gene transcription.

More than 300 different mutations have been identified in AIS to date (http://ww2.mcgill.ca/androgendb/). Extensive structural alterations of the AR can result from complete or partial deletions of the AR gene. Smaller deletions may introduce a frame shift into the open reading frame leading to a premature stop codon downstream of the mutation. Similar molecular consequences arise from the direct introduction of a premature stop codon due to point mutations. Such alterations usually lead to severe functional defects of the AR and are associated with CAIS. Extensive disruption of the AR protein structure can also be due to mutations leading to aberrant splicing of the AR m-RNA (Hellwinkel *et al.* 1999; 2001). However, as aberrant splicing can be partial and thus enable expression of the wild type AR, the AIS phenotype is not necessarily CAIS but may also present with PAIS (Hellwinkel *et al.* 2001). The most common molecular defects of the AR gene are missense mutations. They may either result in CAIS or in PAIS because of complete or partial loss of AR function (Hiort *et al.* 1996). Mutations within the ligand-binding domain may alter androgen binding but may in addition influence dimerization due to disruption of N-C-terminal structural interactions. Mutations within the DNA-binding domain can affect receptor binding to target DNA. Recently, a first female patient with complete AIS without an AR gene mutation but with clear experimental evidence for an AR-coactivator deficiency as the only underlying molecular mechanism of defective androgen action was reported (Adachi *et al.* 2000). Cofactors of the AR will presumably play a pivotal role in the understanding of the phenotypic variability in AIS. So far, only a few mechanisms contributing to the phenotypic diversity in AIS were identified in affected individuals. A striking phenotypic variability in a family with partial AIS has been attributed to differential expression of the 5α reductase type 2 enzyme in genital fibroblasts (Boehmer *et al.* 2001). Another mechanism may be the combination of varying androgen levels during early embryogenesis and partially inactivating mutations of the ligand-binding domain (Holterhus *et al.* 2000).

Moreover, post-zygotic mutations of the AR gene resulting in a somatic mosaicism of mutant and wild-type AR genes can contribute to modulation of the phenotype. This can result in a higher degree of virilization than expected from the AR mutation alone because of the expression of the wild type AR in a subset of somatic cells. Because at least one third of all *de novo* mutations of the AR gene occur at the post zygotic stage, this mechanism is not only important for phenotypic variability in AIS but also crucial for genetic counselling (Hiort *et al.* 1998).

3.4 The role of CAG repeat polymorphisms of the androgen receptor in various target organs

3.4.1 Kennedy syndrome: a pathological expansion of the AR gene CAG repeats

X-linked spinobulbar muscular atrophy (X-SBMA) or Kennedy syndrome, is a rare inherited neurodegenerative disease characterized by progressive neuromuscular weakness being caused by a loss of motor neurons in the brain stem and spinal cord. Disease onset developing in the third to fifth decade of life is likely to be preceded by muscular cramps on exertion, tremor of the hands and elevated muscle creatine kinase. The initial description of one of the individuals affected with Kennedy syndrome also includes gynaecomastia, a hypoandrogenic symptom (Kennedy *et al.* 1968). Subsequent reports emphasized the presence of symptoms indicating the development of androgen insensitivity in men with X-SBMA exhibiting varying degrees of gynaecomastia, testicular atrophy, disorders of spermatogenesis, elevated serum gonadotropins and also diabetes mellitus (e.g. Arbizu *et al.* 1983). Thus, the AR was regarded as candidate gene for X-SBMA and the expansion of the polyglutamine repeat within the N-terminal region was furtheron recognized as the cause (La Spada *et al.* 1991). The longer the CAG repeat in the AR gene, the earlier the onset of the disease is observed and the more severe the symptoms of hypoandrogenicity are (Choong and Wilson 1998; Dejager *et al.* 2002; Doyu *et al.* 1992; Mariotti *et al.* 2000; Mhatre *et al.* 1993). The absence of any neuromuscular deficit or degeneration in patients with complete androgen insensitivity (CAIS) (Quigley *et al.* 1995) suggests that neurological deficits in XBSMA are not caused by a lack of androgen influence but rather by a neurotoxic effect associated with the pathologically elongated number of CAG repeats, which causes irregular processing of the AR protein and accumulation of end products (Abdullah *et al.* 1998).

3.4.2 Ethnic differences

The normal range of CAG repeats is probably 9 to 37 and follows a normal, slightly skewed distribution towards the higher number of triplets (Edwards *et al.* 1992; Hsing 2000a; Kuhlenbäumer *et al.* 2001; Platz *et al.* 2000) and symptoms related to XBSMA seem to start at 38 to 40 CAG repeats (Pioro *et al.* 1994). Within the normal

range of the AR polyglutamine stretch, significant differences between ethnic groups have been observed. For healthy men of African descent the mean number of CAG repeats ranges between 18 and 20 (Edwards *et al.* 1992; Platz *et al.* 2000) and seems to be even shorter in certain African subpopulations (Kittles *et al.* 2001). In healthy Caucasians the mean number of CAG repeats is 21 to 22 (Edwards *et al.* 1992; Platz *et al.* 2000) while in East Asians a mean of 22–23 triplets is found (Hsing *et al.* 2000a; Platz *et al.* 2000, van Houten and Gooren 2000; Wang *et al.* 2001) (also see Chapter 2). These differences can possibly be held responsible for some variations of androgen-dependent diseases and features which are observed among different ethnicities, e.g. beard growth or rate of prostate cancer.

3.4.3 Prostate development and malignancy

The prostate is an androgen-regulated organ and androgen receptor co-activators such as ARA24 and p160 are expressed in prostate tissue (see Chapter 12). Binding of these co-activators to the CAG repeat tract, which represents the androgen receptor's co-activator binding site, is reduced with increasing length of triplet numbers. Hence, the prostate should be an organ, in which effects of the CAG repeat polymorphism are visible. In general, there is a substantial difference in the incidence of prostate cancer between ethnic groups, with African Americans having a 20- to 30-fold higher incidence than East Asians (Hsing *et al.* 2000b). Such disparity cannot be explained entirely by screening bias in different populations. Also after multiple adjustments for ethnic and screening differences a significant contrast in incidence rates between African Americans, Caucasians and Asians is found (Platz *et al.* 2000; Ross *et al.* 1998).

It can be assumed that a polymorphism of the AR with the capacity to modulate androgen effects has an influence on the fate of malignant cells in the prostate. Thus, with shorter CAG repeats, an earlier onset of the disease would be observed, as well as an association with aggressiveness of the tumor. Investigation of a younger study group would then lead to the supposition of an increased risk to develop prostate cancer. While this would hold true for a specific younger age group, it is likely that the effect cannot be observed when older men are also involved since the overall incidence of prostate cancer is high. Stratification for life style factors and multidimensional matching of controls in a sufficient number of subjects is a prerequisite for respective investigations: this is best met by eight studies (Balic *et al.* 2002; Beilin *et al.* 2001; Correa-Cerro *et al.* 1999; Giovannucci *et al.* 1997; Hsing *et al.* 2000a; Latil *et al.* 2001; Platz *et al.* 2000; Stanford *et al.* 1997). Seven of these described an independent contribution of the CAG repeat polymorphism to prostate cancer, either to the age of onset or to the general risk of development. The age of the study group, timepoint and intensity of diagnostic performance varying with the location of the study most likely influence the result as to whether it is

seen as earlier onset or higher risk. The putative association with disease stage is also likely to be influenced by such factors. Each triplet may hence account for a 3 to 14% risk for prostate cancer (Stanford *et al.* 1997). In conclusion, it is likely that the genesis of prostate cancer cells is not induced by androgens, but that stronger androgenicity induced by ARs with shorter polyglutamine stretches contributes to a faster development of these cells and this might be seen either as earlier onset of or as higher risk for prostate cancer, depending on the age of the study group.

Another aspect is the putative relation between benign hyperplasia of the prostate (BPH) and the CAG repeat polymorphism of the AR gene. BPH consists of the overgrowth of tissue within the transition zone and periurethral area of the prostate. This is histologically defined as epithelial and fibromuscular hyperplasia (Price *et al.* 1984). One factor modulating androgenic exposure is the cellular level of androgens, particularly dihydrotestoterone. The influence of the CAG repeat polymorphism causes variations in such effects as demonstrated by several studies. The two largest studies comparing matched healthy controls ($n = 1041$ and $n = 499$) and BPH patients ($n = 310$ and $n = 449$) described the odds ratio for BPH surgery or an enlarged prostate gland to be 1.92 ($p = 0.0002$) when comparing CAG repeat length of 19 or less to 25 or more. For a six-repeat decrement in CAG repeat length, the odds ratio for moderate or severe urinary obstructive symptoms from an enlarged prostate gland was 3.62 ($p = 0.004$) (Giovannucci *et al.* 1999a; 1999b). Similarly, adenoma size was found to be inversely associated with the number of CAG repeats in 176 patients vs. 41 controls (Mitsumori *et al.* 1999). Prostate growth during androgen substitution is also significantly modified by the CAG repeat polymorphism (*see below*).

3.4.4 Reproductive functions

Stimulation of Sertoli cells by FSH is a prerequisite in primate spermatogenesis and intratesticular androgen activity represents an important co-factor with a positive effect on the supporting function of Sertoli cells. Thus, it can be speculated that the CAG repeat polymorphism within the AR gene could have a limited influence on spermatogenesis. Such an effect can be observed as severely impaired spermatogenesis in X-SBMA patients (Arbizu *et al.* 1983). The investigation of the possible influence of a polyglutamine stretch within the normal length on sperm production requires a sample of carefully selected patients in which significant confounders (obstructive symptoms due to infections, congenital aplasia of the vas deferens [CBAVD], impaired spermatogenesis due to hormone disorders, deletions in one of the azoospermia-associated regions of the Y-chromosome) have been ruled out.

Control groups consisting of healthy fertile males should be homogenous in terms of ethnic origin (see above). It should be considered that within the cohort of fertile controls, sperm densities below 20 Mill / ml might occur (Rajpert-De Meyts

et al. 2002). Unfortunately, a fraction of studies on this subject did not strictly exclude patients described above. Therefore, it is not surprising that conflicting results emerged when infertile and fertile men were compared in regard to their number of CAG repeats. Some studies reported higher numbers of CAG triplets in infertile men (Tut *et al.* 1997; Legius *et al.* 1999; Dowsing *et al.* 1999; Yoshida *et al.* 1999; Yong *et al.* 2000; Mifsud *et al.* 2001; Patrizio *et al.* 2001; Wallerand *et al.* 2001; Mengual *et al.* 2003), but some did not (Lundberg Giwercman *et al.* 1998; Hiort *et al.* 1999; Dadze S *et al.* 2000; van Golde *et al.* 2002; Rajpert-De Meyts *et al.* 2002). In contrast to studies from Europe, a relationship between sperm production and CAG repeat length is obviously more likely to be found in mixed populations, especially including men of Asian origin.

When only fertile men covering the whole range of normal sperm concentrations were involved in respective evaluations, a shorter CAG repeat tract was associated with higher sperm numbers (von Eckardstein *et al.* 2001; Rajpert-De Meyts *et al.* 2002). Nevertheless, a marked variation of sperm density in relation to the AR polymorphism was observed. Hence, spermatogenesis is likely to be influenced by the number of CAG repeats within the normal range, but whether this reaches relevance for individuals remains doubtful. The range of sperm concentrations leading to infertility is most likely reached at CAG repeat numbers that are associated with X-SBMA. Furthermore, it can be assumed that the proportion of men with longer CAG repeats among infertile patients may, in case of strict selection criteria excluding all known causes of infertility, appear higher than in a control population. Genetic counselling concerning inheritability of this modulator of spermatogenesis is of very restricted value, since the CAG polymorphism is located on the X-chromosome and the specific tract length will affect spermatogenesis of the offspring only in one half of the grandsons.

3.4.5 Bone tissue

Polymorphisms of the estrogen receptor (ER) have repeatedly been demonstrated to modulate quantity and quality of bone tissue in healthy men (e.g. Sapir-Koren *et al.* 2001). As androgen activity influences bone metabolism (see Chapter 7), respective observations apply to the CAG repeat polymorphism in the AR gene as well: in 110 healthy younger males, a high number of CAG repeats was significantly associated with lower bone density (Zitzmann *et al.* 2001b). This result is corroborated by a negative association between AR CAG repeat length and bone density at the femoral neck in a group of 508 Caucasian men aged over 65 years (Zmuda *et al.* 2000a). The same workgroup also observed a more pronounced bone loss at the hip and increased vertebral fracture risk among older men with longer AR CAG repeat length (Zmuda *et al.* 2000b). In a group of 140 Finnish men aged 50–60 years, lumbar and femoral bone mineral density values were higher in

those men with shorter CAG repeats in comparison to those with longer CAG repeats (Remes *et al.* 2003). The differences reach statistical significance when the groups with CAG repeat length of 15–17 and 22–26 are compared directly. In contrast, in a group of 273 healthy Belgian men aged 71 and 86 years, no influence of the androgen receptor gene polymorphism was seen, but about 30% of these men had androgen levels below the lower limit of normal and were thus lacking sufficient androgen receptor activation (van Pottelbergh *et al.* 2001).

Higher androgenization will lead to higher peak bone mass (Khosla 2002); thus, the AR polymorphism effects on bone density are likely to be visible among healthy younger males, while the difference could be mitigated by the overall age-dependent bone loss and may no longer be visible in old men, in whom confounders have exerted influence on bone tissue. Thus, the longer the CAG repeat in the AR gene, the lower peak bone density in males will be, while it is inconclusive whether this effect reaches clinical significance in terms of higher fracture risk.

In women, low androgen levels and, hence, low activation of the AR are present. In addition, two alleles of the AR gene will cause a less pronounced effect in terms of influence exerted by the CAG repeat polymorphism. Nevertheless, reports concerning such impact on bone density in women exist, demonstrating an association of reduced bone mass and/or osteoporotic fractures in women with longer CAG repeats (Chen *et al.* 2003; Sowers *et al.* 1999; Tofteng *et al.* 2003; Langdahl *et al.* 2003). As can be expected from physiology, the AR polymorphism does not influence the effects of hormone replacement therapy by estrogens on bone tissue in postmenopausal women (Salmen *et al.* 2003).

3.4.6 Cardiovascular risk factors

Testosterone plays an ambiguous role in relation to cardiovascular risk factors and its respective role has not been fully resolved (see Chapter 10). The interactions between the CAG repeat polymorphism, serum levels of sex hormones, lifestyle factors and endothelium-dependent and independent vessel relaxation of the brachial artery as well as lipoprotein levels, leptin and insulin concentrations and body composition were described in over 100 eugonadal men of a homogenous population. In agreement with previously demonstrated androgen effects on these parameters it was demonstrated that androgenic effects were attenuated in persons with longer CAG repeats while testosterone levels themselves played only a minor role within the eugonadal range. Significant positive correlations with the length of CAG repeats were seen for endothelial-dependent vasodilatation, HDL-cholesterol concentrations, body fat content, insulin and leptin levels. These results remained stable in multiple regression analyses correcting for age and life-style factors. It was demonstrated by a 5-factor model that adverse and beneficial components are mutually dependent (Zitzmann *et al.* 2001a; 2003a). Within the investigated range

of androgen-related cardiovascular risk factors and eugonadal testosterone levels, the CAG repeat polymorphism could play a more dominant role than testosterone itself. Concerning lipid concentrations, corresponding results are reported in men with X-SBMA (Dejager *et al.* 2002). Also in agreement, an inclination to develop diabetes mellitus has been described for these patients (Arbizu *et al.* 1983). Hence, adverse or beneficial effects of a longer or shorter CAG repeat chain in regard to cardiovascular risk will most likely strongly depend on co-factors. The implications in terms of modulation of cardiovascular risk by androgens apply especially to hypogonadal men receiving testosterone substitution. The pharmacogenetic role in this respect of the CAG repeat polymorphism has yet to be elucidated.

3.4.7 Psychological implications

Testosterone substitution in hypogonadal men improves lethargic or depressive aspects of mood significantly (Burris *et al.* 1992). Studies exploring the relationship between gonadal function and depressive episodes demonstrated testosterone levels to be markedly decreased in respective patients (Unden *et al.* 1988; Schweiger *et al.* 1999; Barrett-Connor *et al.* 1999). Accordingly, treatment with testosterone gel may improve symptoms in men with refractory depression (Pope *et al.* 2003). The age-dependent decline of testosterone levels is sometimes associated with symptoms of depression. It has been recently demonstrated in 1000 older men that this mood-dependency on androgen levels is modified by the CAG repeat polymorphism of the AR gene. Depression scores were significantly and inversely associated with testosterone levels in subjects with shorter CAG repeats, while this was not observed in men with moderate and longer polyglutamine stretches in the AR protein. Low versus high testosterone in such men was associated with a five-fold increased likelihood of depressive mood (Seidman *et al.* 2001). It can be speculated that the higher activation rate of the AR in this subgroup revealed effects of declining androgen levels more readily than in subgroups with longer CAG repeats.

In a sample of 172 Finnish men aged 41 to 70 years, the length of CAG repeats was significantly positively associated and independent from testosterone levels with symptom scores concerning depression, as expressed by the wish to be dead ($r = 0.45$; $p < 0.0001$), depressed mood ($r = 0.23$; $p = 0.003$), anxiety ($r = 0.15$; $p < 0.05$), deterioration of general well-being ($r = 0.22$; $p = 0.004$) and also decreased beard growth ($r = 0.49$; $p < 0.0001$) (Harkonen *et al.* 2003).

Another aspect of psychological parameters is represented by the group of externalizing behaviours; these are predominantly found in males and have been associated with androgens (Zitzmann *et al.* 2001c; Diagnostic and Statistical Manual of the American Psychiatric Association 1994). Respective personality traits are attention deficit hyperactivity disorder (ADHD); conduct disorder (CD) and oppositional defiant disorder (ODD). A controlled study in 302 younger men concerning these

disorders in relation to the CAG repeat of the AR gene demonstrated a significantly higher prevalance in genotypes with shorter repeat chains. The group also reported an association of short CAG repeats in the AR gene with novelty-seeking behaviour (drug abuse, pathological gambling) (Comings *et al.* 1999).

Similarly, in a sample of 183 healthy Swedish men aged 20–75 years, associations of CAG repeat length and scores in the Karolinska Scales of Personality were described. Tendencies indicated positive relationships between shorter CAG trinucleotide repeats and personality scales connected to dominance and aggression (low "Lack of Assertiveness", high "Verbal Aggression", high "Monotony Avoidance"). Longer polyglutamine tracts were associated with some neuroticism-related personality scales: high "Muscular Tension", high "Lack of Assertiveness" and high "Psychastenia" (Jönsson *et al.* 2001).

In addition, a case report of three Caucasian brothers describes mental retardation, especially demonstrating a delay in speech development, shy but sometimes aggressive behaviour, marfanoid habitus and relatively large testes in combination with abnormally short CAG repeats (8 triplets) (Kooy *et al.* 1999).

3.4.8 Hair growth

Male pattern baldness is described by a loss of scalp hair and affects up to 80% of males by the age of 80 years. A balding scalp is caused by androgens and expression of the AR in the respective hair follicle and is thus known as androgenetic alopecia (see Chapter 6). One can assume that the influence of the CAG repeat polymorphism on androgenicity causes a variation of androgenetic alopecia. In men with such a clinical condition, significantly shorter CAG repeats were described in comparison to controls by two studies (Ellis *et al.* 2001; Sawaya and Shalita 1998). Thus, the CAG repeat polymorphism is likely to play a role in modulation of androgen influence on male hair pattern, but since statistical significance is weak in a reasonable number of patients due to high interindividual variability, the cosmetic consequence for the individual is questionable.

3.4.9 Pharmacogenetic aspects of testosterone therapy

Considering observations in eugonadal men, one can assume that testosterone therapy in hypogonadal men should have a differential impact on androgen target tissue, depending on the number of CAG repeats. In a longitudinal pharmacogentic study in 131 hypogonadal men, prostate volume was assessed before and during androgen substitution. Considered were the length of CAG repeats, sex hormone levels and anthropometric measures. Initial prostate size of hypogonadal men was dependent on age and baseline testosterone levels, but not the CAG repeat polymorphism. However, when prostate size increased significantly during therapy, prostate growth per year and absolute prostate size under substituted

testosterone levels were strongly dependent on the AR polymorphism, with lower treatment effects in longer repeats. Other modulators of prostate growth were age and testosterone levels during treatment. The odds ratio for men with repeats < 20 compared to those with ≥ 20 to develop a prostate size of at least 30 ml under testosterone substitution was 8.7 (95% CI 3.1 – 24.3, $p < 0.001$). This first pharmacogenetic study on androgen substitution in hypogonadal men demonstrates a marked influence of the CAG repeat polymorphism on prostate growth (Zitzmann *et al.* 2003b).

Another retrospective approach concerning pharmacogenetic influences in hormonal male contraception demonstrated sperm counts to be more easily suppressed by various pharmacological regimens in men with longer CAG repeats as spermatogenesis is partially dependent on intratesticular androgen activity. This was only observed in the subgroup with residual gonadotropin activity, causing stimulatory effects on spermatogenesis and Leydig cell production of testosterone which can bind to intratesticular androgen receptors, hence making differences caused by the CAG polymorphism visible (von Eckardstein *et al.* 2002). When the distinction in regard to gonadotropin secretion is not made, the difference between individuals with long or short CAG repeats cannot be observed, as testicular androgen receptors will not be activated in persons lacking LH and, hence, intratesticular testosterone (Yu and Handelsman 2001).

3.4.10 A hypothetical model of androgen action

Testosterone levels within the normal range will more or less saturate present androgen receptors and it has been demonstrated that androgenic effects will reach a plateau at certain levels, which are probably tissue-specific (Zitzmann *et al.* 2002a; 2002b). In agreement, a study applying exponentially increasing doses of testosterone to hypogonadal men shows corresponding results (Bhasin *et al.* 2001): androgen effects on various parameters increased linearly with the logarithm of testosterone levels and linearly with the logarithm of the testosterone dose. In practice, this means more or less a plateau effect. Significant increments of androgenic effects caused by rising testosterone levels within the eugonadal range are only seen beyond the normal range and when clearly supraphysiological levels are reached. Therefore, it can be assumed that within the range of such a plateau of saturation, genetically determined functional differences in androgen receptor activity can be best observed, while in a condition of hypogonadism, androgenicity will be strongly dependent on androgen levels as testosterone binds to androgen receptors and will increase androgen effects until saturation is reached (Figure 3.4). This model explains why androgen effects are found when comparing hypo- and eugonadal men, but can often not be confirmed for various testosterone levels within the eugonadal range. Indeed, the clinical distinction between hypo- and eugonadism

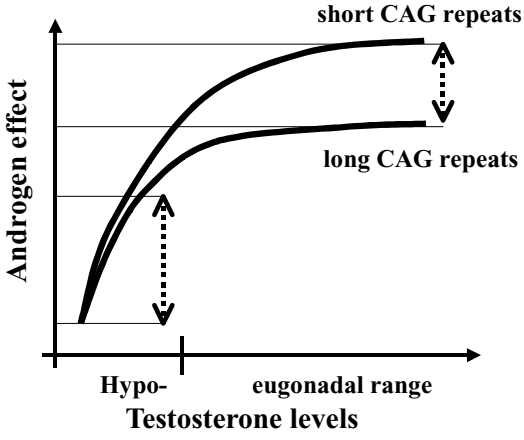


Fig. 3.4 Hypothetical model of androgen effects: Within the hypogonadal range and in comparison to the eugonadal range, differences in androgen effects are determined by testosterone levels. Within the eugonadal range, androgen effects depend rather on the AR polymorphism. As this effect depends on the presence of AR co-activators, the concentrations of which are tissue-specific, the shapes of the curves are putatively variable from organ to organ.

is only possible when androgen effects do not increase in a linear fashion with testosterone levels. During substitution therapy of hypogonadal men, both effects on androgenicity, increment of testosterone levels from the hypo- into the eugonadal range and modulation of androgen effects within the eugonadal range by the androgen receptor polymorphism have to be taken into account.

3.5 Treatment options in androgen insensitivity syndromes

Treatment of patients with an intersex disorder must be directed both towards medical and psychological aspects. Psychological expertise is necessary when gender identity is uncertain, especially in the individual with genital ambiguity. However, questions regarding social and cultural coping with intersexuality should also be asked and answered with a view towards enhancement of the quality of life of this patient.

At present medical treatment is based on the assessment of the individual phenotype in correlation with our knowledge of the underlying AR pathophysiology (Hines *et al.* 2003). The decision of sex of rearing must be made in a thorough discussion between medical specialists of both endocrinology and surgery as well as with specialized psychologists together with the parents (Hiort *et al.* 2003).

In individuals with AIS raised as females, the gonads may be removed at various ages. In patients with complete AIS, gonads should not be removed before puberty, leading to "testicular feminization" with an isosexual pubertal development. The

risk of a malignancy of the gonads should not be underestimated; however, to date there is no report of a prepubertal or pubertal AIS-patient with a gonadal malignancy. In female patients with partial AIS caused by a mosaic mutation of the AR gene or decreased, albeit distinct, receptor activity due to a point mutation of the AR gene, the gonads should be removed before the beginning of puberty (Holterhus *et al.* 2002). Hormone replacement therapy in female patients with AIS will always include estrogens. However, when and if gestagens should be replaced and a cyclic replacement be given is debatable in these patients without Mullerian structures.

In male patients with partial or minimal AIS, only little is known about high-dose androgen therapy for further masculinization. In published cases, additional therapy with 250 mg testosterone enanthate every week led to a marked increase in virilization (Foresta *et al.* 2002; Hiort *et al.* 1993; Radmayr *et al.* 1998; Weidemann *et al.* 1998). Moreover, anabolic effects were also seen, such as increase in bone mineral density. Apparently the site of mutation within the AR does not allow prediction of therapeutic response, as both mutations within the DNA and the hormone binding region are susceptible to high-dose androgen treatment.

Hormonal treatment in AIS is still based on individual case observations and the development and evaluation of guide lines is necessary for the future.

3.6 Outlook

Further decoding of the molecular and biochemical pathways is necessary for a comprehensive understanding of normal and abnormal sexual determination and differentiation. Based on the known molecular defects of impaired human sexual development, recent achievements in the field of functional genomics and proteomics offer unique opportunities to identify the genetic programs downstream of these pathways, which are ultimately responsible for structure and function of a normal or abnormal genital phenotype. Hopefully this knowledge will lead to better medical decisions in patients with androgen insensitivity due to AR defects and will open pathways for the development of individual therapeutic options.

The highly polymorphic nature of glutamine residues within the AR protein, which is encoded by the CAG repeat polymorphism within the AR gene, causes a subtle gradation of androgenicity among individuals. This modulation of androgen effects may be small but continuously present during a man's lifetime, thus exerting effects that are measurable in many tissues as various degrees of androgenicity (Fig. 3.5). It remains to be seen whether these insights are important enough to become part of individually useful laboratory assessments. The pharmacogenetic implication of this polymorphism seems to play an important role as modulator

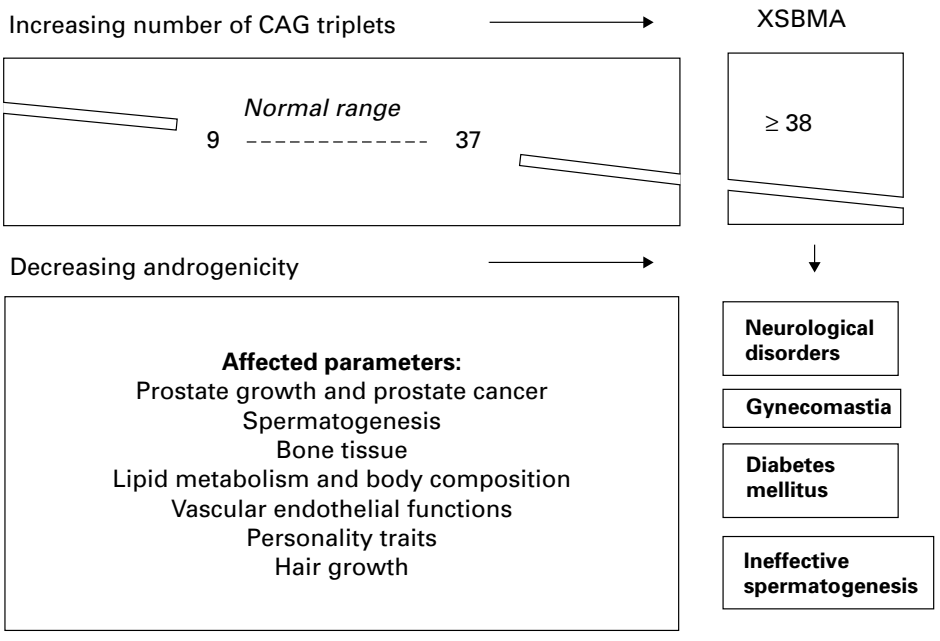


Fig. 3.5 The inverse association between the number of CAG repeats in the AR gene and functionality of the AR protein. Longer CAG tracts result in lower transcription of target genes and, thus, lower androgenicity. Expansion of the encoded polyglutamine stretch to beyond probably 38 leads to the neuromuscular disorder X-linked spinobulbar muscular atrophy (SBMA), a condition in which defective spermatogenesis and undervirilization are observed. Conversely, low numbers of CAG repeats are associated with increased androgenicity of susceptible tissues.

of treatment effects in hypogonadal men. Further studies are required to decide whether these insights should sublimate into individualized aspects of testosterone therapy, e.g. adaptation of dosage or surveillance intervals.

3.7 Key messages

- A defective androgen receptor may lead to variable phenotypes of androgen insensitivity in humans.
- In infants and children stimulation of the gonads with human chorionic gonadotropin is necessary for evaluation of gonadal hormone synthesis.
- In young infants laboratory findings may demonstrate variable testosterone values; thus the discrimination of androgen insensitivity from other causes of ambiguous genitalia is difficult.
- The stanozolol-based sex hormone-binding globulin androgen sensitivity test is a helpful functional test in androgen insensitivity, albeit not discriminatory in infants and patients with somatic mutations of the androgen receptor.

- Definitive diagnosis of androgen insensitivity is based on the analysis of a mutation in the androgen receptor gene.
- Some androgen receptor defects leading to partial androgen insensitivity may be overcome by high-dose androgen therapy.
- The CAG repeat polymorphism in exon 1 of the androgen receptor gene modulates androgen effects: testosterone effects are attenuated according to the length of triplet residues.
- Clinically, the CAG repeat polymorphism causes significant modulations of androgenicity in healthy eugonadal men in various tissues and psychological traits.
- The pharmacogenetic implications of this polymorphism are likely to play a significant role in future testosterone treatment of hypogonadal men as treatment effects are markedly influenced by the number of CAG repeats, at least in the prostate.

3.8 REFERENCES

Abdullah A, Trifiro MA, Panet-Raymond V, Alvarado C, de Tourreil S, Frankel D, Schipper HM, Pinsky L (1998) Spinobulbar muscular atrophy: polyglutamine-expanded androgen receptor is proteolytically resistant in vitro and processed abnormally in transfected cells. Hum Mol Genet 7:379–384

Adachi M, Takayanagi R, Tomura A, Imasaki K, Kato S, Goto K, Yanase T, Ikuyama S, Nawata H (2000) Androgen-insensitivity syndrome as a possible coactivator disease. N Engl J Med 343:856–862. Erratum in: N Engl J Med (2001) 344:696

Ahmed SF, Cheng A, Hughes IA (1999) Assessment of the gonadotrophin-gonadal axis in androgen insensitivity syndrome. Arch Dis Child 80:324–329

Aiman J, Griffin JE, Gazak JM, Wilson JD, MacDonald PC (1979) Androgen insensitivity as a cause of infertility in otherwise normal men. N Engl J Med 300:223–227

Arbizu T, Santamaria J, Gomez JM, Quilez A, Serra JP (1983) A family with adult onset spinal and bulbar muscular atrophy X-linked inheritance and associated testicular failure. J Neurol Sci 59:371–382

Balic I, Graham ST, Troyer DA, Higgins BA, Pollock BH, Johnson-Pais TL, Thompson IM, Leach RJ (2002) Androgen receptor length polymorphism associated with prostate cancer risk in Hispanic men. J Urol 168:2245–2248

Barrett-Connor E, von Mühlen DG, Kritz-Silverstein D (1999) Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J Clin Endocr Metab 84:573–577

Beilin J, Harewood L, Frydenberg M, Mameghan H, Martyres RF, Farish SJ, Yue C, Deam DR, Byron KA, Zajac JD (2001) A case-control study of the androgen receptor gene CAG repeat polymorphism in Australian prostate carcinoma subjects. Cancer 92:941–949

Bertelloni S, Federico G, Baroncelli GI, Cavallo L, Corsello G, Liotta A, Rigon F, Saggese G (1997) Biochemical selection of prepubertal patients with androgen insensitivity syndrome by sex hormone-binding globulin response to the human chorionic gonadotropin test. Pediatr Res 41:266–271

Beuschlein F, Keegan CE, Bavers DL, Mutch C, Hutz JE, Shah S, Ulrich-Lai YM, Engeland WC, Jeffs B, Jameson JL, Hammer GD (2002) SF-1, DAX-1, and acd: molecular determinants of adrenocortical growth and steroidogenesis. Endocr Res 28:597–607

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:1172–81

Boehmer AL, Brinkmann AO, Nijman RM, Verleun-Mooijman MC, de Ruiter P, Niermeijer MF, Drop SL (2001) Phenotypic variation in a family with partial androgen insensitivity syndrome explained by differences in 5 alpha dihydrotestosterone availability. J Clin Endocrinol Metab 86:1240–1246

Bouvattier C, Carel JC, Lecointre C, David A, Sultan C, Bertrand AM, Morel Y, Chaussain JL (2002) Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. J Clin Endocrinol Metab 87:29–32

Burris AS, Banks SM, Carter CS, Davidson JM, Sherins RJ (1992) A long-term prospective study of the physiologic and behavioural effects of hormone replacement in untreated hypogonadal men. J Androl 13:297–304

Chang C, Kokontis J, Liao S (1988) Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 85:7211–7215

Chen HY, Chen WC, Wu MC, Tsai FJ, Tsai CH (2003) Androgen receptor (AR) gene microsatellite polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. Eur J Obstet Gynecol Reprod Biol 107:52–56

Choong CS, Wilson EM (1998) Trinucleotide repeats in the human androgen receptor: a molecular basis for disease. J Mol Endocrinol 21:235–257

Comings DE, Chen C, Wu S, Muhleman D (1999) Association of the androgen receptor gene (AR) with ADHD and conduct disorder. Neuroreport 10:1589–1592

Correa-Cerro L, Wohr G, Haussler J, Berthon P, Drelon E, Mangin P, Fournier G, Cussenot O, Kraus P, Just W, Paiss T, Cantu JM, Vogel W (1999) (CAG)nCAA and GGN repeats in the human androgen receptor gene are not associated with prostate cancer in a French-German population. Eur J Hum Gen 7:357–362

Dadze S, Wieland C, Jakubiczka S, Funke K, Schröder E, Royer-Pokora B, Willers R, Wieacker PF (2000) The size of the CAG repeat in exon 1 of the androgen receptor gene shows no significant relationship to impaired spermatogenesis in an infertile Caucasoid sample of German origin. Mol Hum Reprod 6:207–214

Dejager S, Bry-Gauillard H, Bruckert E, Eymard B, Salachas F, LeGuern E, Tardieu S, Chadarevian R, Giral P, Turpin G (2002) A comprehensive endocrine description of Kennedy's disease revealing androgen insensitivity linked to CAG repeat length. J Clin Endocr Metab 87:3893–3901

Deslypere JP, Young M, Wilson JD, McPhaul MJ (1992) Testosterone and 5 alpha-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. Mol Cell Endocrinol 88:15–22

Diagnostic and Statistical Manual of the American Psychiatric Association. IV (1994) Washington DC: American Psychiatric Association

Dowsing AT, Yong EL, Clark M, McLachlan RI, de Kretser DM, Trounson AO (1999) Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet 354:640–643

Doyu M, Sobue G, Mukai E, Kachi T, Yasuda T, Mitsuma T, Takahashi A (1992) Severity of X-linked recessive bulbospinal neuronopathy correlates with size of the tandem CAG repeat in androgen receptor gene. Annals Neurol 32:707–710.

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253

Ellis JA, Stebbing M, Harrap SB (2001) Polymorphism of the androgen receptor gene is associated with male pattern baldness. J Invest Dermatol 116:452–455

Forest MG, Cathiard AM, Bertrand JA (1973) Evidence of testicular activity in early infancy. J Clin Endocrinol Metab 37:148–151

Foresta C, Bettella A, Ferlin A, Garolla A, Moro E, Baldinotti F, Simi P, Dallapiccola B (2002) Response to local dihydrotestosterone treatment in a patient with partial androgen-insensitivity syndrome due to a novel mutation in the androgen receptor gene. Am J Med Genet 107:259–260

Giovannucci E, Stampfer MJ, Chan A, Krithivas K, Gann PH, Hennekens CH, Kantoff PW (1999a) CAG repeat within the androgen receptor gene and incidence of surgery for benign prostatic hyperplasia in U.S. physicians. Prostate 39:130–134

Giovannucci E, Platz EA, Stampfer MJ, Chan A, Krithivas K, Kawachi I, Willett WC, Kantoff PW (1999b) The CAG repeat within the androgen receptor gene and benign prostatic hyperplasia. Urology 53:121–125

Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Dahl D, Brufsky A, Talcott J, Hennekens CH, Kantoff PW (1997) The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci 94:3320–3323

Harkonen K, Huhtaniemi I, Makinen J, Hübler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F, Pollanen P (2003) The polymorphic androgen receptor gene CAG repeat pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 26:187–194

Harley VR, Clarkson MJ, Argentaro A (2003) The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. Endocr Rev 24:466–487

Hellwinkel OJ, Bull K, Holterhus PM, Homburg N, Struve D, Hiort O (1999) Complete androgen insensitivity caused by a splice donor site mutation in intron 2 of the human androgen receptor gene resulting in an exon 2-lacking transcript with premature stop-codon and reduced expression. J Steroid Biochem Mol Biol 68:1–9

Hellwinkel OJC, Holterhus PM, Struve D, Homburg N, Hiort O (2001) A unique exonic splicing mutation in the human androgen receptor gene demonstrates the physiologic relevance of regular androgen receptor transcript variants. J Clin Endocrinol Metab 86: 2569–2575

Hines M, Ahmed SF, Hughes IA (2003) Psychological outcomes and gender-related development in complete androgen insensitivity syndrome. Arch Sex Behav 32:93–101

Hiort O (2002) Androgens and puberty. Best Pract Res Clin Endocrinol Metab 16:31–41

Hiort O, Holterhus PM (2000) The molecular basis of male sexual differentiation. Eur J Endocrinol 142:101–110

Hiort O, Huang Q, Sinnecker GHG, Sadeghi-Nejad A, Wolfe HJ, Yandell DW (1993) Single strand conformation polymorphism analysis of androgen receptor gene mutations in patients with androgen insensitivity syndromes: Application for diagnosis, genetic counseling, and therapy. J Clin Endocrinol Metab 77:262–266

Hiort O, Gramss B, Klauber G (1995) True hermaphroditism with 46,XY karyotype and a point mutation in the SRY gene. J Pediatr 126:1022

Hiort O, Sinnecker GHG, Holterhus PM, Nitsche EM, Kruse K (1996) The clinical and molecular spectrum of androgen insensitivity. Am J Med Genet 63:218–222

Hiort O, Sinnecker GHG, Holterhus PM, Nitsche EM, Kruse K (1998) Inherited and de novo androgen receptor gene mutations: Investigation of single-case families. J Pediatr 131:939–943

Hiort O, Horter T, Schulze W, Kremke B, Sinnecker GH (1999) Male infertility and increased risk of diseases in future generations. Lancet 354:1907–1908

Hiort O, Holterhus PM, Horter T, Schulze W, Kremke B, Bals-Pratsch M, Sinnecker GHG, Kruse K (2000) Significance of mutations in the androgen receptor gene in males with idiopathic infertility. J Clin Endocrinol Metab 85:2810–2815

Hiort O, Reinecke S, Thyen U, Jürgensen M, Holterhus PM, Schön D, Richter-Appelt H (2003) Aspects of puberty in disorders of somatosexual differentiation. J Pediatr Endoc Met 16:297–306

Holterhus PM, Brüggenwirth HT, Hiort O, Kleinkauf-Houcken A, Kruse K, Sinnecker GHG, Brinkmann AO (1997) Mosaicism due to a somatic mutation of the androgen receptor gene determines phenotype in androgen insensitivity syndrome. J Clin Endocrinol Metab 82:3584–3589

Holterhus PM, Sinnecker GH, Hiort O (2000) Phenotypic diversity and testosterone-induced normalization of mutant L712F androgen receptor function in a kindred with androgen insensitivity. J Clin Endocrinol Metab 85:3245–3250

Holterhus PM, Bruggenwirth HT, Brinkmann AO, Hiort O (2001) Post-zygotic mutations and somatic mosaicism in androgen insensitivity syndrome. Trends Genet 17:627–628

Holterhus PM, Piefke S, Hiort O (2002) Anabolic steroids, testosterone-precursors and virilizing androgens induce distinct activation profiles of androgen responsive promoter constructs. J Steroid Biochem Molec Biol 82:269–75

Holterhus PM, Hiort O, Demeter J, Brown PO, Brooks JD (2003) Differential gene-expression patterns in genital fibroblasts of normal males and 46,XY females with androgen insensitivity syndrome: evidence for early programming involving the androgen receptor. Genome Biol 4:R37

Hsiao PW, Lin DL, Nakao R, Chang C (1999) The linkage of Kennedy's neuron disease to ARA24 the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem 274:20229–20234

Hsiao PW, Thin TH, Lin DL, Chang C (2000) Differential regulation of testosterone vs. 5 alpha-dihydrotestosterone by selective androgen response elements. Mol Cell Biochem 206:169–175

Hsing AW, Gao YT, Wu G, Wang X, Deng J, Chen YL, Sesterhenn IA, Mostofi FK, Benichou J, Chang C (2000a) Polymorphic CAG and GGN repeat lengths in the androgen receptor

gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 60:5111–5116

Hsing AW, Tsao L, and Devesa SS (2000b) International trends and patterns of prostate cancer incidence and mortality. Int J Cancer 85:60–67

Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA (2002) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Gen 9:267–274

Jönsson EG, von Gertten C, Gustavsson JP, Yuan QP, Lindblad-Toh K, Forslund K, Rylander G, Mattila-Evenden M, Asberg M, Schalling M (2001). Androgen receptor trinucleotide repeat polymorphism and personality traits. Psychiatr Genet 11:19–23

Kalloo NB, Gearhart JP, Barrack ER (1993) Sexually dimorphic expression of estrogen receptors, but not of androgen receptors in human fetal external genitalia. J Clin Endocrinol Metab 77:692–698

Kennedy WR, Alter M, Sung JH (1968) Progressive proximal spinal and bulbar muscular atrophy of late onset. Neurology 18:671–680

Khosla S (2002) Oestrogen bones and men: when testosterone just isn't enough. Clin Endocrinol 56:291–293

Kittles RA, Young D, Weinrich S, Hudson J, Argyropoulos G, Ukoli F, Adams-Campbell L, Dunston GM (2001) Extent of linkage disequilibrium between the androgen receptor gene CAG and GGC repeats in human populations: implications for prostate cancer risk. Hum Gen 109:253–261

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R (1991) Male development of chromosomally female mice transgenic for Sry. Nature 351:117–121

Kooy RF, Reyniers E, Storm K, Vits L, van Velzen D, de Ruiter PE, Brinkmann AO, de Paepe A, Willems PJ (1999) CAG repeat contraction in the androgen receptor gene in three brothers with mental retardation. Am J Med Gen 85:209–213

Kuhlenbäumer G, Kress W, Ringelstein EB, Stögbauer F (2001) Thirty-seven CAG repeats in the androgen receptor gene in two healthy individuals. J Neurol 248:23–26

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79.

Langdahl BL, Stenkjaer L, Carstens M, Tofteng CL, Eriksen EF (2003) A CAG repeat polymorphism in the androgen receptor gene is associated with reduced bone mass and increased risk of osteoporotic fractures. Calcif Tissue Int (in press)

Latil AG, Azzouzi R, Cancel GS, Guillaume EC, Cochan-Priollet B, Berthon PL, Cussenot O (2001) Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. Cancer 92:1130–1137

Legius E, Vanderschueren D, Spiessens C, D'Hooghe T, Matthijs G (1999) Association between CAG repeat number in the androgen receptor and male infertility in a Belgian study. Clin Gen 56:166–167

Lim HN, Hawkins JR (1998) Genetic control of gonadal differentiation. Baillieres Clin Endocrinol Metab 12:1–16

Lubahn DB, Joseph DR, Sar M, Tan J-A, Higgs HN, Larson RE, French FS, Wilson EM (1988a) The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. Mol Endocrinol 2:1265–1275

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM (1988b) Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240:327–330

Lundberg-Giwercman Y, Xu C, Arver S, Pousette A, Reneland R (1998) No association between the androgen receptor gene CAG repeat and impaired sperm production in Swedish men. Clin Gen 54:435–436

Lundberg-Giwercman Y, Nikoshkov A, Lindsten K, Bystrom B, Pousette A, Knudtzon J, Alm J, Wedell A (2000) Response to treatment in patients with partial androgen insensitivity due to mutations in the DNA-binding domain of the androgen receptor. Horm Res 53:83–88

Mariotti C, Castellotti B, Pareyson D, Testa D Eoli, M Antozzi C, Silani V, Marconi R, Tezzon F, Siciliano G, Marchini C, Gellera C, Donato SD (2000) Phenotypic manifestations associated with CAG-repeat expansion in the androgen receptor gene in male patients and heterozygous females: a clinical and molecular study of 30 families. Neuromusc Dis 10:391–397

Marshall GR, Wickings E, Nieschlag E (1984) Testosterone can initiate spermatogenesis in an immature nonhuman primate, Macaca fascicularis. Endocrinology 114:2228–2233

Melo KF, Mendonca BB, Billerbeck AE, Costa EM, Inacio M, Silva FA, Leal AM, Latronico AC, Arnhold IJ (2003) Clinical, hormonal, behavioral, and genetic characteristics of androgen insensitivity syndrome in a Brazilian cohort: five novel mutations in the androgen receptor gene. J Clin Endocrinol Metab 88:3241–50

Mengual L, Oriola J, Ascaso C, Ballesca JL, Oliva R (2003) An increased CAG repeat length in the androgen receptor gene in azoospermic ICSI candidates. J Androl 24:279–284

Meschede D, Behre HM, Nieschlag E (2000) Disorders of androgen target organs. In Andrology: Male reproductive health and dysfunction, 2nd ed. (Nieschlag E, Behre HM) (eds), Springer Heidelberg, pp 223–239

Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nature Genetics 5:184–188.

Mifsud A, Sim CK, Boettger-Tong H, Moreira S, Lamb DJ, Lipshultz LI, Yong EL (2001) Trinucleotide (CAG) repeat polymorphisms in the androgen receptor gene: molecular markers of risk for male infertility. Fertil Steril 75:275–281

Mitsumori K, Terai A, Oka H, Segawa T, Ogura K, Yoshida O, Ogawa O (1999) Androgen receptor CAG repeat length polymorphism in benign prostatic hyperplasia (BPH): correlation with adenoma growth. Prostate 41:253–257

Odell WD, Parker LN (1984) Control of adrenal androgen production. Endocr Rev 10:617–630

Ozisik G, Achermann JC, Meeks JJ, Jameson JL (2003). SF1 in the development of the adrenal gland and gonads. Horm Res 59 (Suppl 1):94–98

Patrizio P, Leonard DG, Chen KL, Hernandez-Ayup S, Trounson AO (2001) Larger trinucleotide repeat size in the androgen receptor gene of infertile men with extremely severe oligozoospermia. J Androl 22:444–448

Pioro EP, Kant A, Mitsumoto H (1994) Disease expression in a Kennedy's disease kindred is unrelated to CAG tandem repeat size in the androgen receptor gene: characterization in a symptomatic female. Ann Neurol 36:318

Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E (2000) Racial variation in Prostate Cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 92:2009–2017

Pope HG, Cohane GH, Kanayama G, Siegel AJ, Hudson JI (2003). Testosterone Gel Supplementation for Men with Refractory Depression: A randomized placebo-controlled trial. Am J Psychiatry 160:105–111

Poujol N, Wurtz JM, Tahiri B, Lumbroso S, Nicolas JC, Moras D, Sultan C (2000) Specific recognition of androgens by their nuclear receptor. A structure-function study. J Biol Chem 275:24022–24031

Price P, Wass JA, Griffin JE, Leshin M, Savage MO, Large DM, Bu'Lock DE, Anderson DC, Wilson JD, Besser GM (1984) High dose androgen therapy in male pseudohermaphroditism due to 5 alpha-reductase deficiency and disorders of the androgen receptor. J Clin Invest 74:1496–508

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271–321

Radmayr C, Culig Z, Hobisch A, Corvin S, Bartsch G, Klocker H (1998) Analysis of a mutant androgen receptor offers a treatment modality in a patient with partial androgen insensitivity syndrome. Eur Urol 33:222–226

Rajpert-De Meyts E, Leffers H, Petersen JH, Andersen AG, Carlsen E, Jorgensen N, Skakkebaek NE (2002) CAG repeat length in androgen-receptor gene and reproductive variables in fertile and infertile men. Lancet 359:44–46

Remes T, Vaisanen SB, Mahonen A, Huuskonen J, Kroger H, Jurvelin JS, Penttila IM, Rauramaa R (2003) Aerobic exercise and bone mineral density in middle-aged finnish men: a controlled randomized trial with reference to androgen receptor aromatase and estrogen receptor alpha gene polymorphisms. Bone 32:412–420

Rodien P, Mebarki F, Mowszowicz I, Chaussain JL, Young J, Morel Y, Schaison G (1996) Different phenotypes in a family with androgen insensitivity caused by the same M780I point mutation in the androgen receptor gene. J Clin Endocrinol Metab 81:2994–2998

Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, Stanczyk FZ, Kolonel LN, Henderson BE (1998) Androgen metabolism and Prostate Cancer: establishing a model of genetic susceptibility. Cancer Res 58:4497–4504

Salmen T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, Pallonen H, Saarikoski S, Honkanen R, Maenpaa PH (2003) Relation of androgen receptor gene polymorphism to bone mineral density and fracture risk in early postmenopausal women during a 5-year randomized hormone replacement therapy trial. J Bone Miner Res 18:319–324

Sapir-Koren R, Livshits G, Landsman T, Kobyliansky E (2001) Bone mineral density is associated with estrogen receptor gene polymorphism in men. Anthropol Anzeiger 59:343–353

Sawaya ME, Shalita AR (1998) Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia hirsutism and acne. J Cutan Med Surg 3:9–15

Schweiger U, Deuschle M, Weber B, Korner A, Lammers CH, Schmider J (1999) Testosterone gonadotropin and cortisol secretion in male patients with major depression. Psychosom Med 61:292–296

Seidman SN, Araujo AB, Roose SP, McKinlay JB (2001) Testosterone level androgen receptor polymorphism and depressive symptoms in middle-aged men. Biol Psych 50:371–376

Sinnecker G, Kohler S (1989) Sex hormone-binding globulin response to the anabolic steroid stanozolol: evidence for its suitability as a biological androgen sensitivity test. J Clin Endocrinol Metab 68:1195–1200

Sinnecker GHG, Hiort O, Nitsche E, Holterhus PM, Kruse K (1997) Functional assessment and clinical classification of androgen sensitivity in patients with mutations of the androgen receptor gene. Eur J Pediatr 156:7–14

Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M (1999) Genetic markers bone mineral density and serum osteocalcin levels. J Bone Min Res 14:1411–1419

Stanford JL, Just JJ, Gibbs M, Wicklund KG, Neal CL, Blumenstein BA, Ostrander EA (1997) Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 57:1194–1198

Tilley WD, Marcelli M, Wilson JD, McPhaul MJ (1989) Characterization and expression of a cDNA encoding the human androgen receptor. Proc Natl Acad Sci USA 86:327–331

Tofteng CL, Kindmark A, Brandstrom H, Abrahamsen B, Petersen S, Stiger F, Stilgren LS, Jensen JE, Vestergaard P, Langdahl BL, Mosekilde L (2003) Polymorphisms in the CYP19 and AR genes relation to bone mass and longitudinal bone changes in postmenopausal women with or without hormone replacement therapy: The Danish osteoporosis prevention study. Calcif Tissue Int (*in press*)

Trapman J, Klaassen P, Kuiper GGJM, van der Korput JAGM, Faber PW, van Rooij HCJ, Geurts van Kessel A, Voorhorst MM, Mulder E, Brinkmann AO (1988) Cloning, structure and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Commun 153:241–248

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation impaired sperm production and male infertility. J Clin Endocr Metab 82:3777–3782

Unden F, Ljunggren JG, Beck-Friis J, Kjellman F, Wetterberg L (1988) Hypothalamic-pituitary-gonadal axis in major depressive disorders. Acta Psychiatri Scand 78:138–146

Van Golde R, Van Houwelingen K, Kiemeney L, Kremer J, Tuerlings J, Schalken J, Meuleman E (2002) Is increased CAG repeat length in the androgen receptor gene a risk factor for male subfertility? J Urol 167:621–623

Van Houten ME, Gooren LJ (2000) Differences in reproductive endocrinology between Asian men and Caucasian men – a literature review. Asian J Androl 2:13–20

Van Pottelbergh I, Lumbroso S, Goemaere S, Sultan C, Kaufman JM (2001) Lack of influence of the androgen receptor gene CAG-repeat polymorphism on sex steroid status and bone metabolism in elderly men. Clin Endocrinol 55:659–666

von Eckardstein S, Syska A, Gromoll J, Kamischke A, Simoni M, Nieschlag E (2001) Inverse correlation between sperm concentration and number of androgen receptor CAG repeats in normal men. J Clin Endocr Metab 86:2585–2590

von Eckardstein S, Schmidt A, Kamischke A, Simoni M, Gromoll J, Nieschlag E (2002) CAG repeat length in the androgen receptor gene and gonadotrophin suppression influence the effectiveness of hormonal male contraception. Clin Endocrinol 57:647–655

Wallerand H, Remy-Martin A, Chabannes E, Bermont L, Adessi GL, Bittard H (2001) Relationship between expansion of the CAG repeat in exon 1 of the androgen receptor gene and idiopathic male infertility. Fertil Steril 76:769–774

Wang G, Chen G, Wang X, Zhong J, Lu J (2001) The polymorphism of (CAG)n repeats within androgen receptor gene among Chinese male population. Zhonghua Yi Xue Yi Chuan Xue Za Zhi (Chinese Journal of Medical Genetics) 18:456–458

Weidemann W, Peters B, Romalo G, Spindler KD, Schweikert HU (1998) Response to androgen treatment in a patient with partial androgen insensitivity and a mutation in the deoxyribonucleic acid-binding domain of the androgen receptor. J Clin Endocrinol Metab 83:1173–1176

Weinbauer GF, Nieschlag E (1990) The role of testosterone in spermatogenesis. In: Testosterone: Action, Deficiency and Substitution, ed. Nieschlag E, Behre HM; Springer Berlin, Heidelberg, pp 23–50

Yong EL, Lim LS, Wang Q, Mifsud A, Lim J, Ong YC, Sim KS (2000) Androgen receptor polymorphisms and mutations in male infertility. J Endocrinol Invest 23:573–577

Yoshida KI, Yano M, Chiba K, Honda M, Kitahara S (1999) CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. Urology 54:1078–1081

Yu B, Handelsman DJ (2001) Pharmacogenetic polymorphisms of the AR and metabolism and susceptibility to hormone-induced azoospermia. J Clin Endocrinol Metab 86:4406–4411

Zitzmann M, Brune M, Kornmann B, Gromoll J, von Eckardstein S, von Eckardstein A, Nieschlag E (2001a) The CAG repeat polymorphism in the AR gene affects high density lipoprotein cholesterol and arterial vasoreactivity. J Clin Endocr Metab 86:4867–4873

Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001b) The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. Clin Endocrinol 55:649–657

Zitzmann M, Nieschlag E (2001c) Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. Euro J Endocrinol 144:183–197

Zitzmann M, Brune M, Nieschlag E (2002a) Vascular reactivity in hypogonadal men is reduced by androgen substitution. J Clin Endocr Metab 87:5030–5037

Zitzmann M, Brune M, Vieth V, Nieschlag E (2002b) Monitoring bone density in hypogonadal men by quantitative phalangeal ultrasound. Bone 31:422–429

Zitzmann M, Junker R, Kamischke A, Nieschlag E (2002c) Contraceptive steroids influence the hemostatic activation state in healthy males. J Androl 23:503–511

Zitzmann M, Gromoll J, von Eckardstein A, Nieschlag E (2003a) The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum levels of leptin and insulin in men. Diabetologia 46:31–39

Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E (2003b) Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: a longitudinal pharmacogenetic study. J Clin Endocrinol Metab 88:2049–2054

Zmuda JM, Cauley JA, Kuller LH, Newman AB, Robbins J, Harris T, Ferrell RE (2000a) Androgen receptor CAG repeat polymorphism: a novel marker of osteoporotic risk in men. Osteoporosis Int 11:S151 355

Zmuda JM, Cauley JA, Kuller LH, Zhang X, Palermo L, Nevitt MC, Ferrell RE (2000b) Androgen receptor CAG repeat length is associated with increased hip bone loss and vertebral fracture risk among older men. J Bone Min Res 15:S491 M141

4

Behavioural correlates of testosterone

K. Christiansen

Contents

4.1 Introduction

Behavioural endocrinology is the study of the interaction between hormones and behaviour. This interaction is bidirectional: hormones can affect behaviour, and behaviour can alter hormone levels. Thus, hormonal-behavioural correlations can be due to hormonal effects on behaviour, but certain behaviour (such as physical exercise, stress, sexual behaviour, alcohol consumption, and nutrition) is known to influence hormone levels as well (see below and Christiansen 1999).

Hormones do not cause behavioural changes per se; they can only alter the probability that particular behaviour will occur in the presence of a particular stimulus. Hormones can influence regions of the central nervous system (CNS) which contain hormone receptors by inducing changes in the rate of cellular function. The interaction of a hormone with its receptor begins a series of cellular events that lead to a genomic response wherein the hormone acts directly or indirectly to activate genes that regulate protein synthesis (e.g., Bixo *et al.* 1995; Chalepakis *et al.* 1990; Ford and Cramer 1982; Genazzani *et al.* 1992; Hutchinson 1991; McEwen 1992; McEwen *et al.* 1984; Sekeris 1990; Viru 1991).

Two decisive phases have been named in the discussion about the time of the effects of sex hormones on brain structures and consequently on behaviour. During fetal and neonatal life, relatively high concentrations of hormones, especially testosterone, are said to influence brain development by organizing the undifferentiated brain in a sex-specific manner. It has been shown, according to studies primarily in rodents, but also in primates and other mammals, that the hypothalamus, the hippocampus, the preoptic-septal region, and the limbic system (especially the amygdala) are important target areas for sex steroid action (Bettini *et al.* 1992; Brain and Haug 1992; Collaer and Hines 1995; Ellis 1982; Ford and Cramer 1982; Hutchinson 1991; 1993; Hutchinson and Steimer 1984; McEwen 1992; Michael and Bonsall 1990; Naftolin *et al.* 1990; Simon and Whalen 1987; Whalen 1982). These brain structures and hence the corresponding behavioural repertoires are then thought to be activated at the beginning of puberty when the production of sex hormones increases (Archer 1988; Beatty 1979; Becker *et al.* 1992; Schulkin 1993). However, experimental studies of birds, rodents, and monkeys have demonstrated that an animal's previous experiences in aggressive encounters can sometimes be more important than the testosterone level in determining an individual's aggressiveness or dominance (Archer 1991; Gordon *et al.* 1979; Rejeski *et al.* 1988).

In humans, hormonal influences on behaviour are much less potent than in animals. Quantitative and qualitative behavioural differences in males and females are thought to result mainly from a combination of psychosocial factors which are the end product of differential experience and expectation produced by socialization. Moreover, hormonal influences on human behaviour are difficult to prove as pertinent research has to rely predominantly on correlational studies of endogenous hormone levels and behaviour which cannot ascertain hormonal *influences*. Some knowledge derives from clinical studies on individuals who have been exposed to atypical levels of hormones during some developmental period of their lives, so-called "experiments of nature". Only on very few occasions can scientists ethically manipulate hormone levels in humans to observe subsequent effects on brain and behaviour. But even from these studies on hormone substitution one cannot always draw firm conclusions regarding a particular hormonal-behavioural

relation. If any, results from double-blind, placebo-controlled experimental designs can be interpreted as meaning that a particular hormone is the metabolic agent associated with behaviour. However, in behavioural endocrinology of humans, these are rare exceptions. Therefore, conclusions regarding hormonal effects on human behaviour have to be drawn with great care.

4.2 Sexuality

It is widely acknowledged that sexual behaviour in humans is multifactorial. Although no attempt will be made to deal with these issues here, it should be pointed out that intrapsychic, social, somatic and cultural factors can profoundly influence sexuality. The evidence presented here serves primarily to underline the contribution of sex hormones as a determinant of sexual behaviour.

It has long been recognized that androgens play a critical role in human male sexual behaviour. Prepubescent boys do not engage in sexual activity outside the context of play. After puberty, when the testes begin to secrete androgens, sex drive and the motivation to seek sexual contact become powerful and are overtly expressed. Sexual performance and copulatory ability increase as well. The general pattern of age-dependent rise and decline of androgen levels in men corresponds to average levels of male sexual activity throughout the cycle of life. When blood levels of testosterone, especially non SHBG-bound testosterone, diminish as men age, this mirrors their usually declining sexual interest and potency (Davidson *et al.* 1983). These observations suggest, but do not prove, that male sexual behaviour is influenced by androgens.

Less obvious and difficult to infer from everyday observation is the role of testosterone in female sexual behaviour. Physiological testosterone levels in women, which are one tenth of those in the normal male and to which males are unresponsive, seemed to be negligible. Thus, the idea that androgens could have enhancing effects on female sexual desire and arousal received little attention until synthezised testosterone was discovered to treat (post-) menopausal (Brincat *et al.* 1984) or oophorectomized (Sherwin *et al.* 1985) women.

4.2.1 Influence of testosterone on sexual behaviour in men

The physiological range of testosterone levels (3–12 ng/ml) is considerably higher than that necessary to maintain normal sexual functions. Testosterone levels found to be critical for sexual functions in males lie around 3ng/ml (Nieschlag 1979), and they show a clear intersubject variation. On the other hand, levels at which a decline of androgen-related sexual behaviour in individual subjects occurs appears to be reproducible (Gooren 1987).

Table 4.1 Significantly positive effects of androgens (testosterone, DHT) on various aspects of sexual behaviour in men

Behaviour	Endogenous testosterone/ DHT level	Testosterone substitution
Sexual interest and phantasies	Nilsson *et al.* 1995	Anderson *et al.* 1992 Bancroft 1984 Carani *et al.* 1990a Gooren 1987 Morales *et al.* 1997 O'Carroll and Bancroft 1984 Skakkebaek *et al.* 1981
Sexual arousal	-	Anderson *et al.* 1992 Bancroft 1984 Carani *et al.* 1990a Gooren 1987 Morales *et al.* 1997 Su *et al.* 1993
Spontaneous erections (during sleep, in the morning)	Carani *et al.* 1992 Schiavi *et al.* 1988	Carani *et al.* 1990a Luisi and Franchi 1980 Salmimies *et al.* 1982
Ejaculation	Schiavi *et al.* 1988	Gooren 1987 Salmimies *et al.* 1982 Skakkebaek *et al.* 1981
Sexual activities with partner	Schiavi *et al.* 1988	Carani *et al.* 1990a
Orgasms in sexual activity (masturbation or coitus)	Knussmann *et al.* 1986 Mantzoros *et al.* 1995 Schiavi *et al.* 1988	Davidson *et al.* 1979

Besides evidence from nonhuman primates and clinical case reports on effects of castration in human males (Nelson 1995), studies of hypogonadal men on androgen replacement therapy provide convincing evidence of the essential role of androgens in some aspects of male sexual behaviour (Table 4.1). In patients with induced or spontaneous hypogonadism, pathological withdrawal as well as reintroduction of exogenous androgens affected the frequency of sexual phantasies, sexual arousal and desire, spontanenous erections during sleep and in the morning, ejaculation, sexual activities with and without a partner, and orgasms through coitus or masturbation (Bancroft 1984; 1986; Carani *et al.* 1990a; 1992; Davidson *et al.* 1979; Gooren 1987; Luisi and Franchi 1980; Morales *et al.* 1997; Salmimies *et al.* 1982; Schiavi *et al.* 1988; Skakkebaek *et al.* 1981).

There is only limited evidence on the effects of testosterone administration to eugonadal men with or without sexual problems. In a controlled study of eugonadal men with diminished sexual desire O'Carroll and Bancroft (1984) produced a significant increase in sexual interest with injections of testosterone esters when compared to placebo injections. But in most of the men studied the increase in sexual interest was not translated into an improvement of their sexual relationship – perhaps because psychological problems with their partner had not been resolved with hormonal treatment only. When supraphysiological doses of testosterone used as potential hormonal male contraceptive agents were administered to healthy volunteers, this resulted in a significant increase in psychosexual stimulation or arousal during testosterone substitution, although there was no change in sexual activity or spontaneous erections (Anderson *et al.* 1992; Bagatell *et al.* 1994; Su *et al.* 1993).

As the healthy male produces much higher levels of androgens than necessary to maintain sexual function, lowering serum testosterone levels to the normal low range or increasing them to the high normal range in eugonadal men has no appreciable effect on sexual function (Buena *et al.* 1993). This led to the conclusion that androgens are only beneficial in those men whose endogenous levels are abnormally low. However, Bancroft (1984) pointed out that we cannot be certain on this point because with increasing levels of endogenous androgen supply it becomes more difficult to manipulate the circulating levels with exogenous hormones. The homeostatic mechanisms are powerful and the more testosterone is administered, the more the individual's own supply is suppressed or the metabolic clearance rate is increased. In a study by Benkert *et al.* (1979), who gave eugonadal men testosterone undecanoate daily to treat erectile dysfunction, no increase in circulating hormone levels was achieved. Their failure to produce any behavioural effect on erectile function therefore may not be due to ineffective androgens, but rather a result of their failure to alter hormone levels.

Indeed, in several studies a significant relationship between physiological androgen levels and male sexual behaviour was observed. In a Swedish epidemiological investigation of 500 men aged 51 years low levels of non SHBG-bound testosterone were associated with low sexual interest (Nilsson *et al.* 1995). In young soldiers aged 18 to 22 years serum concentrations of 5α-dihydrotestosterone were a significant hormonal determinant of orgasmic frequency (Mantzoros *et al.* 1995). In young healthy volunteers Knussmann *et al.* (1986) could ascertain significantly positive correlations of salivary and total serum testosterone with the frequency of orgasms during the 48 hours following blood sampling. In their study, the majority of *intra*individual correlation coefficients (from 6 samples per subject) were also positive but some negative and insignificant ones were found as well. This finding points to the great *inter*individual variability of behavioural responses to

hormones, and it could explain contradictory results from other pertinent studies on testosterone levels and frequency of orgasms (Buena *et al.* 1993; Kraemer *et al.* 1976; Persky *et al.* 1978; Raboch and Stárka 1972, 1973; Schwartz *et al.* 1980).

4.2.2 Influence of testosterone on sexual behaviour in women

A variety of models have been used to test the relationship between testosterone and sexuality in women. Because plasma testosterone levels peak around the time of ovulation (Ferin 1996), one investigational strategy involved monitoring changes in several aspects of sexual behaviour at differerent points during the menstrual cycle. As plasma levels of estradiol also reach their highest point at the ovulatory phase, this research design makes it difficult to prove that testosterone alone induces the increase in sexual behaviour during the midcycle portion of the menstrual cycle observed in some studies (Adams *et al.* 1978; Harvey 1987; Dennerstein *et al.* 1994; Matteo and Rissman 1984). But several well-controlled correlational studies measuring circulating testosterone in women found evidence of an androgenic enhancement of sexual behaviour. Higher testosterone levels (midcycle peaks or average levels of plasma testosterone throughout the cycle) were associated with less sexual avoidance (Persky *et al.* 1982); more sexual gratification (Persky *et al.* 1978; 1982), sexual thoughts (Alexander and Sherwin 1993), and initiation of sexual activity (Morris *et al.* 1987); higher levels of sexual interest and desire (Alexander and Sherwin 1993; Alexander *et al.* 1990; Leiblum *et al.* 1983) and vasocongestive responses to erotic films (Schreiner-Engel *et al.* 1981); increased frequency of masturbation (Bancroft *et al.* 1983) and coitus (Morris *et al.* 1987); and a higher number of sexual partners (Cashdan 1995).

The positive relationship between testosterone and various measures of sexual interest and behaviour is intriguing; on the other hand, most studies failed to provide evidence of a peak of sexual behaviour at the time of the midcycle peak in testosterone. However, this is no argument against any testosterone-sexuality relationship in females, as an increase in testosterone does not have to produce an immediate behavioural response. For instance, the latency between androgen administration and increases in sexual desire in hypogonadal men ranges from days to several weeks.

The most powerful design for the study of the specificity of testosterone influence involves hormone replacement therapy in women who are oophorectomized. It is common clinical practise to treat these patients with estrogen replacement, but substitution of testosterone is also sensible as the women are deprived of ovarian androgen production as well. Several studies on naturally or surgically menopausal women have shown – without contradictory evidence – that administration of testosterone, either alone or in addition to an estrogen replacement regimen, is more effective than estrogens alone or a placebo. In particular, an increase in sexual

desire and phantasies was elicited, but also in sexual arousal, sexual sensation, and in coital or orgasmic frequency, and masturbation frequency (Davis and Tan 2001; Sarrel *et al.* 1998; Sherwin, 2002; Sherwin and Gelfand 1987; Sherwin *et al.* 1985; Shifren *et al.* 2000).

Although there is converging evidence from these correlational and experimental investigations that testosterone enhances male and female sexual behaviour, such as sexual desire or sexual phantasies, the underlying behavioural mechanism is not fully understood. Testosterone might have direct effects on cognitive behaviour, e.g., influence the awareness of sexual cues (Alexander and Sherwin 1993), or may act peripherally to enhance sexual pleasure and, thereby increase sexual desire (Davidson *et al.* 1982). Myers and Morokoff (1986) could show that serum testosterone levels in women correlated with genital responses and subjective physical sensation (i.e., vaginal lubrication and breast sensation) in response to erotic visual stimulation.

Whether the effects of androgen were mediated by direct action on the nervous system, by an effect on the genital organs, or both had not been investigated in their study. At present, only data obtained from investigations of ovarectomized animals are available. Traish *et al.* (2002) characterized androgen receptor expression in rabbit vaginal tissues from control and ovariectomized animals treated with or without androgen replacement therapy. They found that vaginal tissues express androgen receptors and that the expression of these receptors is regulated differently in the proximal and distal vagina by androgens and estrogens. They concluded that androgens appear to play a role in vaginal and clitoral function during genital sexual arousal.

4.2.3 Influence of sexual behaviour on testosterone

The general concept that behaviour can feed back to hormone levels was first described with regard to sexual behaviour in an often cited publication (Anonymous 1970). A man working on an island attributed his increased beard growth immediately prior and during his visits to his girlfriend on the mainland to elevated androgen levels induced by sexual anticipation and sexual activity. Since then, numerous empirical studies dealt with effects of sexual behaviour (e.g., sexual stimulation, masturbation and coitus with or without orgasm) on testosterone levels. It could be demonstrated that almost any sexual behaviour can significantly alter sex hormone levels; however, cognitive factors and emotional involvement of the subjects produced mixed results. The majority of data on eugonadal men reports on effects of ejaculation. Orgasmic frequency in males, whether through masturbation or coitus, correlated positively with free, non SHBG-bound testosterone and serum testosterone (Christiansen *et al.* 1984; Dabbs and Mohammed 1992; Knussmann *et al.* 1986; Kraemer *et al.* 1976), while earlier investigations (Lee *et al.* 1974; Monti

et al. 1977; Stearns *et al.* 1973) could not ascertain behavioural-hormonal interactions. A significant rise in testosterone and DHT after masturbation was measured in blood samples of young males (Brown *et al.* 1978; Monti *et al.* 1977; Purvis *et al.* 1976) while a case study by Fox *et al.* (1972) found a testosterone increase in one male subject only after ejaculation during sexual intercourse but not after masturbation. This was explained by the man's lack of emotional involvement with his autoerotic behaviour.

Endocrine effects of erotic stimulation were investigated by Christiansen *et al.* (1984) who detected a significant increase of testosterone levels after more or less accidental sexual stimulation through attractive people, erotic pictures and movies – not sexual activities – during 24 hours before the blood sampling. Even closer correlations were found in controlled laboratory experiments showing erotic or sexually neutral films (Carani *et al.* 1990b; Evans and Distiller 1979; Hellhammer *et al.* 1985; Lincoln 1974; Pirke *et al.* 1974; Stoléru *et al.* 1993).

Up to now, very little attention has been paid to behavioural-androgenic effects in women. Only Dabbs and Mohammed (1992) measured salivary testosterone levels in women after sexual intercourse and detected a significant increase of testosterone compared to a baseline value. Samples taken in the evenings without preceding coitus did not show such an increase.

4.3 Stress

Activation of the hypothalamic-pituitary-adrenal axis and the subsequent release of cortisol is considered one of the major components of the physiological stress response in humans (Rose 1984). Stress responses of the pituitary-gonadal axis are not as well known although their sensibility and specifity are impressive.

Earliest studies investigated young military trainees in extremely stressful situations during combat training (Kreuz *et al.* 1972; Rose *et al.* 1969) and observed a significant decline of testosterone levels under psychologic and somatic stress. Twenty years later, Opstad (1992) studied Norwegian military cadets during five days of training involving strenuous exercise and almost total deprivation of food and sleep. They confirmed the previous findings of significantly decreased testosterone levels. Opstad attributed the hormonal response to extreme endurance training, sleep deficit and psychic stress (cf. Christiansen *et al.* 1984; Cortés-Gallegos *et al.* 1983; Guezennec *et al.* 1994; Singer and Zumoff 1992). In a similar study design, Bernton *et al.* (1995) investigated young male soldiers during eight weeks. They lived under extreme psychosomatic stress with long exposure to rough environments, caloric deprivation, four hours of sleep per week and psychologic stressors including constant risk of academic failure and the threat of simulated attack. The soldiers' testosterone levels decreased to clearly hypogonadal levels.

Even under less extreme conditions, psychosomatic stressors exert an influence on gonadal hormones: decreased concentrations of testosterone were found in males after surgery with anaesthesia (Carstensen *et al.* 1973; Matsumoto *et al.* 1970; Nakashima *et al.* 1975), driving heavy goods vehicles (Cullen *et al.* 1979), and routine flying missions in fighter type aircraft (Leedy and Wilson 1985) as well as significantly higher serum concentrations in female pilots flying a transport plane in comparison to female members of the ground crew (Dongyun and Yumin 1990).

Psychic stressors, e.g., financial difficulties, examinations, serious quarrels, loss of close friends or relatives, dissatisfaction, boredom, or watching a movie with a stressful theme were generally followed by decreasing testosterone levels in males (Christiansen *et al.* 1985; Francis 1981; Hellhammer *et al.* 1985; Nilsson *et al.* 1995). Even anticipation of a stressful event, a final exam at the university, leads to a decrease of salivary testosterone in males and to an increase in females the morning before the stressful situtation (Christiansen and Hars 1995). From the viewpoint of evolutionary biology, the sex-specific response to stress makes sense. While spermatogenesis in males is testosterone-dependent, in females, high levels of androgens are usually associated with anovulatory menstrual cycles. Thus, both reactions suppress fertility in order to increase the chance of survival of the individual and its family or group who are living under threatening or other stressful circumstances, which could be hazardous for pregnancies and infants, and the raising of children.

4.4 Physical exercise

A great deal of research on the effects of exercise upon the male and female reproductive system has taken place, and it has been demonstrated that some similarities, especially with regard to testosterone, exist between the sexes in the physiological outcomes of physical training when intrinsic gender differences in the endocrine system are acknowledged (Hackney 1989; Shangold 1984).

Despite their athletic appearance male athletes have lower androgen levels than untrained men in a resting state. The results of comparative studies suggest significantly lower free and total testosterone concentrations in chronically (several years) endurance-trained runners, weight lifters, rowers, cyclists, and swimmers (Arce and De Souza 1993; Arce *et al.* 1993; Hackney 2000; Hackney *et al.* 1988; Wheeler *et al.* 1984; 1991). In these studies, testosterone concentrations of trained subjects were only 60–85% of the age-matched untrained men. It should be noted that these low testosterone levels are typically not outside the clinical norm, but at the very low end of this range.

With few exceptions (Elias *et al.* 1991; 1993; MacConnie *et al.* 1986) LH and FSH concentrations and pulsatile frequency-amplitude were unaffected by the training,

even though testosterone was significantly reduced. Elias and Wilson (1993) emphasize that exercise effects on gonadotropic and gonadal hormones are independent of each other, and it is still uncertain what exact mechanisms cause the change in testosterone levels. Arce and De Souza (1993) attribute the decline in testosterone to alterations in hepatic and extrahepatic (muscles, skin) metabolism of testosterone which cannot be compensated by the athletes' gonads.

Acute effects of submaximal, prolonged (>60 min) exercise in marathon runners or cross-country skiers resemble hormonal changes found in endurance-trained men during resting state. After a 42-km marathon run or cross-country skiing over a distance of 75 km a highly significant decline in testosterone concentrations compared to pre-competition baselines was observed which could last as long as 5 days (Cook *et al.* 1986; Dessypris *et al.* 1976; Marinelli *et al.* 1994; Tanaka *et al.* 1986; Vasankari *et al.* 1993). After a long distance run (21 km or marathon), young sportswomen had significantly increased testosterone levels (Baker *et al.* 1982; De Crée *et al.* 1990; Hale *et al.* 1983). Even after a 30-min run significantly higher testosterone levels were observed (Shangold *et al.* 1981).

Regardless of the kind of sport, maximal or submaximal exercise (5–30 min) normally results in significant increases of testosterone levels in males independent of LH and FSH secretion. LH and FSH remain either relatively unchanged throughout the short exercise period (Sutton *et al.* 1973; Tegelman *et al.* 1988) or show an increase (Adlercreutz *et al.* 1976; Cumming *et al.* 1986; Kuoppasalmi *et al.* 1978).

The apparent disagreement between the effects of submaximal, prolonged exercise and 5–30 min exercise on testosterone levels in males are explained by the early (non-LH dependent) rise in testosterone concentrations (Adlercreutz *et al.* 1976) and a decreased metabolism of testosterone due to a drop in hepatic blood flow (Cadoux-Hudson *et al.* 1985).

As typical as the quick increase in testosterone concentration is the rapid decline below baseline levels 15–60 minutes later (Adlercreutz *et al.* 1976; Elias *et al.* 1993). The reduction can last up to three days (Adlercreutz *et al.* 1976; Häkkinen and Pakarinen 1993; Kuoppasalmi *et al.* 1978; Tegelman *et al.* 1988) and its duration was found to depend on the intensity of the exercise (Häkkinen and Pakarinen 1993).

4.5 Aggression

For many decades, scientists have tried to capture and explain the phenomenon of human aggression and the observed sex differences (Archer 2000; Eagly and Steffen 1986; Frodi *et al.* 1977; Fry 1998; Gladue 1991a; Hyde 1984; Knight *et al.* 2002; Richardson and Green 1999; Tieger 1980; Wynn *et al.* 1996). Among biological

factors, the endocrine system, especially testosterone, has been most intensively studied. A substantial body of data on subhuman primates has demonstrated a causative role of sex hormones in the development of sex-dimorphic aggressive behaviour (Archer 1988; Barfield 1984; Bernstein *et al.* 1983; Goy *et al.* 1988; Keverne 1979; Rose *et al.* 1971; 1975), and these results have focused attention on how endocrine activity and human aggression can interact with one another.

4.5.1 Prenatal hormones and aggression

Animal studies generally indicate that the presence of androgens in early life is important in establishing a biological readiness for future aggressive behaviour (Archer 1988). Much of the pertinent research on psychological effects of sex hormones in human studies consists of naturally occurring syndromes which result either from spontaneous endocrine excess or deficiency during fetal and early postnatal life (e.g., congenital adrenal hyperplasia) or prenatal sex hormone treatment of the pregnant mother. It is important to emphasize that true experimental design is precluded when studying potentially harmful hormone treatments in humans. Therefore, the researchers had to rely almost exclusively on these "experiments of nature" or existing clinical conditions to investigate how early exposure to hormones influences the potential for aggressive behavior in humans.

Overall, the results of pertinent studies show a slight effect of exposure to testosterone, progesterone with androgenic potential or diethylstilbestrol (a synthetic, nonsteroidal estrogen which exerts organizational effects similar to those of androgens converted to estrogens): an increase in physical aggressiveness, play with fighting figures, and intense energy expenditure (e.g., vigorous play and athleticism) but not on verbal aggression during childhood years. The positive effects of early exposure to sex hormones are significant for both boys and girls, yet these influences seem subtle (Berenbaum and Hines 1992; Ehrhardt and Baker 1974; Ehrhardt and Meyer-Bahlburg 1981; Ehrhardt *et al.* 1989; Hines 1982; Jacklin *et al.* 1983; Nordenström *et al.* 2002; Reinisch 1981; Reinisch and Sanders 1984). In a recent study, Hines *et al.* (2002) found that the mothers' endogenous testosterone level measured once between gestational weeks 15 to 36 (mean 16th week) related linearly to masculine-typical gender role behaviour of their child during preschool age in girls but not in boys.

Exogenous estrogens, usually given in combination with progestagens and progestin-based progestagens can counteract endogenous androgenic effects and thus demasculinize certain aspects of aggressive behaviour in early childhood and adolescence (Ehrhardt *et al.* 1984; Meyer-Bahlburg and Ehrhardt 1982; Yalom *et al.* 1973; Zussman *et al.* 1975).

Archer (1991) explained the rather small or even insignificant effects of prenatal hormone excess with results obtained from animal studies. He suggests that

Table 4.2 Endogenous testosterone and aggression in men and women

Nature of relationship between circulating testosterone and aggression	Studies based on behavioural measures	Studies based on self-ratings
Significant positive	Aromäki *et al.* 1997; Banks and Dabbs 1996[a]; Brooks and Reddon 1996; Christiansen and Winkler 1992; Dabbs and Hargrove 1997[b]; Dabbs *et al.* 1987, 1988[b], 1991, 1995; Ehlers *et al.* 1980[b]; Ehrenkranz *et al.* 1974; Elias *et al.* 1981; Gladue *et al.* 1989; Inoff-Germain *et al.* 1998[b]; Kedenburg 1977; Kreuz and Rose 1972; Lindman *et al.* 1987; Mattson *et al.* 1980; Olweus *et al.* 1980, 1988; Rada *et al.* 1976; Scaramella and Brown 1978	Aromäki *et al.* 1997; Cashdan 2003[b]; Christiansen and Knussmann 1987; Ehrenkranz *et al.* 1974; Gladue 1991b; Gray *et al.* 1991; Harris *et al.* 1996[a]; Houser 1979; Mattson *et al.* 1980; Olweus *et al.* 1980; Persky *et al.* 1971; Rada *et al.* 1983; Van Goozen *et al.* 1994[b]; von der Pahlen *et al.* 2002[b]
Insignificant positive	Kedenburg 1977[b]; Lindman *et al.* 1992; Meyer-Bahlburg *et al.* 1974a, 1974b; Rada *et al.* 1983; Susman *et al.* 1987	Bateup *et al.* 2002[b]; Campbell *et al.* 1997; Dabbs *et al.* 1991; Doering *et al.* 1975; Meyer-Bahlburg *et al.* 1974b; Persky *et al.* 1977; Rada *et al.* 1976; Udry and Talbert 1988[a]
Insignificant negative	Susman *et al.* 1987[b]	Kreuz and Rose 1972; Monti *et al.* 1977; Persky *et al.* 1982[b]
Significant negative	–	Gladue 1991b[b]

Note: [a] These citations involved female and male subjects
[b] These citations involved female subjects

organizing effects may only be detected clearly after puberty in the presence of adult sex hormone levels. In human studies the possibility that pubertal sex hormones are required to detect hormonal influences on behaviour remained untested except for the study by Yalom *et al.* (1973). He found stronger correlations of prenatal anti-androgen exposure to behaviour in adolescents after reaching puberty.

4.5.2 Adult testosterone levels

4.5.2.1 Aggressive behaviour

A number of studies provide evidence for an influence of circulating levels of androgens on aggression in males and females after puberty (Table 4.2). The hormonal effect is now referred to as *activational* as opposed to *organizational* effects in early

life. In the beginning, several pertinent studies were carried out in prison where usually some of the inmates are highly aggressive. Here, the researchers expected a significant relationship between current testosterone levels and aggression, a hypothesis that was confirmed.

There is consistent data from eight studies carried out on different types of violent male offenders who showed substantially higher testosterone levels than those found in selected samples of less violent prison inmates. Kreuz and Rose (1972) were the first to find that prisoners with a history of violent crime during adolescence showed higher testosterone levels than prisoners lacking such a history. Similar positive findings were reported by Aromäki *et al.* (1999); Banks and Dabbs (1996); Brooks and Reddon (1996); Dabbs *et al.* (1987; 1991; 1995), Ehrenkranz *et al.* (1974) as well as by Mattsson *et al.* (1980) who also found in their study of adolescent offenders that verbal aggression and impulsive behaviour in prison correlated significantly positively with testosterone levels. Studies of female prison inmates (Dabbs *et al.* 1988; Dabbs and Hargrove 1997) confirmed the results obtained from male offenders. Even the low testosterone concentrations in women, about 10 to 15% of circulating testosterone levels in men, exerted an influence on aggression or unprovoked violence in women. These findings, however, should not lead to the conclusion that testosterone unconditionally illicits violence in humans. It can only alter the probability that aggressive behaviour will occur under a specific combination of external and internal cues. Moreover, even if many findings suggest that testosterone is related to antisocial and offending behaviour, a significant positive correlation of testosterone with violence does not correspond necessarily with a high degree of aggression in all probands of the sample. There may also be individuals with relatively low testosterone levels and a high degree of aggression in the same study group.

With less severe offences the evidence for testosterone influence on aggressive behaviour is not as consistent. Neither Meyer-Bahlburg and his co-workers (1974a; 1974b), who investigated eight men with XYY-syndrome in comparison to normal XY-males nor Lindman *et al.* (1992) with their study on men who had been arrested for battering their wife under alcohol influence could find significantly higher testosterone levels in the study group in comparison to their controls, in the latter case non-violent pub patrons with a similar amount of alcohol consumption.

On the other hand, aggressive behaviour which was not a punishable offence also showed significant correlations with androgens in men and women. Under experimentally controlled alcohol intake, aggressively predisposed students were more dominant in a discussion and had higher free testosterone levels than nonaggressively predisposed students (Lindman *et al.* 1987). In male hockey players the pre-play testosterone levels correlated positively with reactive aggression during the tournament (Scaramella and Brown 1978). Male patients in a clinic for nervous

diseases showed more destructive aggression with higher levels of testosterone (Kedenburg 1977); in female patients he also detected a positive, however insignificant relation. The latter result was confirmed by Ehlers *et al.* (1980) who found that female outpatients of a neurobehavioural clinic who displayed more aggressive behaviour had significantly higher testosterone levels than women who were low in aggression.

In pubescent boys peer's and mothers' ratings of aggressive behaviour correlated positively with testosterone and several other androgens (Olweus *et al.* 1980; 1988; Susman *et al.* 1987). Testosterone-aggression relations based on hormone levels and observational measures of female adolescents while interacting with their parents were significantly positive with regard to their expression of anger when aggressed against by the parents (Inoff-Germain *et al.* 1988).

In a group of traditionally living !Kung San hunter-gatherers (so-called bushmen) from the Kalahari desert in Namibia, Christiansen and Winkler (1992) found that within a subgroup of physically aggressive San men violent behaviour correlated significantly and positively with free testosterone and 5α-dihydrotestosterone (DHT) levels. As the physically aggressive men also exhibited higher mean values in body measurements of robustness of the face and trunk, this finding may point to a possible pathway of *indirect* androgen action on human aggression, in addition to the widely accepted influence of testosterone on aggressive behaviour via its action on specific sites in the central nervous system.

4.5.2.2 Sexual aggression

Many aspects of sexual behaviour in the normal male are testosterone-dependent. With pathologically low serum testosterone levels a significant decrease in the frequency of sexual fantasies, sexual arousal and desire, spontaneous nocturnal or morning erections, ejaculations, sexual activity with and without a partner has been observed and also successfully treated with androgen replacement. Even among eugonadal men, some evidence for a positive relationship of endogenous testosterone with sexual behaviour has been found. This gave rise to the supposition that abnormally high androgen levels in men might elicit rape or other types of sexual aggression.

The first pertinent study was carried out on 52 sex offenders by Rada *et al.* (1976). A group of brutally violent rapists (according to clinical classification, police records and interviews) showed significantly higher levels of plasma testosterone than a combination of three other groups consisting of less overtly violent rapists, convicted child molesters and adult male volunteers. Taken together as one subgroup, rapists (both brutal and less violent) did not differ significantly from the child molesters in testosterone levels. Their mean serum testosterone values of

6.1 ng/ml (rapists) and 5.0 ng/ml (child molesters) both fell within the range of normal populations.

In a follow-up study Rada *et al.* (1983) failed to confirm differences in testosterone concentrations between a subsample of violent rapists and non-violent sex offenders or normal controls, while the group of child molesters had significantly lower testosterone levels than the rapists' group.

In a study of healthy normal young men Christiansen and Knussmann (1987a) found that interest in sexual aggression – assessed by measuring the viewing times of relevant slides – exhibited a low positive correlation with free testosterone and a significantly negative correlation with the hormone ratio DHT to testosterone. The correlation coefficients of free testosterone levels and interest in aggressive sexuality rose slightly as the aggressiveness illustrated in the slides increased.

4.5.2.3 Self-ratings of aggression

While the positive link between testosterone and past or present aggressive behaviour is fairly consistent, self-report measures of aggression, irritability, and hostility exhibit as many insignificant as significant relations with endogenous testosterone levels. Table 4.2 gives an overview of the distribution of significant and insignificant findings over the last twenty years, both for aggressive behaviour and self-ratings of aggression.

The inconsistent results for questionnaire data may be explained by several factors. First the selection of subjects and sample size: the age of the volunteers ranged between 10 to over 70 years; the sample sizes varied between 5 and 1709 subjects. Furthermore, great differences exist with regard to the number of serum or saliva samples collected for the hormone assays. In most studies only a single sample was used to determine the individual's sex hormone level; other investigators preferred to rely on up to 30 samples collected over a two-month period in order to find *the* typical sex hormone level of a subject. Additional influence on the outcome of psychoendocrinological studies stems from the choice of the questionnaire. As the studies originate from countries all over the world, it was impossible always to use the same aggression inventory and thus quite diverse scales are supposed to represent the same personality trait, e.g., dominance, hostility or reactive aggression.

A positive relation of testosterone and questionnaire data of aggressiveness was found in both sexes from puberty to old age (Aromäki *et al.* 1999; Cashdan 2003; Christiansen and Knussmann 1987a; Ehrenkranz *et al.* 1974; Gladue 1991b; Gray *et al.* 1991; Harris *et al.* 1996; Houser 1979; Mattsson *et al.* 1980; Olweus *et al.* 1980; Persky *et al.* 1971; Rada *et al.* 1983; van Goozen *et al.* 1994a; von der Pahlen *et al.* 2002). Gladue (1991b) is the only one who found significantly negative correlations between testosterone and self-report measures of physical and verbal aggression in

females – in contrast to the results of van Goozen *et al.* (1994a) and Harris *et al.* (1996).

Several researchers could not detect any significant testosterone-aggressiveness relationship in men or women (Bateup *et al.* 2002; Campbell *et al.* 1997; Dabbs *et al.* 1991; Doering *et al.* 1975; Kreuz and Rose 1972; Meyer-Bahlburg *et al.* 1974b; Monti *et al.* 1977; Persky *et al.* 1977, 1982; Rada *et al.* 1976; Susman *et al.* 1987; Udry and Talbert 1988).

Thus it seems to be worth considering whether perhaps the relationship between testosterone and aggressiveness might be obscured by the interindividual variability in environmental, familial, and cognitive characteristics as well as personality traits that promote learning and emission of aggressive behaviour.

4.5.3 Testosterone administration

Correlational data reviewed in the previous chapters suggest that aggressive behaviour and presumably also aggressiveness in men and women are related to current endogenous testosterone levels – but they do not prove a cause-effect relationship. In addition to studies on prenatal hormone treatment, research on the effects of testosterone intake in the adult female and male could possibly clarify the question whether aggression is actually testosterone-dependent.

Testosterone replacement therapy for hypogonadal males (Finkelstein *et al.* 1997; Skakkebaek *et al.* 1981) or exogenous testosterone as potential hormonal male contraceptive (Anderson *et al.* 1992; Bagatell *et al.* 1994; Nieschlag 1992; see Chapter 23 by Nieschlag and Behre in this volume) failed to show such an effect (Table 4.3). In a double-blind study by Björkqvist *et al.* (1994) male university students were given either testosterone, placebo, or no treatment for one week. After treatment the placebo group scored higher than both the control and testosterone group on self-evaluated anger, irritability, and impulsivity. Unfortunately, the authors of this well-controlled study did not assess aggressiveness with any of the standardized aggression questionnaires. A double-blind, cross-over study (Sherwin and Gelfand 1985) provided further evidence of a positive effect on hostility scores in surgically menopausal women during testosterone replacement therapy.

With increasing anabolic-androgenic steroid abuse a new field of research opened up. Adverse behavioural effects such as increased irritability and aggressiveness have been reported in several field studies on men and women (Bahrke *et al.* 1992; Brower *et al.* 1991; Perry *et al.* 1990; Strauss *et al.* 1983; 1985). A double-blind study by Hannan *et al.* (1991) confirmed these findings. Increased aggressiveness (resentment, hostility, and aggression) occurred, even more so in high-dose anabolic steroid users. In a review of pertinent research Uzych (1992) concludes that the possibility of increased aggression after steroid abuse cannot be excluded, but claiming that aggression in anabolic-androgenic steroid users is only testosterone-dependent is too simple. Factors such as unstable personality may be the source of willingness

Table 4.3 Effects of exogenous testosterone on aggression in men and women

Nature of relationship between circulating testosterone and aggression	Studies based on behavioural measures	Studies based on self-ratings or interviews
Significant positive	van Goozen *et al.* 1995a[a]	Brower *et al.* 1991 Finkelstein *et al.* 1997 Hannan *et al.* 1991 Perry *et al.* 1990 Sherwin and Gelfand 1985[a] Strauss *et al.* 1983, 1985[a] van Goozen *et al.* 1991, 1994[a], 1995b
Insignificant positive	–	Anderson *et al.* 1992 Bagatell *et al.* 1994 Bahrke *et al.* 1990, 1992 Nieschlag 1992 Skakkebek *et al.* 1981
Insignificant negative	–	Anderson *et al.* 1992 Björkqvist *et al.* 1994

Note: [a] These citations involved female subjects

to abuse steroids, as well as of aggressiveness. Bahrke *et al.* (1990) observed that irritability was slightly increased in many male steroid users but that only in a few, who were premorbid, might steroid use have been sufficient "to push them over the edge" and contribute to irrational or violent behaviour. Björkqvist *et al.* (1994) also conclude that steroid abuse may, for some, be a mediating factor enhancing aggressive tendencies by producing states of elated emotionality.

Further support of testosterone influence on aggression was published by van Goozen *et al.* (1994a; 1995a; 1995b) who studied female-to-male and male-to-female transsexuals. After three months of cross-sex hormone treatment female-to-male transsexuals responded with more anger and aggression on a questionnaire describing hypothetical aversive situations. In male-to-female transsexuals anger and aggression proneness significantly decreased after androgen deprivation (van Goozen *et al.* 1995a; 1995b).

In order to understand the complexity of the relation between sex hormones and aggression one further aspect has to be considered: testosterone and aggression seem to be mutually dependent. In addition to sex hormone influences on human aggression, several studies have shown that assertive or aggressive behaviour (e.g., in sport competitions or game contests) followed by a rise in status leads to an increase in testosterone levels (Booth *et al.* 1989; Elias 1981; Gladue *et al.* 1989; Gonzalez-Bono *et al.* 2000; Mazur and Lamb 1980; Mazur *et al.* 1992; McCaul *et al.* 1992).

However, the rise in testosterone following a win seems to be associated with the subject's elevated mood of victory or elation. Active participation in a competition is not necessarily required: testosterone levels increased among spectators watching their favourite sports team win and decreased for fans of the losing teams (Bernardt *et al.* 1989). If the mood elevation is absent or subjects do not regard the win as important, than the rise in testosterone did not occur (Mazur *et al.* 1997; Salvadore *et al.* 1987). Booth *et al.* (1989) also found that testosterone rose in tennis players 15 minutes before the next match – if the individual had won the previous match and probably anticipated winning again. Thus, the experience of winning and of a rise in status seemed to produce a rise in testosterone or to maintain an already elevated level, sustaining the winner's activation and readiness to enter subsequent competitions for higher status. Mazur's "Biosocial theory of status" (1985) incorporates these findings by hypothesizing a feedback loop between an individual's testosterone level and his or her assertiveness in attempting to achieve or maintain interpersonal status or dominance rank. This feedback loop may account for winning and losing "streaks" because each win reinforces a high testosterone level, which in turn reinforces further assertiveness or aggression.

This model of reciprocal effects between sex hormones and environment, although being more complex than simple hormone or experiential factors, still does not fully explain the variation in aggressive behaviour between individuals. New research perspectives will be necessary and helpful. A recent study within the theoretical framework of evolutionary psychobiology by Neave and Wolfson (2003) set an example. They argued that human males are more dominant and activated, attacking more readily within a territory defined as their own – a typical behaviour shown by males (and females) of various species, including subhuman primates. As antagonistic behaviour is related to testosterone levels, with an invasion triggering a subsequent rise in this hormone, they investigated the hypothesis that territoriality in male soccer players is positively related to levels of testosterone. They found that salivary testosterone levels in soccer players were significantly higher before home games than away games. Moreover, testosterone levels were higher before playing against an extreme rival than a moderate rival which could be a reaction to the extent of the perceived threat. However, in this study the self-report mood rating before the matches (e.g., dominant, confident, anxious, aggressive) did not relate to the players' testosterone levels.

4.6 Mood

Depressive illness comprises a group of disorders that have different clinical symptoms and that respond to different treatment modalities. The symptoms of depression include reduced mood; low self-esteem; general fatigue; feelings of guilt; sleep and sex drive disturbances; absence of pleasure; agitated or retarded motor

symptoms. Thus, depression covers a wide range of emotional and clinical states, and as a normal mood, depression is ubiquitous in human existence. It is an uncontradicted finding that depression is more prevalent in women than in men. The higher incidence of depression in women is primarily seen from puberty on and is less marked in the years after menopause with the exception of the perimenopausal time (Steiner *et al.* 2003; Weissman and Olfson 1995). This phenomenon could in part be explained by the hypothesis (Maggi and Perez 1985) that sex hormones are involved in the etiology of some types of depression.

The relationship between cyclic hormonal changes and social behaviour has been examined in great detail (Bäckström 1992; Bäckström *et al.* 1983; Bains and Slade 1988; Bancroft 1993; Lewis 1990). Besides the contradictory, however significant roles of estradiol and progesterone, testosterone was found to be associated with higher levels of premenstrual dysphoria (Dunn *et al.* 2001; Eriksson *et al.* 1992). Corresponding observations were made in female depressives who had higher testosterone levels than healthy controls (Hartmann *et al.* 1996; Vogel *et al.* 1978). Reduction of premenstrual dysphoria with androgen-antagonists in women with premenstrual syndrome (PMS) also supports the idea of increased androgenicity (Rowe and Sasse 1986). In non-clinical studies on adolescent girls not suffering from depression either significantly positive (Paikoff *et al.* 1991; Warren and Brooks-Gunn 1989) or insignificantly positive (Susman *et al.* 1987) testosterone-dyshoria relations were found.

In males, the study of testosterone in depressive illness was prompted by the observation that in castrates and hypogonadal men treated with androgens the incidence of depressed mood and emotional instability appreciably decreased. Beach (1948) reported several cases of involutional melancholia in older men which were explained by a reduction of testicular hormones below the level necessary for mental stability. Improvement in mood and alertness was described in 65% of such cases when treated with testosterone propionate. However, more recent approaches to the psychoendocrinology of depression, using radioimmunoassays for determination of androgen levels in body fluids, were less successful in demonstrating anti-depressive properties of endogenous testosterone in male patients. Only Vogel *et al.* (1978) and Yesavage *et al.* (1985) found a significantly negative relation of testosterone with depression. The majority of clinical studies failed to ascertain significant effects of testosterone or testosterone production rate on depression (Levitt and Joffe 1988; Persky *et al.* 1968; 1971; Rubin *et al.* 1981; 1989; Sachar *et al.* 1973; Unden *et al.* 1988). Although some data suggested a dysfunction of the hypothalamo-pituitary-gonadal (HPG) axis, the majority of resarchers concluded that there appears to be no major dysregulation of HPG axis activity in male endogenous depressives, even if dysregulation might be related to reduced energy levels and sexual interest occurring in many depressive men (Angst 1983; Freedman and Carter 1982; Matussek 1980; Rubin *et al.* 1989; von Zerssen *et al.* 1984).

Parallel to clinical studies, research was extended to sex hormone levels and mood in healthy males with symptoms of severe depression. Besides nonconfirmatory evidence (Anderson *et al.* 1992; Persky *et al.* 1977; Susman *et al.* 1987), suprisingly, quite a number of significant, however controversial, relationships between mood and sex hormone status could be demonstrated, although hormonal and psychological variables lay within the normal range (Table 4.4). In a two-month study of testosterone cycles and affective states among 20 healthy young males Doering *et al.* (1974) observed significant positive correlations between testosterone and self-ratings of depression. Houser (1979) tested five healthy men three times a week over a 10-week period and confirmed their findings: a significantly positive relationship between testosterone and self-ratings of depression and a negative correlation with elation. Christiansen and Knussmann (unpublished data) observed a corresponding trend in a large sample ($n = 117$) of unselected volunteers. Men with a high testosterone level had higher depression scores ($p < 0{,}06$) on a self-rating scale than low-testosterone males.

On the other hand, there also exists some evidence for an association of high endogenous testosterone levels with emotional well-being, as was found in early studies on testosterone substitution. A sample of 21 healthy young males was investigated and reported significantly negative correlations of salivary testosterone with depression and anxiety and a positive correlation with joyfulness (Hubert 1990; compare: Barrett-Connor *et al.* 1999; Christiansen *et al.* 1984; Daitzman and Zuckerman 1980; Diamond *et al.* 1989; Grinspoon *et al.* 2000; Wang *et al.* 1996). Furthermore, testosterone supplementation in elderly males with low or borderline low serum testosterone levels (Barrett-Connor *et al.* 1999; Tenover 1992), in healthy men without hypogonadism (Wang *et al.* 1996), and in hypogonadal immunodeficiency virus-infected men (Grinspoon *et al.* 2000) resulted in a significant improvement in sense of wellbeing and a significant reduction in negative mood scores. Azad *et al.* (2003) demonstrated that testosterone replacement therapy in elderly males with very low free testosterone levels not only improved their mental well-being and social interactions – but more importantly – they found significant increases of cerebral perfusion in selected areas of the CNS involved in emotional behaviour, general arousal reaction, and wakefulness, which could be caused by selective responsiveness of these areas to androgen. Similar beneficial effects of androgen replacement in women were first reported by Sherwin and Gelfand (1985). Oophorectomized women under testosterone substitution had significantly lower depression scores compared to both their baseline and placebo-phase scores (see also Shifren *et al.* 2000; Zweifel and O'Brien 1997).

Research in anabolic steroid abuse supported these findings. Pope and Katz (1988, 1989) contacted bodybuilding studios and offered members using steroids

Table 4.4 Significant relationships of testosterone and mood (non-clinical depression/mania) in men and women

Study (authors)	Endogenous testosterone	Testosterone substitution
Doering *et al.* 1974	positive with depression	
Houser 1979	positive with depression	
	negative with elation	
Daitzman and Zuckerman 1980	negative with depression	
Christiansen *et al.* 1984	negative with anxiety	
Christiansen and Knussmann (unpublished results)	positive with depression	
Sherwin and Gelfand 1985[a]		▼ depression
Rowe and Sasse 1986[a]		▲ premenstrual dysphoria
Pope and Katz 1988, 1989		▲ elation or mania
Brower *et al.* 1989		▼ depression
Diamond *et al.* 1989	negative with anxiety	
Hubert 1990	negative with anxiety	
	negative with depression	
	positive with joyfulness	
Perry *et al.* 1990		▼ depression
Paikoff *et al.* 1991[a]	negative with depression	
Tenover 1992		▲ well-being
Ericksson *et al.* 1992[a]	positive with premenstrual dysphoria	
Wang *et al.* 1996	positive with well-being	▲ well-being
	negative with nervousness	▼ nervousness
	negative with irritability	▼ irritability
		▼ depression
Barrett-Connor *et al.* 1999	negative with depression	
Grinspoon *et al.* 2000	negative with depression	▼ depression
Shifren *et al.* 2000[a]		▲ well-being
		▼ depression
Dunn *et al.* 2001[a]	positive with premenstrual dysphoria	
Azad *et al.* 2003		▲ well-being

▲ increase ▼ decrease

[a] these citations involved female subjects

a cash payment to engage in confidential interviews about their steroid use. One third of 41 individuals reported to be manic or near manic following steroid use. Most symptoms subsided when anabolic steroid use was discontinued (Pope and Katz 1989). Perry *et al.* (1990) investigated 20 competitive and noncompetitive weight lifters who consistently practised anabolic steroid abuse in cycles lasting 7 and 14 weeks. Accompanying the changes in physical parameters 70% of the men experienced depression more frequently while cycling. Clinical symptoms of depression were noted in 40% to 50% of the subjects, including low energy levels and excessive worrying.

According to Kashkin and Kleber (1989), anabolic steroids might have directly rewarding properties. Increasing numbers of reports on unexpected suicides in previously non-depressed young men who had abruptly stopped using anabolic steroids have been noted. Thus, withdrawal symptoms such as fatigue and depressed mood manifested by anabolic steroid abusers (Brower *et al.* 1989) and the symptoms of postpartum depression in women may result from a common underlying cause, dependence upon elevated steroid hormone levels (Nelson 1995).

4.7 Cognitive function

For many decades consistent sex differences in tests of cognitive abilities have been widely reported. During certain developmental stages – in particular during the first years of life and from puberty to early adulthood – girls surpass boys in several verbal skills. In contrast, males excel after about the tenth year and in adulthood at nonverbal skills, especially at spatial rotation and manipulation, at the related concept of field independence, and at mathematical reasoning (Halpern 2000; Hyde and Linn 1988; Hyde *et al.* 1990; Kimura 1996; Linn and Petersen 1985; Maccoby and Jacklin 1974; Masters and Sanders 1993; Wittig and Petersen 1979). In recent meta-analyses it was found that gender differences in cognitive functioning have been decreasing since the seventies; however, they are still significant (Hyde and Linn 1988; Hyde *et al.* 1990; Linn and Petersen 1985; Stumpf and Klieme 1989).

Many sources of variance contribute to the gender differences which have been observed: culture, physical environment, socialization practices, life experiences, X-chromosome linkage of spatial and partly of verbal skills as well as the degree of hemispheric lateralization of verbal and nonverbal processes (Baenninger and Newcombe 1989; Blatter 1982; Hahn 1987; Vandenberg and Kuse 1979; Waber 1977; Wynn *et al.* 1996; Zitzmann *et al.* 2001). It is also generally accepted that sex hormones play a critical role in sex-typical cognitive abilities as well as in interindividual differences within the sexes.

4.7.1 Clinical studies and testosterone substitution

Evidence of a connection between sex hormones and spatial abilities came first from studies of phenotypic women with Turner's syndrome (X0 karyotype, no gonadal hormones) or testicular-feminization syndrome (XY karyotype; here the body tissues are unable to respond to normal levels of testosterone; see Chapter 2 in this volume). These patients have female external genitalia, they are raised as girls, and develop a feminine gender identity. With regard to cognitive functioning such individuals' verbal skills surpass their spatial abilities, the typical pattern of cognitive abilities in women. Moreover, on average they are also below healthy women in terms of their nonverbal performance but not of their verbal abilities (Collaer and Hines 1995; Dellantonio *et al.* 1984; Garron and van der Stoep 1969; Imperato-McGinley *et al.* 1991; Masica *et al.* 1969; Rovet and Netley 1979; 1982; Serra *et al.* 1978; Silbert *et al.* 1977). The strikingly poor nonverbal abilities are mainly explained by their low sex hormone levels.

Feminized prepubertal boys suffering from a kwashiorkor-induced endocrine dysfunction have been found to exhibit a more feminine cognitive style than a male control group. Dawson (1972) attributed this finding to a two-way relationship between sex hormones and socialization – because of their physical effeminateness, the boys tended to be raised into more feminine roles, including feminine cognitive behaviour.

Studies on men with idiopathic or aquired hypogonadotrophic hypogonadism appear to confirm the importance of testosterone for spatial abilities (Alexander *et al.* 1998; Buchsbaum and Henkin 1980; Hier and Crowley 1982; O'Connor *et al.* 2001). A group of men with idiopathic hypogonadotrophic hypogonadism, and presumably a lifelong testosterone deficiency, performed significantly poorer than a group of men with late onset of pathologically reduced testosterone levels or normal controls on a number of spatial tests, but not on verbal tests. As short-term androgen therapy did not restore spatial function, these findings suggest that pre- and perinatal hormonal environments have lifelong effects on intellectual function in humans.

Although controversial, data from children with congenital adrenal hyperplasia (CAH) more or less support this hypothesis. Beginning in the third month of gestation, these patients are exposed to high levels of fetal androgens as a result of an enzymatic defect of the adrenal cortex. As a result, androgen production is greatly and continuously increased until treatment is initiated sometime in early postnatal life (Lauritzen 1987). While Lewis *et al.* (1968), Baker and Ehrhardt (1974), and McGuire *et al.* (1975) could not ascertain any effect of the prenatal exposure to higher-than-normal levels of androgens in CAH-children, Perlman (1973), Resnick *et al.* (1986), and Berenbaum *et al.* (1995) found that CAH-girls were superior to their siblings or normal controls in spatial tasks. However, in boys

Table 4.5 Androgen treatment and cognitive functioning in men and women

Study	Sample	Cognitive task
Simonson *et al.* 1941	castrates, eunuchoids	critical flicker frequency ▲
Simonson *et al.* 1944	older men	critical flicker frequency ▲
Düker 1957	men in a state of mental exhaustion	Arithmetical problem solving ▲
Klaiber *et al.* 1971	college students	verbal and nonverbal repetitive tasks ▲
Stenn *et al.* 1972	poorly androgenized male adolescents	verbal repetitive tasks ▲
Sherwin 1988	oophorectomized women	short-term memory ▲ abstract reasoning ▲ perceptual speed ▲
van Goozen *et al.* 1994b	female-to-male transsexuals	visual-spatial skills ▲ verbal fluency ▼
Janowsky *et al.* 1994	older men	visual-spatial ability ▲
Alexander *et al.* 1998	hypogonadal men	word fluency ▲
Slabbekoorn *et al.* 1999	female-to-male transsexuals	visual-spatial skills ▲
Janowsky *et al.* 2000	elderly men	verbal and spatial memory ▲
Cherrier *et al.* 2001	elderly men	verbal and spatial memory ▲

▲ significant increase ▼ significant decrease

with CAH the prenatal increase of androgens did not significantly influence their visual-spatial abilities (Hampson *et al.* 1998; Resnick *et al.* 1986; Trautmann *et al.* 1995). This lack of androgen influence on cognition in boys is consistent with two studies measuring fetal or neonatal androgens. Neither Jacklin *et al.* (1988), who measured testosterone in umbilical cord blood obtained at birth, nor Finegan *et al.* (1992), who measured testosterone in second trimester amniotic fluid obtained via amniocentesis, detected a significant relationship to cognitive skills of boys at age six or age four.

Direct *manipulation of steroid hormones* supports the conclusion that androgens play a role in cognition. Studies on the effects of hormone treatment on cognitive functioning in adult males date back to 1941 and 1944 when Simonson and his co-workers published their experiments using methyl testosterone (Table 4.5). Administration of testosterone to castrated human males, eunuchoids, and older men improved their ability to perceive flicker (critical flicker frequency), a measure of attention and alertness, as long as the androgen treatment lasted. Düker (1957) using either testosterone, estradiol, or a combination of testosterone and estradiol produced a significant rise in concentration and speed solving simple arithmetical problems in a group of men with severe mental exhaustion. Similar results were reported by Stenn *et al.* (1972) who treated three poorly androgenized male

adolescents. Intramuscular injections of testosterone enhanced their concentration and performance in a verbal fluency task. However, testosterone replacement therapy in hypogonadal men does not necessarily enhance cognitive speed and memory functions. While Alexander *et al.* (1998) obtained significant improvements in their subjects with either hyper- or hypogonadotropic hypogonadism, Sih *et al.* (1997) did not find such positive effect on word fluency or memory in older hypogonadal men.

A double-blind, cross-over, placebo-controlled study on substitution of sex steroids in oophorectomized women also demonstrated a causal link between testosterone and cognition (Sherwin 1988). Patients treated after surgery with either testosterone enanthate or a combination of testosterone enanthate, estradiol dienanthate, and estradiol benzoate showed stability both in sex hormone concentrations and in aspects of cognitive performance which are typically the most prominent deficits in menopausal women, short- and long-term memory and perceptual speed. Similar improvements of verbal and spatial memory were found in healthy elderly males under testosterone substitution (Cherrier *et al.* 2001; Janowsky *et al.* 2000). When normal, aging men were given testosterone to enhance sexual functioning, as a side-effect they showed improved performance on a visual-spatial task, relative to a placebo group (Janowsky *et al.* 1994). Zitzmann *et al.* (2001) investigated whether cerebral structures previously described to be involved in mental rotation (Gur *et al.* 2000) are influenced by testosterone substitution in hypogonadal males who reached eugonadal levels during the treatment. Using a positron emission tomography (PET) during a mental rotation task before and after testosterone substitution they could demonstrate in four out of six males between ages 20 to 63 years distinct changes in cerebral glucose metabolism in specific areas, however, with marked interindividual variability. Two men who did not improve their spatial ability score showed no change in cerebral glucose metabolism.

Direct effects of sex steroids on spatial performance could not be ascertained in 35 boys with delayed puberty (Liben *et al.* 2002). In a placebo-controlled study the adolescent boys (mean age 13.7 years) did not show an improvement of visual-spatial abilities under testosterone supplementation used to approximate the normal hormone environments of early, middle and late puberty, as the spatial performance of the adolescents did not vary with levels of actively circulating sex steroids.

Androgen treatment was also given to female-to- male transsexuals who received high doses of testosterone esters intramuscularly in preparation for sex therapy. Their spatial skills improved dramatically and their verbal fluency skills declined considerably within three months (van Goozen *et al.* 1994b). A more recent study (Slabbekoorn *et al.* 1999) involving 16 female-to- male and 14 male-to-female transsexuals before, during and after cross-sex hormone therapy confirmed the earlier findings. Testosterone treatment showed an enhancing, slowly reversible effect

on spatial performance in adult female-to male transsexuals, but no deteriorating effect on their verbal fluency. In contrast, anti-androgen treatment of male-to-female transsexuals in combination with estrogen therapy had no declining effect on spatial ability, nor an enhancing one on verbal fluency. For ethical reasons, today manipulation of gonadal hormones is restricted to patients in clinical studies. Thus, it is over 25 years ago that Klaiber *et al.* (1971) tested effects of infused testosterone on mental performance in healthy male college students. After a 4-hour infusion of testosterone or a saline infusion in the control group, performance of the control group on a repetitive mental task showed a significantly greater decline than the testosterone-infused group. The result indicates that testosterone acted to prevent the typical decline from morning to afternoon in the performance of simple repetitive verbal or number tasks.

4.7.2 Endogenous testosterone levels

In the early seventies, determination of sex hormones using radioimmunoassays was established as a highly specific and reliable method. This enabled scientists to obtain quantitative data on circulating hormones in blood or saliva and to investigate the relationship of current sex steroids and cognitive abilities in normal, healthy subjects (Table 4.6). Komnenich *et al.* (1978) were the first to investigate healthy young women ($n = 24$) and men ($n = 10$). Four times within a month they measured the concentration of FSH, LH, testosterone, estradiol, and progesterone in plasma. On each of the days, simple repetitive tasks and a nonverbal test of field-independence were administered. Only the performance on the verbal tasks was positively related to estradiol in males. None of the other hormones exhibited a significant relationship to cognitive performance in men or women.

In a relatively large sample of 43 men between 20 and 40 years Shute *et al.* (1983) detected a distribution of visual-spatial test scores as a function of androgen levels with the best-fitting third-order polynomial function describing the curve. Shute *et al.* reported that normal males selected for low plasma androgens were superior on certain spatial tests, while in their sample of 48 females the reverse was true, that is, highest-androgen females were superior to low-androgen women. However, due to the high cross-reactivity of the antibody used in their radioimmunoassays the authors speak of "general androgen" level instead of free, non SHBG-bound testosterone, which they originally intended to measure. Gouchie and Kimura (1991) found an effect similar to that of Shute and co-workers, using not only the extremes of the group, but a simple median split to divide all subjects on the basis of saliva testosterone levels in normal men and women. For one of the two spatial tests (paper folding test) there is a significant sex-by-hormone level interaction, indicating that low levels of testosterone in males and high levels of testosterone in females are associated with superior performance. A composite score for tests on which males

Table 4.6 Serum and saliva androgens and cognitive abilities in normal males and females

Study	Cognitive task	Hormonal-cognitive relation
Komnenich *et al.* 1978	verbal	non-significant (m) (f)
	visual-spatial	curvilinear (m+f)
	visual-spatial	non-significant linear (m)
	visual-spatial	positively linear (f)
Shute *et al.* 1983	visual spatial	curvilinear (m)
	visual-spatial	positively linear (f)
Gordon and Lee 1986	visual-spatial	positively linear (m)
Christiansen and	visual-spatial	positively linear (m)
Knussmann 1987b	field-independence	positively linear (m)
	verbal fluency	negatively linear (m)
McKeever and Deyo 1990	visual-spatial	positively linear (m)
Gouchie and Kimura 1991	visual-spatial (paper folding)	curvilinear (m+f)
	visual-spatial (paper folding)	positively linear (f)
	visual-spatial + mathematical reasoning	curvilinear (m+f)
		non-significant (m) (f)
	verbal	non-significant (m) (f)
	perceptual speed	
Tan and Akgün 1992	non-verbal	positively linear (m)
		non-significant (f)
Christiansen 1993	tactual-spatial	positively linear (m)
	field independence	positively linear (m)
	verbal fluency	negatively linear (m)
Janowsky *et al.* 1998	spatial recall	positively linear (m)
	verbal recall	positively linear (m)
	visual-spatial	non-significant (m) (f)
Silverman *et al.* 1999	mental rotation	positively linear (m)
Davison and Susman 2001	mental rotation	positively linear (m) (f)
	block design	positively linear (m)
	verbal meaning	non-significant (m) (f)

m = males f = females m+f = mixed group of males and females

normally excel (paper folding, mathematical reasoning, mental rotations) shows a similar relationship. On the other hand, multiple regression analyses of the male data alone revealed a trend in men for a positive linear relationship between saliva testosterone levels and visual spatial abilities ($R = 0.29$; $p < 0.06$). Gouchie and Kimura noted no evidence or consistent relationship between testosterone concentrations in men or women and their performance on perceptual speed tasks or vocabulary tests (at which females usually excel over males).

The interpretation of such findings is complicated by the fact that testosterone may exert some of its effects through aromatization to estradiol in the brain. The suggestion has been made that it may in fact be the estrogen level which is related in a curvilinear fashion to spatial ability (Nyborg 1988). However, his hypothesis of a correlation between circulating serum estradiol and cognitive functioning could not be supported by the only two studies which, in addition to testosterone, measured serum estradiol (Christiansen 1993; Shute *et al.* 1983).

In contrast to Shute *et al.* (1983) and Gouchie and Kimura (1991), several studies have shown a significant linear testosterone-cognitive relationship. Gordon and Lee (1986) investigated 32 men with four visual-spatial and four verbal tests and determined testosterone levels of their subjects. Testosterone concentrations correlated significantly positively with one spatial orientation task, but not with any of the other spatial or verbal tests. The study by Christiansen and Knussmann (1987b) attempted a broader investigation of the effects of androgens on spatial and nonspatial cognitive abilities in a larger sample of 117 men in their twenties. They collected blood and saliva samples to determine serum concentrations of testosterone, non SHBG-bound saliva testosterone, and 5α-dihydrotestosterone (DHT). Cognitive functioning was ascertained by 11 spatial and verbal ipsative test scores, reflecting intraindividual variance in the performance of these tasks, independent of the person's general level of achievement. The relationship between androgens and cognitive performance exhibited a clear pattern. All significant correlations between hormone values and verbal tests were negative. In contrast, significant correlations between androgens and spatial and field independence tests were all positive. Correspondingly, a more "masculine" cognitive pattern (superior skills on spatial tests as compared to verbal tests) was positively correlated to all three androgens. It should be noted that total serum testosterone clearly showed the greatest number of significant relations with verbal and spatial test scores. Three years later, McKeever and Deyo (1990) confirmed these findings of a positive correlation between androgen levels and spatial tasks with regard to DHT. Further evidence for a linear androgen-cognitive relationship comes from Tan and Akgün (1992) who noted a positive correlation between nonverbal tasks and testosterone in a sample of Turkish university students. This was only the case for a subsample of right-handed men with right eye preference, but mixed-dominant males and young females showed no relationship. Janowsky *et al.* (1998) who investigated healthy male and female volunteers between 23–34 years of age with a comprehensive cognitive test battery found their male subjects with higher free testosterone levels excelled in verbal and spatial recall tasks, but spatial cognition (block design, card rotation) did not relate to salivary testosterone in males or females (compare Anderson *et al.*'s (1995) study involving boys at the onset of puberty). Nevertheless, recent studies confirm that visual-spatial abilities seem to have a rather stable relationship to endogenous testosterone levels in males, but also in females.

Silverman *et al.* (1999) measured salivary testosterone in men and found a general linear relation between individual differences in testosterone levels and performance on a three-dimensional mental rotation task. Davison and Susman (2001) tested boys and girls ranging in age from 9 to 14 years. Testosterone and estradiol were assessed at three test sessions every 6 months. The results showed positive relations between spatial scores and testosterone in boys at all three sessions and in girls at the third session. In addition, the data supported a link between longitudinal change in testosterone levels and longitudinal change in spatial performance in both girls and boys.

As all the previous data were collected from individuals living in Western cultures, Christiansen (1993) tried to validate the findings in a non-Western group of healthy males. Althogether 256 !Kung San hunter-gatherers ("bushmen") and Kavango farmers from Nambia (southern Africa) were investigated. They lived mainly on the subsistence level in their traditional lifestyle with a low degree of transition to Western culture. Testosterone, DHT, estradiol, and "free" salivary testosterone were determined. In order to make a sensible comparison of previous findings of hormone-related cognitive performance, spatial and verbal tests were the same or similar to those used in the study by Christiansen and Knussmann (1987b), but were adapted for testing of illiterate subjects with no experience in paper and pencil tasks. The African data yielded the same hormonal cognitive pattern as was found in Western samples. Total and salivary testosterone showed the greatest number of significant relationships, a positive one with visual- or tactual-spatial tasks and a negative association with verbal tests.

The summary of sex hormone effects on cognitive abilities makes it reasonable to conclude that testosterone plays a role in cognitive functioning throughout life – from the prenatal period through adulthood till old age. But it has to be noted that explanations of inter- and intraindividual differences in cognitive abilities are complex and any causal model will have to recognize the reciprocal effects that environment and biology have on each other.

4.8 Key messages

- The interaction of testosterone with behaviour is bidirectional: testosterone can influence behaviour, and behaviour can alter testosterone levels.
- Testosterone affects brain development by organizing certain brain regions during fetal and neonatal life. At puberty, these brain structures and hence the behavioural repertoire are thought to be activated with increasing sex hormone concentrations. However, in humans behaviour is predominantly determined by intrapsychic, social and cultural factors; hormonal influences are less powerful than in animals.
- Male sexual behaviour is related to androgens. It becomes overt and powerful in puberty when the testes begin to secrete androgens.

- In hypogonadal males testosterone replacement provides convincing evidence of its role in some aspects of sexual behaviour. In eugonadal males testosterone substitution has only very limited influence.
- Endogenous testosterone levels in eugonadal men may be positively correlated to sexual interest and frequency of orgasms, but the findings are contradictory.
- In women, correlational and experimental studies show that testosterone is associated with an enhancement of sexual behaviour.
- Due to the endocrine effects of sexual stimulation and activities, an increase in testosterone was observed in both sexes.
- Stress responses of the pituitary-gonadal axis show a remarkable sensibility. In males, testosterone levels decrease under psychosomatic and psychic stress, even under anticipation of stressful events, while testosterone concentrations in females rise.
- Compared to untrained men, well-trained athletes have lowered testosterone levels in a resting state.
- During and after prolonged submaximal exercise decreased testosterone levels in males and increased levels in females were measured. Acute effects of short exercise are a rise in testosterone concentrations, followed by a decline below baseline levels.
- Human aggression and endocrine activity are mutually dependant. Prenatal exposure to exogenous androgenic steroids results in slight increases of aggressive behaviour in boys and girls.
- Aggressive behaviour and self-report measures of aggression are predominantly positively correlated to endogenous testosterone levels in men and women although the aggressive act is generally removed in time from the hormone assessment.
- In both sexes, testosterone replacement therapy or anabolic steroid abuse can result in increased aggressiveness, but this effect is not universal.
- Assertive or aggressive behaviour followed by a rise in status – even more so when associated with the person's elevated mood or elation – leads to a rise in testosterone levels.
- The relationship between mood and androgens is less clear. Neither clinical research on depressive men nor studies on mood and endogenous testosterone concentrations in normal males and females provided consistent results. However, early reports on castrates and studies involving mostly hypogonadal men and menopausal women described an improvement in emotional stability following treatment with testosterone.
- Androgens play a critical role in sex-typical cognitive functioning throughout life in normal men and women. Several studies have shown both linear and curvilinear effects of testosterone on visual-spatial abilities. Direct manipulation of testosterone supports the conclusion of its important role in cognition in females and males, predominantly positive effects on visual-spatial tasks, perceptual speed and memory.

4.9 REFERENCES

Adams DB, Gold AR, Burt AD (1978) Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives. N Engl J Med 299:1145–1150

Adlercreutz H, Härkönen M, Kuoppasalmi K, Kosunen K, Näveri H, Rehunen S (1976) Physical activity and hormones. Adv Cardiol 18:144–157

Alexander GM, Sherwin BB (1993) Sex steroids, sexual behavior, and selection attention for erotic stimuli in women using oral contraceptives. Psychoneuroendocrinology 18:91–102

Alexander GM, Sherwin BB, Bancroft J, Davidson DW (1990) Testosterone and sexual behavior in oral contraceptive users and nonusers: A prospective study. Horm Behav 24:388–402

Alexander GM, Swerdloff RS, Wang C, Davidson T, McDonald V, Steiner B, Hines M (1998) Androgen-behavior correlations in hypogonadal men and eugonadal men. Horm Behav 33: 85–94

Anderson RA, Bancroft J, Wu FC (1992) The effect of exogenous testosterone on sexuality and mood of normal men. J Clin Endocrinol Metab 75:1505–1507

Anderson A, Harrison GA, Brush G, Jewell TMK (1995) Testosterone and physical and mental development in adolescent boys. J Hum Ecol 4:141–146

Angst J (1983) The origins of depression: Current concepts and approaches. Springer, Berlin

Anonymous (1970) Effects of sexual activity on beard growth in man. Nature 226:869–870

Arce JC, De Souza MJ (1993) Exercise and male factor infertility. Sports Med 15:146–169

Arce JC, De Souza MJ, Pescatello LS, Luciano AA (1993) Subclinical alterations in hormone and semen profile in athletes. Fertil Steril 59:398–404

Archer J (1988) The behavioral biology of aggression. Cambridge University Press, Cambridge

Archer J (1991) The influence of testosterone on human aggression. Brit J Psychol 82:1–28

Archer J (2000) Sex differences in aggression between heterosexual partners: A meta-analytic review. Psychol Bull 126:651–680

Aromäki AS, Lindman RE, Ericksson, CJP (1999) Testosterone, aggressiveness, and antisocial personality. Aggress Behav 25:113–123

Azad, N, Pitale S, Barnes WE, Friedman N (2003) Testosterone treatment enhances regional brain perfusion in hypogonadal men. J Clin Endorinol Metab 88:3064–3068

Bäckström T (1992) Neuroendocrinology of premenstrual syndrome. Clin Obstet Gynecol 35:612–628

Bäckström T, Sanders D, Leask R, Davidson D, Warner P, Bancroft J (1983) Mood, sexuality, hormones, and the menstrual cycle. II. Hormone levels and their relationship to the premenstrual syndrome. Psychosom Med 45:503–507

Baenninger M, Newcombe N (1989) The role of experience in spatial test Performance: A meta-analysis. Sex Roles 20:327–344

Bagatell CJ, Heiman JR, Matsumoto AM, Rivier JE, Bremner WJ (1994) Metabolic and behavioral effects of high-dose, exogenous testosterone in healthy men. J Clin Endocrinol Metab 79:561–567

Bahrke MS, Wright JE, O'Connor JS, Strauss RH, Catlin DH (1990) Selected psychological characteristics of anabolic-androgenic steroid users. New Engl J Med 12:834–835

Bahrke MS, Wright JE, Strauss RH, Catlin DH (1992) Psychological moods and subjectively perceived behavioral and somatic changes accompanying anabolic-androgenic steroid use. Am J Sports Med 20:717–724

Bains GK, Slade P (1988) Attributional patterns, moods, and the menstrual cycle. Psychosom Med 50:469–476

Baker ER, Mathur RS, Kirk RF (1982) Plasma gonadotropins, prolactin and steroid concentration in female runners immediately after a long distance run. Fertil Steril 38:38–41

Baker SW, Ehrhardt AA (1974) Prenatal androgen, intelligence, and cognitive sex differences. In: Friedman RC, Richart RM, Vande Wiele RL (eds) Sex differences in the brain. Wiley, New York, pp 53–76

Bancroft J (1984) Hormones and human sexual behavior. J Sex Mar Ther 10:3–21

Bancroft J (1986) Low sexual desire. In: Dennerstein L, Fraser I (eds) Hormones and behavior. Elsevier, Amsterdam, pp 396–405

Bancroft J (1993) The premenstrual syndrome – A reappraisal of the concept and the evidence. Psychol Med 24 (Suppl):1–47

Bancroft J, Sanders D, Davidson D, Warner P (1983) Mood, sexuality, and the menstrual cycle. II. Sexuality and the role of androgens. Psychosom Med 45: 509–517

Banks T, Dabbs JM Jr (1996) Salivary testosterone and cortisol in a delinquent and violent urban subculture. J Soc Psychol 136:49–56

Barfield RJ (1984) Reproductive hormones and aggressive behavior. In: Flannelly KJ, Blanchard RJ, Blanchard DC (eds) Biological perspectives on aggression. Progress in clinical and biological research, Vol 169. Liss, New York, pp 105–134

Barrett-Connor E, Von Muhlen DG, Kritz-Silverstein D (1999) Bioavailable testosterone and depressed mood in older men: The Rancho Bernado Study. J Clin Endocrinol Metab 81:3578–3583

Bateup H, Booth A, Shirtcliff, EA, Granger DA (2002) Testosterone, cortisol, and women's competition. Evol Hum Behav 23:181–192

Beach FA (1948) Hormones and behavior (2nd ed 1949) Hoeber, New York

Beatty WW (1979) Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. Horm Behav 12:112–163

Becker JB, Breedlove, JM, Crews D (1992) Behavioral endocrinology. MIT Press, Cambridge

Benkert O, Witt W, Adam W, Leitz A (1979) Effects of testosterone undecanoate on sexual potency and the hypothalamic-pituitary-gonadal axis of impotent males. Arch Sex Behav 8:471–480

Berenbaum SA, Hines M (1992) Early androgens are related to childhood sex-typed toy preferences. Psychol Sci 3:203–206

Berenbaum SA, Korman K, Leveroni C (1995) Early hormones and sex differences in cognitive abilities. Learn Individ Diff 7:303–321

Bernhardt P, Dabbs J, Fielden J, Lutter C (1989) Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. Physiol Behav 65:59–62

Bernstein I, Gordon TP, Rose RM (1983) The interaction of hormones, behavior and social context in nonhuman primates. In: Svare BB (ed) Hormones and aggressive behavior. Wiley, New York pp 535–561

Bernton E, Hoover D, Galloway R, Popp K (1995) Adaptation to chronic stress in military trainees. Ann NY Acad Sci 774:217–231

Bettini E, Pollio G, Santagati S, Maggi A (1992) Estrogen receptor in rat brain: Presence in the hippocampal formation. Neuroendocrinology 56:502–508

Bixo M, Bäckström T, Winblad B, Andersson A (1995) Estradiol and testosterone in specific regions of the human female brain in different endocrine states. J Steroid Biochem Mol Biol 55:297–303

Björkqvist K, Nygren T, Björklund A-C, Björkqvist S-E (1994) Testosterone intake and aggressiveness: Real effect or anticipation? Aggress Behav 20:7–26

Blatter P (1982) Sex differences in spatial ability: the X-linked gene theory. Percept Mot Skills 55:455–462

Booth A, Shelly G, Mazur A, Tharp G, Kittok R (1989) Testosterone, and winning and losing in human competition. Hormon Behav 23:556–571

Brain PF, Haug M (1992) Hormonal and neurochemical correlates of various forms of animal aggression. Psychoneuroendocrinology 17:537–551

Brincat M, Studd JWW, O'Dowd T, Magos A, Cardozo, LD, Wardle, PJ, Cooper D (1984) Subcutaneous hormone implants for the control of climacteric symptoms: A prospective study. Lancet 1:16–18

Brooks JH, Reddon JR (1996) Serum testosterone in violent and nonviolent young Offenders. J Clin Psychol 52:475–473

Brower KJ, Blow FCX, Beresford TP, Fuelling VC (1989) Anabolic-androgenic steroid dependence. J Clin Psychol 50:31–33

Brower KJ, Blow FCX, Young YP, Hill EM (1991) Symptoms and correlates of anabolic-androgenic steroid dependence. Brit J Addict 86:759–768

Brown WA, Monti PM, Corriveau DP (1978) Serum testosterone and sexual activity and interest in men. Arch Sex Behav 7:97–103

Buchsbaum MS, Henkin RI (1980) Perceptual abnormalities in patients with chromatin negative gonadal dysgenesis and hypogonatropic hypogonadism. Int J Neurosci 11:201–209

Buena F, Swerdloff RS, Steiner BS, Lutchmansingh P, Peterson MA, Pandian MR, Galmarini M, Bhasin S (1993) Sexual function does not change when serum testosterone levels are pharmologically varied within the normal male range. Fertil Steril 59:1118–1123

Cadoux-Hudson TA, Few JD, Imms FJ (1985) The effects of exercise on the production and clearance of testosterone in well trained young men. Eur J Appl Physiol 54:321–325

Campbell A, Muncer S, Odber J (1997) Aggression and testosterone: Testing a bio- social model. Aggress Behav 23:229–238

Carani C, Zini D, Baldini A, Della Casa L, Ghizzani A, Marrama P (1990a). Effects on androgen treatment in impotent men with normal and low levels of free testosterone. Arch Sex Behav 19:223–234

Carani C, Bancroft J, Del Rio G, Granata ARM, Facchinetti F, Marrama P (1990b) The endocrine effects of visual erotic stimuli in normal men. Psychoneuroendocrinology 15:207–216

Carani C, Bancroft J, Granata A, Del Rio G, Marrama P (1992) Testosterone and erectile function, nocturnal penile tumescence and rigidity, and erectile response to visual erotic stimuli in hypogonadal and eugonadal men. Psychoneuroendocrinology 17:647–654

Carstensen, H, Amer I, Wide L, Amer B (1973) Plasma testosterone, LH and FSH during the first 24 hours after surgical operations. J Steroid Biochem 4:605–611

Cashdan E (1995) Hormones, sex and status in women. Horm Behav 29:354–366

Cashdan E (2003) Hormones and competitive aggression in women. Aggress Behav 29:107–115

Chalepakis G, Schauer M, Slater EP, Beato M (1990) Gene regulation by steroid hormones. In: Alexis MN, Sekeris CE (eds) Activation of hormone and growth factor receptors. Kluver Academic Publishers, Dordrecht/Boston/London, pp 151–172

Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, Raskind MA, Brodkin K, Bremner W, Petrova A, Latendresse S, Craft S (2001) Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 57:80–88

Christiansen K (1993) Sex-hormone related variations of cognitive performance in !Kung San hunter-gatherers of Namibia. Neuropsychobiology 27:97–107

Christiansen K (1999) Hypophysen-Gonaden-Achse Mann. In: Kirschbaum C, Hellhammer D (eds) Psychoneuroendokrinologie und Psychoimmunologie. Enzyklopädie der Psychologie, Biologische Psychologie. Hogrefe, Göttingen, pp 141–222

Christiansen K, Hars O (1995) Effects of stress anticipation and stress coping strategies on salivary testosterone levels. J Psychophysiol 3:264

Christiansen K, Knussmann R (1987a) Androgen levels and components of aggression in men. Horm Behav 21:170–180

Christiansen K, Knussmann R (1987b) Sex hormones and cognitive functioning in men. Neuropsychobiology 18:27–36

Christiansen K, Knussmann R, Couwenbergs C (1984) Zusammenhänge zwischen Sexualhormonen des Mannes und Ernährung, Streß und Sexualverhalten. Homo 35:251–272

Christiansen K, Knussmann R, Couwenbergs C (1985) Sex hormones and stress in the human male. Horm and Behav 19:426–440

Christiansen K, Winkler EM (1992) Hormonal, anthropometrical, and behavioral correlates of physical aggression in !Kung San men of Namibia. Aggr Behav 18:271–280

Collaer ML, Hines M (1995) Human behavioral sex differences: A role for gonadal hormones during early development? Psychol Bull 118:55–107

Cook NJ, Read GF, Walker RF, Harris B, Riad-Fahmy D (1986) Changes in adrenal and testicular activity monitored by salivary sampling in males throughout marathon runs. Eur J Appl Physiol 55:634–638

Cortés-Gallegos V, Castaneda G, Alonso R, Sojo I, Carranco A, Cervantes C, Parra A (1983) Sleep deprivation reduces circulating androgens in healthy men. Arch Androl 10:33–37

Cullen J, Fuller R, Dolphin C (1979) Endocrine stress responses of drivers in a "real-life" heavy-goods vehicle driving task. Psychoneuroendocrinology 4:107–115

Cumming DC, Brunsting LA, Strich G, Ries AL, Rebar RW (1986). Reproductive hormone increases in response to acute exercise in men. Med Sci Sports Exerc 18369–373

Dabbs JM Jr, Hargrove MF (1997) Age, testosterone, and behavior among female prison inmates. Psychosom Med 59:477–480

Dabbs JM Jr, Mohammed S (1992) Male and female salivary testosterone concentrations before and after sexual activity. Physiol Behav 52:195–197

Dabbs JM Jr, Frady RL, Carr TS, Besch NF (1987) Saliva testosterone and criminal violence in young adult prison inmates. Psychosom Med 49:174–182

Dabbs JM Jr, Ruback RB, Frady RL, Hopper CH, Sgoutas DS (1988) Saliva testosterone and criminal violence among women. Pers Indiv Differ 9:269–275

Dabbs JM Jr, Jurkovic GJ, Frady RL (1991) Salivary testosterone and cortisol among late adolescent male offenders. J Abnorm Child Psychol 19:469–478

Dabbs JM, Carr Ts, Frady RL, Riad JK (1995) Testosterone, crime and misbehavior among 692 prison inmates. Pers Indiv Differ 18:627–633

Daitzman R, Zuckerman M (1980) Desinhibitory sensation seeking, personality, and gonadal hormones. Pers Indiv Diff 1:103–110

Davidson JM, Camargo CA, Smith ER (1979) Effects of androgens on sexual behavior in hypogonadal men. J Clin Endocrinol Metab 48:955–958

Davidson JM, Kwan M, Greenleaf WJ (1982) Hormonal replacement and sexuality in men. Clin Endocrinol Metab 11:599–623

Davidson JM, Chen JJ, Crapo L, Gray GD, Greenleaf WJ, Catania JA (1983) Hormonal changes and sexual function in aging men. J Clin Endocrinol Metab 57:71–77

Davis SR, Tan J (2001) Testosterone influences libido and well being in women. Trends Endocrinol Metab 12:33–37

Davison KK, Susman EJ (2001) Are hormone levels and cognitive ability related during early adolescence? Int J Behav Develop 25:416–428

Dawson JML (1972) Effects of sex hormones on cognitive style in rats and men. Behav Genet 2:21–42

De Crée C, Lewin R, Ostyn M (1990) The monitoring of the menstrual status of female athletes by salivary steroid determination and ultrasonography. Eur J Appl Physiol 60:472–477

Dellantonio A, Lis A, Saviolo N, Rigon F, Tenconi R (1984) Spatial performance and hemispheric specialization in the Turner syndrome. Acta Med Auxol 16: 193–203

Dennerstein L, Gotts G, Brown JB, Morse, CA, Farley TMM, Pinol A (1994) The relationship between the menstrual cycle and female sexual interest in women with PMS complaints and volunteers. Psychoneuroendocrinology 19:293–304

Dessypris A, Kuoppasalmi K, Adlercreutz H (1976) Plasma cortisol, testosterone, androstenedione and luteinizing hormone (LH) in a non-competitive Marathon run. J Steroid Biochem 7:33–37

Diamond P, Brisson GR, Caudas B, Péronnet F (1989) Trait anxiety, submaximal exercise and blood androgens. Eur J Applied Physiol Occup Physiol 58:699–704

Doering CH, Brodie HKH, Kraemer H, Becker H, Hamburg DA (1974) Plasma testosterone levels and psychologic measures in men over a 2-month period. In: Friedman RC, Richart RM, Vande Wiele RL, Stern LO (eds) Sex differences in behavior. Wiley, New York, pp 413–432

Doering CH, Brodie KH, Kraemer HC, Moos RH, Becker HB, Hamburg DA (1975) Negative affect and plasma testosterone: A longitudial human study. Psychosom Med 37:484–491

Dongyun S, Yumin W (1990) Flight influence on plasma levels of sex hormones of women pilots. Milit Med 155:262–264

Düker H (1957) Leistungsfähigkeit und Keimdrüsenhormone. Barth, München

Dunn E, Macdougall M, Coote M, Steiner M (2001) Biochemical correlates of Symptoms associated with premenstrual disphoric disorder Arch Womens Ment Health 3 (Suppl. 2):1

Eagly AH, Steffen VJ (1986) Gender and aggressive behavior: A meta-analytic review of the social psychological literature. Psychol Bull 100:309–330

Ehlers CL, Rickler KC, Hovey JE (1980) A possible relationship between plasma testosterone and aggressive behavior in a female outpatient population. In: Girgis M, Kiloh LG (eds) Limbic epilepsy and dyscontrol syndrome. Elsevier/North Holland Biomedical Press, New York, pp 183–194

Ehrenkranz J, Bliss E, Sheard MH (1974) Plasma testosterone: Correlation with aggressive behavior and social dominance in man. J Psychosom Med 36:469–475

Ehrhardt AA, Baker SW (1974) Fetal androgens, human central nervous system differentiation and behavior sex differences. In: Friedman RC, Richart RM, Vande Wiele RL (eds) Sex differences in behavior. Wiley, New York, pp 33–51

Ehrhardt AA, Meyer-Bahlburg HFL (1981) Effects of prenatal sex hormones on gender related behavior. Science 211:1312–1318

Ehrhardt AA, Meyer-Bahlburg HFL, Feldman JF, Ince SE (1984) Sex-dimorphic behavior in childhood subsequent to prenatal exposure to exogenous progestogens and estrogens. Arch Sex Behav, 13:457–478

Ehrhardt AA, Meyer-Bahlburg HFL, Rosen LR, Feldman JF, Veridiano NP, Elkin EJ, McEwen BS (1989) The development of gender-related behavior in females following prenatal exposure to diethylstilbestrol (DES). Horm Behav 23:526–541

Elias M (1981) Serum cortisol, testosterone, and testosterone-binding globulin responses to competitive fighting in human males. Aggress Behav 7:215–224

Elias AN, Wilson AF, Pandian MR, Chune G, James N, Stone SC (1991) CRH and gonadotropin secretion in physically active males after acute exercise. Eur J Appl Physiol 62:171–174

Elias AN, Wilson AF (1993) Exercise and gonadal function. Hum Reprod 8:1747–1761

Elias AN, Wilson AF, Pandian MR, Rojas FP, Kayalek R, Stone SC, James N (1993) Melatonin and gonadotropin after acute exercise in physically active males. Eur J Appl Physiol 66:357–361

Ellis L (1982) Developmental androgen fluctuations and the five dimensions of mammalian sex (with emphasis upon the behavioral dimension and the human species). Ethology Sociobiol 3:171–197

Eriksson E, Sundblad C, Lisjö P, Modigh K, Andersch B (1992) Serum levels of androgens are higher in women with premenstrual irritability and dysphoria than in controls. Psychoneuroendocrinology 17:195–204

Evans IM, Distiller LA (1979) Effects of luteinizing hormone-releasing hormone on sexual arousal in normal men. Arch Sex Behav 8:385–396

Ferin MJ (1996) The menstrual cycle: An integrative view. In: Adashi EY, Rock JA, Rosenwaks Z (eds) Reproductive endocrinology, surgery, and technology. Lippincott-Raven, Philadelphia, pp 103–121

Finegan JK, Niccols GA, Sitarenios G (1992) Relations between prenatal testosterone levels and cognitive abilities at age 4 years. Develop Psychol 28:1075–1089

Finkelstein JW, Susman EJ, Chinchilli VM, Kunselman SJ, D'Arcangelo MR, Schwab J, Demers LM, Liben LS, Lookingbill G, Kulin HE (1997) Estrogen and testosterone increases self-reported aggressive behaviors in hypogonadal adolescents. J Clin Endocrinol Metab 82:2433–2438

Ford DH, Cramer EB (1982) The anatomical distribution of hormones in the central nervous system. In: Vernadakis A, Timiras PS (eds.) Hormones in development and aging. MTP Press, Lancaster, pp 151–180

Fox CA, Ismail AAA, Love DN, Kirkham KE, Loraine JA (1972) Studies on the relationship between plasma testosterone levels and human sexual activity. J Endocrinol 52:51–58

Francis KT (1981) The relationship between high and low trait psychological stress, serum testosterone, and serum cortisol. Experienta 37:1296–1297

Freedman R, Carter DB (1982) Neuroendocrine strategies in psychiatric research. In: Vernadakis A, Timiras PS (eds) Hormones in development and aging. MTP Press, Lancaster pp 619–636

Frodi A, Macaulay J, Thome PR (1977) Are women always less aggressive than men? A review of the experimental literature. Psychol Bull 84:634–660

Fry DP (1998) Anthropological perspectives on aggression. Sex differences and cultural variation. Aggress Behav 24:81–95

Garron DC, van der Stoep LP (1969) Personality and intelligence in Turner syndrome. A critical review. Arch Gen Psychiatry 21:339–346

Genazzani AR, Gastaldi M, Bidzinska B, Mercuri N, Genazzani AD, Nappi RE, Segre A, Petraglia F (1992) The brain as a target organ of gonadal steroids. Psychoneuroendocrinology 17:385–390

Gladue BA (1991a) Qualitative and quantitative sex differences in self-reported aggressive behavioral characteristics. Psychol Reports 68:675–684

Gladue BA (1991b) Aggressive behavioral characteristics, hormones, and sexual orientation in men and women. Aggress Behav 17:313–326

Gladue BA, Boechler M, McCaul KD (1989) Hormonal response to competition in human males. Aggress Behav 15:409–422

Gonzales Bono E, Salvador A, Ricarte MA, Serrano, Arnedo M (2000) Testosterone and attribution of successful competition. Aggress Behav 26:235–240

Gooren LJG (1987) Androgen levels and sex functions in testosterone-treated hypogonadal men. Arch Sex Behav 16:463–473

Gordon HW, Lee PA (1986) A relationship between gonadotropins and visuospatial function. Neuropsychologia 24:563–576

Gordon TP, Rose RM, Grady CL, Bernstein I (1979) Effects of increased testosterone secretion on the behavior of adult male rhesus monkeys living in a social group. Folia Primatol 32:149–160

Gouchie CT, Kimura D (1991) The relation between testosterone levels and cognitive ability patterns. Psychoneuroendocrinology 16:323–334

Goy RW, Bercovitch FB, McBrair MC (1988) Behavioral masculinization is independent masculinization in prenatally androgenized female rhesus macaques. Horm Behav 22:552–571

Gray A, Jackson DN, McKinlay JB (1991) The relation between dominance, anger, and hormones in normally aging men – Results from the Massachusetts male aging study. Psychosom Med 53:375–385.

Grinspoon S, Corcoran C, Stanley T, Baaj A, Basgoz N, Klibanski A (2000) Effects of hypogonadism and testosterone administration on depression indices in HIV – infected men. J Clin Endocrinol Metab 85:60–65

Guezennec CY, Satabin P, Legrand H, Bigard AX (1994) Physical performance and metabolic changes induced by combined prolonged exercise and different energy intakes in humans. Eur J Appl Physiol 68:525–530

Gur RC, Alsop D, Glahn D, Petty R, Swanson CL, Maldjian JA, Turetsky BI, Detre JA, Gee J, Gur RE (2000) An fMRI study of sex differences in regional activation to a verbal and a spatial task. Brain Lang 74:157–170

Hackney AC (1989) Endurance training and testosterone. Sports Med 8:117–127

Hackney AC (2000) Endurance exercise training and reproductive endocrine dysfunction in men: Alterations in the hypothalamic-pituitary-testicular axis. Curr Pharmaceut Design 7:261–273

Hackney AC, Dolny DG, Ness RJ (1988) Comparison of resting reproductive hormonal profiles in select athletic groups. Biol Sport 4:200–204

Häkkinen K, Pakarinen A (1993) Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. J Appl Physiol 74:882–887

Hahn WK (1987) Cerebral lateralization of function: From infancy through childhood. Psychol Bull 101:376–392

Hale RW, Kosasa T, Krieger J (1983) A marathon: the immediate effect on female runners' luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and cortisol levels. Am J Obstet Gynecol 146:550–554

Halpern DF (2000) Sex differences in cognitive abilities (3rd ed) Lawrence Erlbaum, Hillsdale (NJ)

Hampson E, Rovet JF, Altman D (1998) Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Develop Neuropsychol 14:299–320

Hannan CJ Jr, Friedl KE, Zold A, Kettler TM., Plymate SR (1991) Psychological and serum homovanillic acid changes in man administered androgenic steroids. Psychoendocrinology 16:335–343.

Harris JA, Rushton JP, Hampson E, Jackson D (1996) Salivary testosterone and self-report aggressive and prosocial personality characteristics in men and women. Aggress Behav 22:321–331

Hartmann B, Baischer W, Koinig G, Albrecht A, Kirchengast S, Huber J, Langer G (1996) Disturbances in the hypothalamic-pituitary-gonadal axis of depressed fertile women compared to normal controls. In: Genazzani AR, Petraglia F, D'Ambrogio G, Genazzani AD, Artini PG (eds) Recent developments in gynecology and obstetrics. Parthenon, New York, pp 225–230

Harvey SM (1987) Female sexual behavior: Fluctuations during the menstrual cycle. J Psychosom Res 32:101–110

Hellhammer DH, Hubert W, Schürmeyer T (1985) Changes in saliva testosterone after psychological stimulation in men. Psychoneuroendocrinology 10:77–81

Hier DB, Crowley WF (1982) Spatial ability in androgen-deficient men. New Engl J Med 306: 1202–1205

Hines M (1982) Prenatal gonadal hormones and sex differences in human behavior. Psychol Bull 92:56–80

Hines M, Golombok S, Rust J, Johnston KJ, Golding J (2002) Testosterone during pregnancy and gender role behavior of preschool children: A longitudinal population study. Child Develop 73:1678–1687

Houser BB (1979) An investigation of the correlation between hormonal levels in males and mood, behavior and physical discomfort. Horm Behav 12:185–197

Hubert W (1990) Psychotropic effects of testosterone. In: Nieschlag E, Behre HM (eds) Testosterone: Action, deficiency, substitution. Springer, Berlin pp 51–71

Hutchinson JB (1991) Hormonal control of behaviour: Steriod action in the brain. Curr Opin Neurobiol 1:562–570

Hutchinson JB (1993) Sex hormone action on brain mechanisms of aggression. Aggress Behav 19:5

Hutchinson JB, Steimer T (1984) Androgen metabolism in the brain: Behavioural correlates. In: de Vries GJ, de Bruin J, Corner M (eds) Sex differences in the brain. Progress in brain research, Vol 61. Elsevier, Amsterdam, pp 23–51

Hyde JS (1984) How large are gender differences in aggression? A developmental meta-analysis. Develop Psychol 20:722–734

Hyde JS, Linn MC (1988) Gender differences in verbal ability: A meta-analysis. Psychol Bull 104:53–69

Hyde JS, Fennema E, Lamon SJ (1990) Gender differences in mathematics performance: A meta-analysis. Psychol Bull 107:139–155

Imperato-McGinley J, Pichardo M, Gautier T, Voyer D, Bryden MP (1991) Cognitive abilities in androgen-insensitive subjects: Comparison with control males and females from the same kindred. Clin Endocrinol 34:341–347

Inoff-Germain G, Arnold SA, Nottelman ED, Susman EJ, Cutler GB, Chrousos GP (1988) Relations between hormone levels and observational measures of aggressive behavior of young adolescents in family interactions. Develop Psychol 24:129–139

Jacklin CN, Maccoby EE, Doering CH (1983) Neonatal sex-steroid hormones and timidity in 6–18 month-old boys and girls. Dev Psychobiol 16:163–168

Jacklin CN, Wilcox KT, Maccoby EE (1988) Neonatal sex-steroid hormones and cognitive abilities at six years. Dev Psychobiol 21:567–574

Janowsky JS, Oviatt SK, Orwoll ES (1994) Testosterone influences spatial cognition in older men. Behav Neurosci 108:325–332

Janowsky JS, Chavez B, Zamboni BD, Orwoll E (1998) The cognitive neuropsychology of sex hormones in men and women. Develop Neuropsychol 14:421–440

Janowsky JS, Chavez B, Orwoll E (2000) J Cognitive Neurosci 12:407–414

Kashkin KB, Kleber HD (1989) Hooked on hormones? An anabolic steroid addiction hypothesis. J Am Med Assn 262:3166–3170

Kedenburg HD (1977) Androgens and aggressive behavior in man. University Microfilms International, Ann Arbor (Michigan)

Keverne EB (1979) Sexual and aggressive behaviour in social groups of talapoin monkeys. In: Ciba Foundation Symposium 62, Sex hormones and behaviour. Excerpta Medica, Amsterdam, pp 271–297

Kimura D (1996) Sex, sexual orientation and hormones influence human cognitive function. Curr Opin Neurobiol 6:259–263

Klaiber EL, Broverman DM, Vogel W, Abraham GE, Cone FL (1971) Effects of Infused testosterone on mental performances and serum LH. J Clin Endocrinol Metab 32:341–349

Knight GP, Guthrie IK, Page MC, Fabes RA (2002) Emotional arousal and gender differences in aggression: A meta-analysis. Aggress Behav 28:366–393

Knussmann R, Christiansen K, Couwenbergs C (1986) Relations between sex hormone levels and sexual behavior in men. Arch Sex Behav 15:429–445

Komnenich P, Lane DM, Dickey RP, Stone SC (1978) Gonadal hormones and cognitive performance. Physiol Psychol 6:115–120

Kraemer HC, Becker HB, Brodie HKH, Doering CH, Moos RH, Hamburg DA (1976) Orgasmic frequency and plasma testosterone levels in normal human males. Arch Sex Behav 5:125–132

Kreuz LE, Rose RM (1972) Assessment of aggressive behavior and plasma testosterone in a young criminal population. J Psychosom Med 34:321–332

Kreuz LE, Rose RM, Jennings JR (1972) Suppression of plasma testosterone levels and psychological stress. Arch Gen Psychiatry 26:479–482

Kuoppasalmi K, Näveri H, Rehunen S, Härkönen M (1978) Effect of strenous anaerobic running exercise on plasma growth hormone, cortisol, LH, testosterone, androstenedione, estrone and estradiol. J Steroid Biochem 7:2029–2034

Lauritzen C (1987) Intersexualität. In: Wulf K-H, Schmidt-Matthiesen (eds) Gynäkologische Endokrinologie. Urban & Schwarzenberg, München, pp 35–94

Lee PA, Jaffe RB, Midgley AR Jr (1974) Lack of alteration of serum gonadotropins in men and women following sexual intercourse. Am J Obstet Gynecol 120:985–987

Leedy MG, Wilson MS (1985) Testosterone and cortisol levels in crewmen of U.S. Air Force fighter and cargo planes. Psychosom Med 47:333–338

Leiblum S, Bachmann G, Kemmann E, Colburn D, Schwartzman I (1983) Vaginal atrophy in the postmenopausal woman: The importance of sexual activity and hormones. J Am Med Assn 249:2195–2198

Levitt AJ, Joffe RT (1988) Total and free testosterone in depressed men. Acta Psychiatr Scand 77:346–348

Lewis JW (1990) Premenstrual syndrome as a criminal defense. Arch Sex Behav 19:425–441

Lewis VG, Money J, Epstein R (1968) Concordance of verbal and nonverbal ability in the adrenogenital syndrome. John Hopkins Med J 122:192–195

Liben LS, Susman EJ, Finkelstein JW, Chinchilli VM, Kunselman S, Schwab J, Dubas JS, Demers LM, Lookingbill G, D'Arcangelo MR, Krogh HR, Kulin HE (2002) The effects of sex steroids on spatial performance: A Review and an experimental clinical investigation. Develop Psychol 38:236–253

Lincoln GA (1974) Luteinizing hormone and testosterone in man. Nature 252:232–233

Lindman R, Järvinen P, Vidjeskog J (1987) Verbal interactions of aggressively and nonaggressively predisposed males in a drinking situation. Aggress Behav 13:187–196

Lindman R, von der Pahlen B, Öst B, Eriksson CJP (1992) Serum testosterone, cortisol, glucose, and ethanol in males arrested for spouse abuse. Aggress Behav 18:393–400

Linn MC, Petersen AC (1985) Emergence and characterization of sex differences in spatial ability: A meta-analysis. Child Develop 56:1479–1498

Luisi M, Franchi F (1980) Double-blind group comparative study of testosterone undecanoate and mesterolone in hypogonadal male patients. J Endocrinol Invest 3:305–308

Maccoby EE, Jacklin CN (1974) The psychology of sex differences. Stanford University Press, Stanford (CA)

MacConnie SE, Barkan A, Lampman RM, Schork MA, Beitins IZ (1986) Decreased hypothalamic gonadotropin-releasing hormone in male marathon runners. New Engl J Med 315:411–417

Maggi A, Perez J (1985) Role of female gonadal hormones in the CNS: Clinical and experimental aspects. Life Sci 37:893–906

Mantzoros CS, Georgiadis EI, Trichopoulos D (1995) Contribution of dihydrotestosterone to male sexual behaviour. Brit Med J 310:1289–1291

Marinelli M, Roi GS, Giacometti M, Bonini P, Banti G (1994) Cortisol, testosterone, and free testosterone in athletes performing a marathon in 4000m altitude. Horm Res 41:225–229

Masica DN, Money J, Ehrhardt AA, Lewis VG (1969) IQ, fetal sex hormones and cognitive patterns: Studies in the testicular feminizing syndrome of androgen insensitivity. John Hopkins Med J 124:33–43

Masters MS, Sanders B (1993) Is the gender difference in mental rotation disappearing? Behav Genet 23:337–341

Matsumoto K, Takeyasu K, Mizutani S, Hamanaka Y, Uozomi T (1970) Plasma testosterone levels following surgical stress in male patients. Acta Endocrinol 65:11–17.

Matteo S, Rissman EF (1984) Increased sexual activity during the midcycle portion of the human menstrual cycle. Horm Behav 18:249–255

Mattsson A, Schalling D, Olweus D, Löw H, Svensson J (1980) Plasma testosterone, aggressive behavior, and personality dimensions in young male delinquents. J Am Acad Child Psychiatry 19:476–490.

Matussek N (1980) Zur Biochemie und Neuroendokrinologie der Depression. In: Heimann H, Giedke H (eds) Neue Perspektiven in der Depressionsforschung. Huber, Bern, pp 64–72

Mazur A (1985) A biosocial model of status in face-to-face primate groups. Soc Forces 64:377–402

Mazur A, Lamb TA (1980) Testosterone, status, and mood in human males. Horm Behav 14:236–246

Mazur A, Booth A, Dabbs JM (1992) Testosterone and chess competition. Social Psychol 55:70–77

Mazur A, Susman EJ, Edelbrock S (1997) Sex difference in testosterone response to a video game contest. Evol Hum Behav 18:317–326

McCaul K, Gladue B, Joppa M (1992) Winning, losing, mood, and testosterone. Horm Behav 26:486–505

McEwen BS (1992) Steroid hormones: Effect on brain development and function. Horm Res 37, Suppl 3:1–10

McEwen BS, Biegon A, Fischette CT, Luine VN, Parsons B, Rainbow TC (1984) Toward a neurochemical basis of steroid hormone action. In: Martini L, Ganong WF (eds) Frontiers in neuroendocrinology, Vol 8. Raven Press, New York, pp 153–176

McGuire LS, Ryan KO, Omenn GS (1975) Congenital adrenal hyperplasia: II. Cognitive and behavioral studies. Behav Genet 5:175–188

McKeever WF, Deyo RA (1990) Testosterone, dihydrotestosterone, and spatial task performances of males. Bull Psychonomic Soc 28:305–308

Meyer-Bahlburg HFL, Ehrhardt AA (1982) Prenatal sex hormones and human aggression: A review and new data on progestogen effects. Aggress Behav 8:39–62

Meyer-Bahlburg HFL, Boon DA, Sharma M, Edwards JA (1974a) Aggression und Androgene bei männlichen Individuen. Bericht über den 28. Kongreß der DGP, Bd. 4, Klinische Psychologie. Hogrefe, Göttingen pp 57–65

Meyer-Bahlburg HFL, Boon DA, Sharma M, Edwards JA (1974b) Aggressiveness and testosterone measures in man. J Psychosom Med 36:269–273

Michael RP, Bonsall RW (1990) Androgens, the brain and behavior in male primates. In: Balthazart J (ed) Hormones, brain and behavior in vertebrates, 2. Karger, Basel, pp 15–26

Monti P, Brown W, Corriveau D (1977) Testosterone and components of aggressive and sexual behavior in man. Am J Psychiatry 134:692–694

Morales A, Johnston B, Heaton JPW, Lundie M (1997) Testosterone supplementation for hypogonadal impotence: Assessment of biochemical measures and therapeutic outcomes. J Urol 157:849–854

Morris MJ, Udry JR, Kahn-Dawood F, Dawood MY (1987) Marital sex frequency and midcycle female testosterone. Arch Sex Behav 16:27–37

Myers LS, Morokoff PJ (1986) Physiological and subjective arousal in pre- and postmenopausal women taking replacement therapy. Psychophysiology 23:283–292

Naftolin F, Perez J, Lerauth CS, Redmond DE, Garcia-Segura LM (1990) African green monkeys have sexually dimorphic and estrogen-sensitive hypthalamic neuronal membranes. Brain Res Bull 25:575–579

Nakashima A, Koshiyama K, Uozumi T, Monden J, Hamanaka Y, Kurachi K, Aono T, Mizutani S, Matsumoto K (1975) Effects of general anaesthesia and severity of surgical stress on serum LH and testosterone in males. Acta Endocrinol 78:258–269

Neave N, Wolfson S (2003) Testosterone, territoriality, and the home advantage. Psychol Behav 78:269–275

Nelson RJ (1995) An introduction to behavioral endocrinology. Sinauer, Sunderland (Mass)

Nieschlag E (1979) The endocrine function of the human testis in regard to sexuality. In: Ciba Foundation Symposium: Sex, hormones and behavior. Experta Medica, Amsterdam, pp 182–208

Nieschlag E (1992) Testosteron, Anabolika und aggressives Verhalten bei Männern. Dtsch Ärzteblatt 89:1663–1666

Nilsson P, Moller L, Solstad K (1995) Adverse effects of psychosocial stress on gonadal function and insulin levels in middle-aged males. J Internal Med 237: 479–486

Nordenström A, Servin A, Bohlin G, Larsson A, Wedell A (2002) Sex-typed Toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 87:5119–5124

Nyborg H (1988) Mathematics, sex hormones, and brain function. Behav Brain Sci 11:206–207

O'Carrol R, Bancroft J (1984) Testosterone therapy for low sexual interest and erectile dysfunction in men: A controlled study. Brit J Psychiatry 145:146–151

O'Connor DB, Archer J, Hair WM, Wu FCW (2001) Activational effects of testosterone on cognitive function in men. Neuropsychologia 39:1385–1394

Olweus D, Mattsson A, Schalling D, Löw H (1980) Testosterone, aggression, physical and personality dimensions in normal adolescent males. Psychosom Med 42:253–269

Olweus D, Mattsson A, Schalling D, Löw H (1988). Circulating testosterone levels and aggression in adolescent males: A causal analysis. Psychosom Med 50:261–272

Opstad PK (1992) The hypothalamo-pituitary regulation of androgen secretion in young men after prolonged physical stress combined with energy and sleep deprivation. Acta Endocrinol 127:231–236

Paikoff RL, Brooks-Gunn J, Warren MP (1991) Effects of girls' hormonal status on depressive and aggressive symptoms over the course of one year. J Youth Adolescence 20:191–215

Perlman SM (1973) Cognitive abilities of children with hormone abnormalities: Screening by psychoeducational tests. J Lear Disabil 6:21–29

Perry PJ, Andersen KH, Yates WR (1990) Illicit anabolic steroid use in athletes. A case series analysis. Am J Sports Med 18:422–428

Persky H, Zuckerman M, Curtis GC (1968) Endocrine function in emotionally disturbed and normal men. J Nerv Ment Dis 146:488–497

Persky H, Smith KD, Basu GK (1971) Relation of psychological measures of aggression and hostility to testosterone production in man. Psychosom Med 33: 265–277

Persky H, O'Brien C, Fine E, Howard W, Khan M, Beck R (1977) The effects of alcohol and smoking on testosterone function and aggression in chronic alcoholics. Am JPsychiatry 134:621–625

Persky H, Lief HI, Strauss D, Miller WR, O'Brien CP (1978) Plasma testosterone level and sexual behavior of couples. Arch Sex Behav 7:157–173

Persky H, Dreisbach L, Miller WR, O'Brien CP, Khan MA, Lief HI, Charney N, Strauss D (1982) The relation of plasma androgen levels to sexual behavior and attitudes of women. Psychosom Med 44:305–319

Pirke KM, Kockott G, Dittmar F (1974) Psychosexual stimulation and plasma testosterone in man. Arch Sex Behav 3:577–584

Pope HG, Katz DL (1988) Affective and psychotic symptoms associated with anabolic steroid use. Am JPsychiatry 145:487–490

Pope HG, Katz DL (1989) Homicide and near-homicide by anabolic steroid users. J Clin Psychiatry 51:28–31

Purvis K, Landgren B, Cekan Z, Diczfalusy E (1976) Endocrine effects of masturbation. J Endocrinol 70:439–444

Raboch J, Stárka L (1972) Coital activity of men and the levels of plasmatic testosterone. J Sex Res 8:219–224

Raboch J, Stárka L (1973) Reported coital activity of men and levels of plasma testosterone. Arch Sex Behav 2:309–316

Rada RT, Laws DR, Kellner R (1976) Plasma testosterone levels in the rapist. Psychosom Med 38:257–268

Rada RT, Laws DR, Kellner R, Stivastava L, Peake G (1983) Plasma androgens in violent and nonviolent sex offenders. Bull Am Acad Psychiatry Law 11:149–158

Reinisch JM (1981) Prenatal exposure to synthetic progestins increases potential for aggression in humans. Science 211:1171–1173

Reinisch JM, Sanders SA (1984) Prenatal gonodal steroidal influences on gender-related behavior. In: De Vries GH, de Bruin J, Corner M (eds) Sex differences in the brain. Progress in brain research, Vol 61. Elsevier, Amsterdam, pp 407–416

Rejeski WP, Brubaker PH, Herb RA, Kaplan JR, Korituik D (1988) Anabolic steroids and aggressive behavior in cynomolgus monkeys. J Behav Med 11:95–105

Resnick SM, Berenbaum SA, Gottesman II, Bouchard TJ Jr (1986) Early hormonal influences on cognitive functioning in congenital adrenal hyperplasia. Develop Psychol 22:191–198

Richardson DR, Green LR (1999) Social sanction and threat explanations of gender effects on direct and indirect aggression. Aggress Behav 25:425–434

Rose RM (1984) Overview of endocrinology of stress. In: Brown GM, Koslow SH, Reichlin S (eds) Neuroendocrinology and psychiatric disorder. Raven Press, New York, pp 95–122

Rose RM, Bourne PG, Poe RO, Mougey EH, Collins DR, Mason JW (1969) Androgen responses to stress II. Excretion of testosterone, epitestosterone, androsterone and eticholanolone during basic combat training and under threat of attack. Psychosom Med 31:418–436.

Rose RM, Holaday JW, Bernstein IS (1971) Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. Nature 231:366–368

Rose RM, Bernstein IS, Gordon TP (1975) Consequences of social conflict on plasma testosterone levels in rhesus monkeys. Psychosom Med 37:50–60

Rovet J, Netley C (1979) Phenotypic vs. genotypic sex and cognitive abilities. Behav Genet 9:317–322

Rovet J, Netley C (1982) Processing deficits in Turner's syndrome. Develop Psychol 18:77–94

Rowe T, Sasse V (1986) Androgens and premenstrual symptoms – the response to therapy. In: Dennerstein L, Frazer I (eds) Hormones and behaviour. Elsevier, New York, pp 160–165

Rubin RT, Poland RE, Tower BB, Hart PA, Blodgett ALN, Forster, B (1981) Hypothalamo-pituitary-gonadal function in primary endogenously depressed men: Preliminary findings. In: Fuxe K, Gustafsson JA, Wetterberg L (eds) Steroid hormone regulation of the brain. Pergamon, Oxford, pp 387–396

Rubin RT, Poland RE, Lesser IM (1989) Neuroendocrine aspects of primary endogenous depression VIII. Pituitary-gonadal axis activity in male patients and matched control subjects. Psychoneuroendocrinology 14:217–229

Sachar EJ, Halpern F, Rosenfeld RS, Gallagher TF, Hellman L (1973) Plasma and urinary testosterone levels in depressed men. Arch Gen Psychiatry 28:15–18

Salmimies P, Kockott G, Pirke KM, Vogt HJ, Shill WB (1982) Effects of testosterone replacement on sexual behavior in hypogonadal men. Arch Sex Behav 11:345–353

Salvadore A, Simon V, Suay F, Llorens L (1987) Testosterone and cortisol responses to competitive fighting in human males. Aggress Behav 13:9–13

Sarrel P, Dobay B, Wiita B (1998) Estrogen and estrogen-androgen replacement in post-menopausal women dissatisfied with estrogen-alone therapy. J Reprod Med 43:847–856

Scaramella TJ, Brown WA (1978). Serum testosterone and aggressiveness in hockey players. Psychosom Med 40:262–265

Schiavi RC, Schreiner-Engel P, White D, Mandeli J (1988) Pituitary – gonadal function during sleep in men with hypoactive sexual desire and in normal controls. Psychosom Med 50:304–318

Schreiner-Engel P, Schiavi RC, Smith H, White D (1981) Sexual arousability and the menstrual cycle. Psychosom Med 43:199–214

Schulkin J (1993) Hormonally induced changes in mind and brain. Academic Press, San Diego

Schwartz MF, Kolodny RC, Masters WH (1980) Plasma testosterone levels of sexually functional and dysfunctional men. Arch Sex Behav 9:355–366.

Sekeris CE (1990) The mitochondrial genome: A possible primary site of action of steroid hormones. In Vivo 4:317–320

Serra A, Pizzamiglio L, Boari A, Spera S (1978) A comparative study of cognitive traits in human sex chromosome aneuploids and sterile and fertile euploids. Behav Genet 8:143–154

Shangold MM (1984) Exercise and the adult female: Hormonal and endocrine effects. In: Terjung R (ed) Exercise and sports science reviews, Vol 12. Collamore Press, Lexington (MA), pp 53–79

Shangold MM, Gatz ML, Thysen B (1981) Acute effects of exercise on plasma concentrations of prolactin and testosterone in recreational women runners. Fertil Steril 35:699–702

Sherwin BB (1988) Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. Psychoneuroendocrinology 13:345–357

Sherwin BB (2002) Randomized clinical trials of combined estrogen-androgen preparations: effects on sexual functioning Fertil Steril 77:49–54

Sherwin BB, Gelfand MM (1985) Sex steroids and affect in the surgical menopause: A double-blind, cross-over study. Psychoneuroendocrinology 10:325–335

Sherwin BB, Gelfand MM (1987) The role of androgen in the maintenance of sexual functioning in oophorectomized women. Psychosom Med 49:397–409

Sherwin BB, Gelfand MM, Brender W (1985) Androgen enhances sexual motivation in females: A prospective, crossover study of sex steroid administration in the surgical menopause. Psychosom Med 47: 339–351

Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA (2000) Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688

Shute VJ, Pellegrino JW, Hubert L, Reynolds RW (1983) The relationship between androgen levels and human spatial abilities. Bull Psychonomic Soc 21:465–468

Sih R, Morley JE, Kaiser FE, Perry III HM, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: A 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Silbert AR, Wolff PH, Lilienthal J (1977) Spatial and temporal processing in patients with Turner's syndrome. Behav Genet 7:11–21

Silverman I, Kastuk D, Choi J, Phillips K(1999) Testosterone levels and spatial ability in men. Psychoneuroendocrinology 24:813–822

Simon NG, Whalen RE (1987) Sexual differentiation of androgen-sensitive and estrogen-sensitive regulatory systems for aggressive behavior. Horm Behav 21:493–500

Simonson E, Kearns WM, Enzer N (1941) Effect of oral administration of methyltestosterone on fatigue in eunuchoids and castrates. Endocrinology 28:506–512

Simonson E, Kearns WM, Enzer N (1944) Effect of methyl testosterone treatment on muscular performance and the central nervous system of older men. J Clin Endocrinol 4:528–534

Singer F, Zumoff B (1992) Subnormal serum testosterone levels in male internal medicine residents. Steroids 57:86–89

Skakkebaek N, Bancroft J, Davidson D, Warner P (1981) Androgen replacement with oral testosterone undecanoate in hypogonadal men: A double blind controlled study. Clin Endocrinol 14:49–61

Slabbekoorn D, van Goozen SHM, Megens J, Gooren LJG, Cohen-Kettenis PT (1999) Activating effects of cross-sex hormones on cognitive functioning: A study of short-term and long-term hormone effects in transsexuals. Psychoneuroendocrinology 24:423–447

Stearns EL, Winter JSD, Faiman C (1973) Effects of coitus on gonadotropin, prolactin and sex steroid levels in man. J Clin Endocrinol Metab 37:687–691

Steiner M, Dunn E, Born L (2003) Hormones and mood: from menarche to menopause and beyond. J Affective Disord 74:67–83

Stenn PO, Klaiber EL, Vogel W, Broverman DM (1972) Testosterone effects on photic stimulation of the EEG and mental performances of humans. Percept Mot Skills 34:371–378

Stoléru SG, Ennaji A, Cournot A, Spira A (1993) LH pulsatile secretion and testosterone blood levels are influenced by sexual arousal in human males. Psychoneuroendocrinology 18:205–218

Strauss RH, Wright JE, Finerman GAM, Catlin DH (1983) Side effects of anabolic steroids in weight-trained men. Physician Sportsmed 11:86–98

Strauss RH, Liggett MT, Lanese RR (1985) Anabolic steroid use and perceived effects in tenweight-trained men. Physician Sportsmed 253:2871–2873

Stumpf H, Klieme E (1989) Sex-related differences in spatial ability: More evidence for convergence. Percep Mot Skills 69:15–921

Su TP, Pagliaro M, Pickar D, Wolkowitz W, Rubinow DR (1993) Neuropsychiatirc effects of anabolic steroids in male normal volunteers. J Am Med Assoc 269: 2760–2764

Susman EJ, Inoff-Germain G, Nottelmann ED, Loriaux DL, Cutler GB Jr, Chrousos GP (1987) Hormones, emotional dispositions, and aggressive attributes in young adolescents. Child Develop 58:114–1134

Sutton JR, Coleman MJ, Casey J, Lazarus L (1973) Androgen responses during physical exercise. Brit Med J 1:520–522

Tan Ü, Akgün A (1992) There is a direct relationship between nonverbal intelligence and serum testosterone level in young men. Int J Neurosci 64:213–216

Tanaka H, Cleroux J, de Champlain J, Ducharme J, Collu R (1986) Persistent effects of a marathon run on the pituitary-testicular axis. J Endocrinol Invest 9:97–101

Tegelman R, Carlström K, Pousette A (1988) Hormone levels in male ice hockey players during a 26-hour cup tournament. Int J Androl 11:361–368

Tenover JL (1992) Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Tieger T (1980) On the biological basis of sex differences in aggression. Child Develop 51:943–963

Traish AM, Kim N, Min K Munarriz R, Goldstein J (2002) Role of androgens in female genital sexual arousal: Receptor expression, structure, and function. Fertil Steril 77:11–18

Trautmann PD, Meyer-Bahlburg HFL, Postelnek J, New MI (1995) Effects of early prenatal dexamethasone on the cognitive and behavioral development of young children: Results of a pilot study. Psychoneuroendocrinology 20:439–449

Udry JR, Talbert LM (1988) Sex hormone effects on personality at puberty. J Pers Soc Psychol 54:291–295

Unden F, Ljunggren JG, Beck-Friis J, Kjellman BF, Wetterberg L (1988) Hypothalamic-pituitary-gonadal axis in major depressive disorders. Acta Psychiatr Scand 78:138–146

Uzych L (1992) Anabolic-androgenic steroids and psychiatric-related effects: A review. Can J Psychiatry 37:23–28

Vandenberg SG, Kuse AR (1979) Spatial ability: A critical review of the sex-linked major gene hypothesis. In Wittig MC, Petersen AC (eds) Determinants of sex-related differences in cognitive functioning. Academic Press, New York, pp 67–95

van Goozen SHM, Frijda NH, Van de Poll NE (1994a) Anger and aggression in women: Influence of sports choice and testosterone administration. Aggress Behav 20:213–222

van Goozen SHM, Cohen-Kettenis PT, Gooren LJG, Frijda NH, van de Poll NE (1994b) Activating effects of androgens on cognitive performance: Causal evidence in a group of female-to-male-transsexuals. Neuropsychologia 32:1153–1157

van Goozen SHM, Frijda NH, Van de Poll NE (1995a) Anger and aggression during role-playing: Gender differences between hormonally treated male and female transsexuals and controls. Aggress Behav 21:257–273.

van Goozen SHM, Cohen-Kettenis PT, Gooren LJG, Frijda NH, Van de Poll NE (1995b) Gender differences in behavior: Activating effects of cross-sex hormones. Psychoneuroendocrinology 20:343–363

Vasankari TJ, Kujala UM, Heinonen OJ, Huhtaniemi JT (1993) Effects of endurance training on hormonal responses to prolonged physical exercise in males. Acta Endocrinol 129:109–113

Viru A (1991) Adaptive regulation of hormone interaction with receptor. Exp Clin Endocrinol 97:13–28

Vogel W, Klaiber EL, Broverman D (1978) Roles of the gonadal steroid hormones in psychiatric depression in men and women. Prog Neuro-Psych 2:487–503

von der Pahlen B, Lindman R, Sarkola T, Mäkisalo H, Eriksson CJP (2002) An exploratory study on self-evaluated aggression and androgens in women. Aggress Behav 28:273–280

von Zerssen D, Berger M, Doerr P (1984) Neuroendocrine dysfunction in subtypes of depression. In: Shah NS, Donald AG (eds) Psychoneuroendocrine dysfunction in psychiatric and neurological illnesses: Influence of psychopharmacological agents. Plenum, New York, pp 357–382

Waber DP (1977) Sex differences in mental abilities, hemispheric lateralization, and rate of physical growth at adolescence. Develop Psychol 13:29–38

Wang C, Alexander G, Berman N, Salehian B, Davidson T, McDonald V, Steiner B, Hull H, Callegari C, Swerdloff RS (1996) Testosterone replacement therapy improves mood in hypogonadal men: A clinical research center study. J Clin Endocrinol Metab 81:3578–3583

Warren MP, Brooks-Gunn J (1989) Mood and behavior at adolescence: Evidence for hormonal factors. J Clin Endocrinol Metab 69:77–83

Weissman MM, Olfson M (1995) Depression in women: implications for health care research. Science 269:799–801

Whalen RE (1982) Current issues in the neurobiology of sexual differentiation. In: Vernadakis A, Timiras PS (eds) Hormones in development and aging. MTP Press, Lancaster, pp 273–304

Wheeler GD, Wall SR, Belcastro AN, Cumming DC (1984) Reduced serum testosterone and prolactin levels in male distance runners. J Am Med Assn 252:514–516

Wheeler GD, Singh M, Pierce WD, Epling WF, Cumming DC (1991) Endurance training decreases serum testosterone levels in men without change in luteinizing hormone pulsatile release. J Endocrinol Metab 72:422–425

Wittig MC, Petersen AC (1979). Determinants of sex-related differences in cognitive functioning. Academic Press, New York

Wynn TG, Tierson FD, Palmer CT (1996) Evolution of sex differences in spatial cognition. Yearbook Phys Anthropol 39:11–42

Yalom ID, Green R, Fisk N (1973) Prenatal exposure to female hormones: Effect on psychosexual development in boys. Arch Gen Psychiatry 28:554–561

Yesavage JA, Davidson J, Widrow L, Berger PA (1985) Plasma testosterone levels, depression, sexuality, and age. Biol Psychiatry 20:222–225

Zitzmann M, Weckesser M, Schober O, Nieschlag E (2001) Changes in cerebral glucose metabolism and visuospatial capability in hypogonadal males under testosterone substitution therapy. Exp Clin Endocrinol Diabetes 109:302–304

Zussman JU, Zussman PP, Dalton K (1975) Postpubertal effects of prenatal administration of progesterone. Paper presented at the meeting of the Society for Research in Child Development, Denver (Col)

Zweifel JE, O'Brien WH (1997) A meta-analysis of the effect of hormone replacement therapy upon depressed mood. Psychoneuroendocrinology 22:189–212

5

The role of testosterone in spermatogenesis

G.F. Weinbauer, M. Niehaus and E. Nieschlag

Contents

5.1 Rationale

The endocrine control of testicular function is under investigation for many years. Once it became clear that LH/testosterone and FSH are the key factors in the control of the spermatogenic process, considerable efforts were spent in order to

unravel the relative roles of testosterone and FSH for gametogenesis. A variety of experimental approaches was applied covering selective replacement of either hormone in hormone-deficient animal models, selective immunization against LH and FSH or their respective receptors, gene targeting of the reproductive hormones and their receptors, and selective elimination of testosterone-producing Leydig cells by toxicants.

The first edition of this book was published in 1990 followed by an update in 1998. It is interesting that major questions continue to remain an enigma, e.g. the precise mechanism of testosterone and FSH actions and how these hormones cooperate at the testicular level. On the other hand, significant new insights were gained, e.g. that, at least in primates, spermatogonia are the initial target of testosterone and FSH rather than meiotic cells or spermatids and that spermatid release (spermiation) is under endocrine control. Unlike a few years ago, when the relevance of FSH in adult spermatogenesis was questioned, it has become clear that FSH – probably more so than testosterone – is the key regulator of primate spermatogenesis.

The present chapter aims at providing a state-of-the-art review of our current understanding of the role of testosterone in spermatogenesis. Inevitably, we also review the role of FSH in spermatogenesis. A variety of species has been studied over the years and it became evident that certain nonhuman primate species are the most predictive preclinical animal model. Comparative analysis across species also revealed interesting insights such as the predominance of FSH for spermatogenesis in photoperiodic species and the observation that Leydig cells in marmosets normally operate with an "inactive" LH receptor.

5.2 Organisation and kinetics of spermatogenesis

5.2.1 Basic and common features

Spermatogenesis comprises the development of sperm from stem spermatogonia. This process encompasses the multiplication and differentiation of stem cells into differentiated and proliferating germ cells, the redistribution of genetic information during meiosis and the maturation and differentiation of haploid germ cells. Following proliferation of A-type spermatogonia into B-type spermatogonia, these cells enter meiosis and are termed spermatocytes and after completion of reduction divisions, the emerging haploid germ cells are denoted as spermatids. These spermatids undergo a major and complex morphological, structural and functional maturation and development process resulting in the production of spermatozoa. Terminally elongated spermatids (testicular spermatozoa) do not exhibit progressive motility but are capable of fertilization as evidenced by in vitro fertilization techniques.

Among mammals, the entire process of spermatogenesis is topographically determined and during the various developmental phases, only specific germ cell associations assemble. The specific germ cell associations are known as stages of spermatogenesis. Every stage is thought to derive from one stem cell and hence represents a cell clone with intercellular bridges remaining that allow continued cellular communication (Alastalo *et al.* 1998; Ren and Russell 1991). The commonly used staging system is based upon the morphology of the developing acrosome in spermatids (Clermont 1972). It is obvious that the subdivision of the spermatogenic process into various stages is somewhat arbitrary and, hence, that the number of spermatogenic stages varies between species. Dividing the spermatogenic process into stages is critical since many processes and actions occur physiologically in a stage-specific manner. Conversely, disturbances of spermatogenesis imposed by endocrine deficiencies or exposure to toxicants – at least initially – become frequently manifest in a stage-specific response pattern.

Germ cell development is tightly coupled to intratubular somatic cells, the Sertoli cells. These cells possess highly specialised cytological and structural features enabling them to functionally and also physically support germ cell development and movement from the basement to the tubular lumen. Sertoli cells divide during the prepubertal period until the establishment of the blood testis barrier. Two major functions are assigned to the Sertoli cell: to determine adult testis size and sperm production and to enable and coordinate germ cell proliferation and development. Evidence for the former is compelling since it has been demonstrated under normal and pathological conditions that the number of Sertoli cells correlates precisely with the number of sperm produced (de Franca *et al.* 1995).

Whereas previously it was believed that the Sertoli cells govern the spermatogenic process it is now thought that this ability may well reside in the germ cell itself. Modulation and elimination of specific germ cell types by administration of specific toxins provoked stage-specific alterations of Sertoli cell inhibin secretion (Jegou 1993; Sharpe 1994). This view is further corroborated by xenogeneic germ cell transplantation studies in mice testes being injected with germ cells derived from rats (Clouthier *et al.* 1996). In these testes – although only mouse Sertoli cells could be found (Russell and Brinster 1996) – mouse and rat spermatozoa were produced simultaneously and the mouse-specific and rat-specific timing of spermatogenesis was retained (Franca *et al.* 1998). The latter is particularly interesting given the fact that the entire spermatogenic process requires approx. 35 days in mice but approx. 50 days in rats.

During spermatogenesis the developing germ cells are relocated from the basement towards the lumen of the seminiferous tubule, and during spermiation, the spermatozoa are released into the lumen. In order to be able to propel the sperm within the testis and into the epididymis via the excurrent testicular ducts,

the seminiferous tubules contract in a peristaltic manner (Assinder *et al.* 2002; Santiemma *et al.* 2001; Tripiciano *et al.* 1999). These contractions are believed to proceed along the length of the seminiferous tubules and are induced by the peritubular cells. These cells exhibit the features of myoid cells and contain α-smooth muscle actin, panactin, smooth muscle myosin and desmin (Holstein *et al.* 1996) and their contractions are controlled by oxytocin and endothelin. Isolated rat spermatogenic stages VII–VIII segments were most responsive to oxytocin (Harris and Nicholson 1998). Oxytocin and endothelin have also been found in human testes (Ergun *et al.* 1998).

Description and evaluation of the spermatogenic process can be qualitative and quantitative. Qualitatively normal spermatogenesis refers to the presence of all germ cell types and spermatogenic stages. Quantitatively normal spermatogenesis implies that the numbers of all germ cell types are produced and present in normal quantity. This distinction is very important for the discussion of the relative role of testosterone and FSH in spermatogenesis and for the assessment of toxic actions on spermatogenesis.

5.2.2 Species-specific features

Although the spermatogenic process has common and universal features, substantial differences must also be kept in mind. For the purpose of this chapter, the discussion of species-specific aspects is largely confined to a comparison between rodents (mouse, rat, hamster) and primates (nonhuman primates and man).

The system of spermatogonial renewal is quite different between rodents and primates. Rodent stem spermatogonial development is well described and several generations of differentiating and dividing A-type spermatogonia exist prior to formation of B-type spermatogonia (de Rooij and Grootegoed 1998). In the primate, stem spermatogonia ($A_{d(ark)}$-type spermatogonia) are easily recognized and only one generation of renewing spermatogonia, i.e. ($A_{p(ale)}$-type spermatogonia), has been described (Meistrich and van Beek 1993). The precise relationship between A_d-type and A_p-type spermatogonia and their kinetics are still under investigation. Currently the view predominates that the A_p-type spermatogonia – following division – provide one daughter cell to enter the spermatogenic process and the other daughter cell to replenish A_d-type population if needed. Conversely, A_d-type spermatogonia – which rarely divide in the intact testis – are considered to replenish A_p-type spermatogonia in case of severe spermatogonial depletion, e.g. following testicular irradiation (van Alphen *et al.* 1989).

Among rodents, a tubular cross-section is occupied by a single spermatogenic stage (single-stage arrangement), whereas in primates a full range of arrangements comprising predominantly single-stage tubules, predominantly multi-stage tubules (>1 spermatogenic stage/tubular cross-section) and intermediate arrangements

have been described (Wistuba *et al.* 2003). In New World monkeys, hominoids and man, tubules are predominantly multi-stage but are predominantly single-stage in macaques and intermediate in baboon. The human multi-stage arrangement has been suggested to derive from a helical arrangement of spermatogenic stages (Schulze and Rehder 1984; Zannini *et al.* 1999) but this view has also been challenged (Johnson *et al.* 1996). Alternatively, it might merely be the clonal size that determines whether a particular spermatogenic stage entirely occupies a tubule cross-section or not, i.e. whether the tubule is single- or multi-stage. The observation that neotropical primate testes – unlike those from Old World monkeys but similar to man – have predominantly multi-stage tubules implies that this feature has been developed several times during evolution and does not represent a selection criterion for spermatogenesis.

Interestingly, and contrary to previous beliefs, the single-stage vs multi-stage arrangement is not related at all to spermatogenic efficacy, i.e. germ cell loss during meiosis and spermatid maturation (Wistuba *et al.* 2003). For cynomolgus monkeys and man this has also been shown earlier by the use of unbiased stereological techniques for cell enumeration (Zhengwei *et al.* 1998). Hence the differences in testicular germ cell production and sperm output are now believed to be determined by the number of spematogonia entering meiosis and this aspect can be species-specific.

In terms of the number of spermatogenic stages, 14 stages are used for the rat, 12 for the hamster, 8 for the mouse, 6 or 12 for marmoset, 12 for macaques and 6 for chimpanzee and man (Clermont 1972; Millar *et al.* 2000; Smithwick *et al.* 1996; Weinbauer *et al.* 2001a). Originally 12 spermatid development stages were described for man (Clermont and Leblond 1955) and this has been reduced to 6 spermatogenic stages for practical reasons (Clermont, 1969; Fig 5.1). The human 6-stage classification has been applied to other primates and is useful for comparative studies (Dietrich *et al.* 1986; Wistuba *et al.* 2003).

The succession of all given stages is denoted as the cycle of spermatogenesis and the duration of a spermatogenic cycle has been determined using ^{3}H-thymidine, 5-bromodeoxyuridine or the depopulation/repopulation of germ cells following testicular irradiation. In terms of the duration of one spermatogenic cycle, it is 12–14 days for the rat, 10 days for the hamster, 7–9 days for the mouse, 17 days in the Chinese hamster, 10 days for marmosets, 9–11 days for macaques, 14 days for chimpanzees and 16 days for man (Clermont 1972; Millar *et al.* 2000: Smithwick *et al.* 1996; Weinbauer and Korte 1999). For the completion of the entire spermatogenic process, i.e. formation of sperm from stem cell, between 4 and 4.5 spermatogenic cycles are needed.

Reproductive hormones do not influence the frequency of spermatogenic stages and the duration of the spermatogenic cycle (Aslam *et al.* 1999) whereas

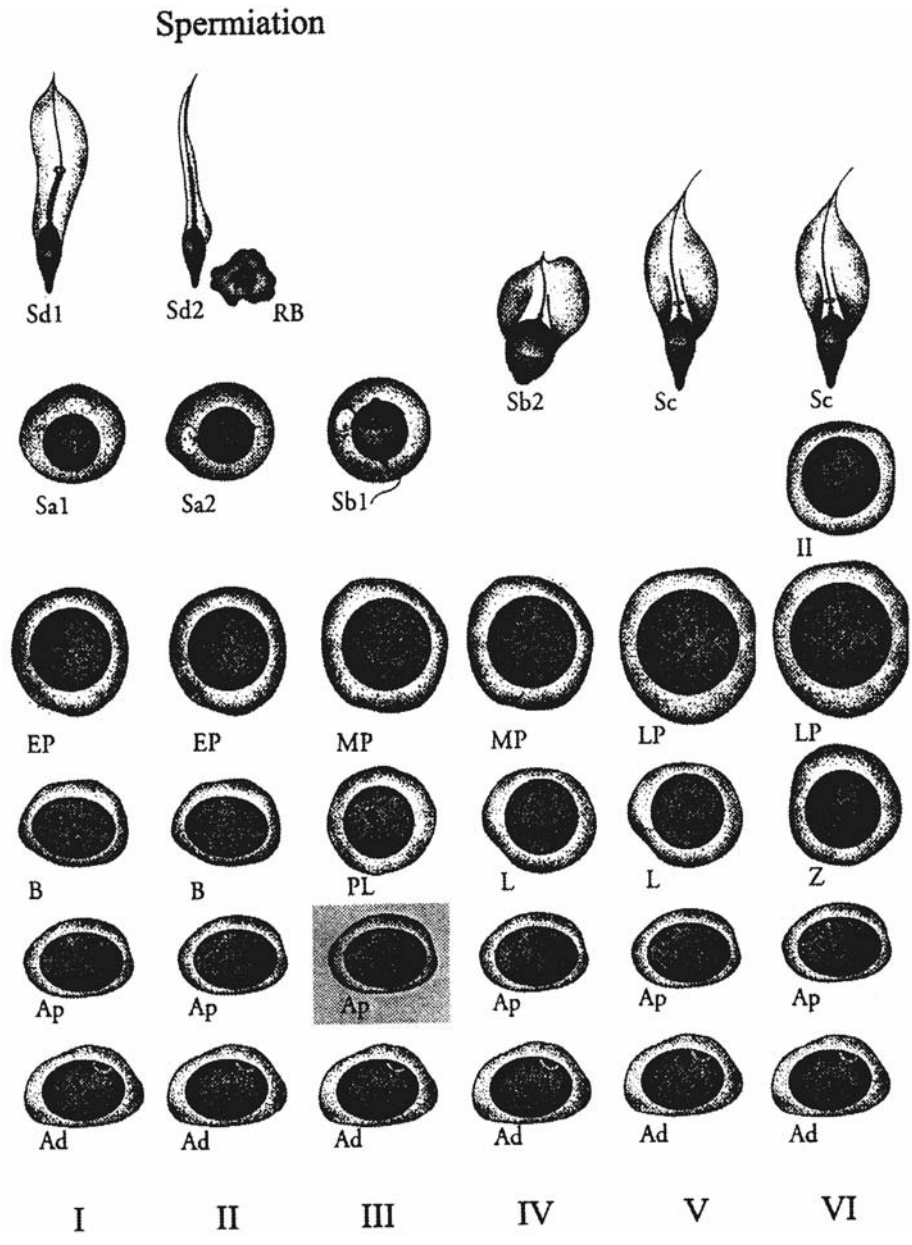


Fig. 5.1 Schematic representation of the spermatogenic process and the six spermatogenic stages in men. The succession of all six stages requires approx. 16 days. Spermiation takes place during stage II→III transition. If stage III is taken as the starting point for the next spermatogenic cycle, the duration of the entire spermatogenic process from renewing spermatogonium (Ap, hatched box) to fully elongated spermatid (Sd2) would require four spermatogenic cycles, i.e. approx. 64 days. If the renewing spermatogonia in stage I are taken as starting point, the duration of the spermatogenic process would require 4.4 – 4.6 spermatogenic cycles. The authors favour the former approach. The human stage classification system has been used for other nonhuman primates (Aslam *et al*. 1999; Dietrich *et al*. 1986; Wistuba *et al*. 2003).

toxicants (Rosiepen *et al.* 1995) or vitamin A deficiency/replenishment (Bartlett *et al.* 1990b; Siiteri *et al.* 1992) can do so. For example, 2,5-hexandione, a neurotoxin and Sertoli cell toxicant disrupting microtubule arrangements (Boekelheide *et al.* 2003), prolonged the duration of one spermatogenic cycle in the rat by one day. In the vitamin A depleted model, germ cell progression is arrested at the level of preleptotene spermatocytes but is restarted in most tubules simultaneously during vitamin A replacement. As a consequence, at a given later time point, most of the seminiferous tubules are in the same spermatogenic stage quite different from the normal stage distribution. However, this synchrony is lost over approximately 10 spermatogenic cycles and frequency distribution of spermatogenic stages returned to normal, strongly suggesting a change in the timing of the relative duration.

5.3 The hypothalamo-hypophyseal-testicular circuit

The hypothalamus-pituitary-testis circuit represents the core unit for the maintenance of the endocrine balance and fertility. Testicular functions, i.e. production of testosterone and of spermatozoa, are entirely subject to regulation by endocrine factors derived from the brain. Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus and stimulates the synthesis and release of the gonadotropic hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (Fig. 5.2). LH acts on testicular Leydig cells and governs the production and secretion of testosterone by these cells. Within the testis, testosterone acts on peritubular cells that surround the seminiferous tubules and on the somatic Sertoli cells within the seminiferous epithelium. Beyond that, testosterone exerts a variety of physiological effects in the periphery and, in fact, androgen receptors have been detected in about 40 organs of the cynomolgus monkey (Dankbar *et al.* 1995).

FSH acts directly within the seminiferous tubules. In the immature testis FSH can also stimulate Leydig cell production. These stimulatory effects have been observed in the absence of endogenous LH (Haywood *et al.* 2003) and are mediated via the FSH receptor. It seems that the FSH receptor is involved in Leydig cell functional maturation and reduced peripheral testosterone levels, but increased testicular testosterone concentrations were observed in FSH receptor-deficient mice (Krishnamurthy *et al.* 2001). In combination with LH/hCG activity, FSH potentiates Leydig cell testosterone production in the immature primate testis (Schlatt *et al.* 1995). The factor(s) that mediate the effects of FSH on immature Leydig cells are yet unknown.

The secretion of GnRH and gonadotropic hormones is controlled by testicular steroid and protein factors. Testosterone is the major steroid eliciting a negative feedback effect on LH and FSH secretion in the male. An additional feedback loop

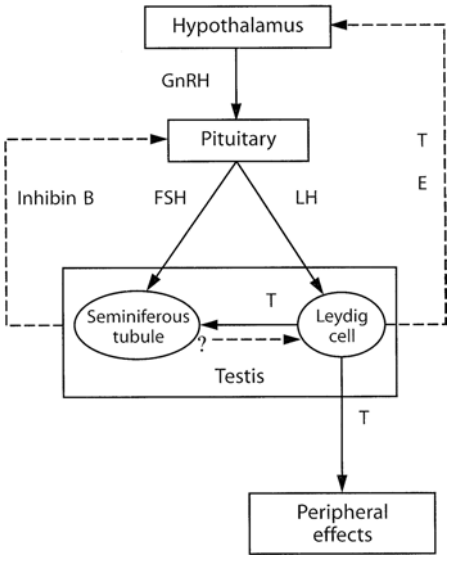


Fig. 5.2 Hypothalamic-hypophyseal-testicular communication in primates. Gonadotropin-releasing hormone (GnRH) stimulates the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary. LH acts on Leydig cells and induces the synthesis and release of testosterone (T). Testosterone acts within the testis and – in the periphery – exerts an inhibitory feedback effect on hypothalamic GnRH secretion. Hence testosterone is the main regulator of LH secretion and also of FSH secretion. In part, the inhibitory effects of testosterone are mediated via aromatization of testosterone into estradiol (E) in the brain. For FSH an additional inhibitory feedback loop operates via testicular inhibin B at the level of the pituitary. Unlike LH, FSH acts on spermatogenesis directly. In the immature testis, FSH activity also stimulates Leydig cell differentiation and testosterone production via yet unknown factor(s) (denoted by dashed line and question mark).

has been described for FSH that is mediated by the effects of inhibins (de Kretser and Phillips 1998). Activin and follistatin are also involved in FSH feedback regulation but act more as local regulators rather than endocrine factors. The actions of testosterone can follow 5α-reduction to dihydrotestosterone (DHT) or aromatization to estradiol. In primates, negative feedback actions can be exerted at the hypothalamic and at the pituitary levels. It would appear, however, that testosterone predominantly acts via hypothalamic action (Fingscheidt *et al.* 1998; Veldhuis *et al.* 1997) whereas inhibin directly influences gonadotropins at the hypophyseal level in vivo and in vitro (Fingscheidt *et al.* 1998; Ramaswamy *et al.* 1998). Activins selectively stimulate FSH secretion and follistatin binds to activin and presumably determines and regulates activin-associated effects through this mechanism (McConnell *et al.* 1998). The physiological relevance of activin for spermatogenesis

is strongly indicated by the observation that over-expression of follistatin is associated with spermatogenic defects, reduced testis size and reduced fertility in mice (Guo *et al.* 1998).

5.4 Androgen dependence of spermatogenesis

5.4.1 Neonatal androgen secretion

A distinct peak of testosterone synthesis and secretion occurs perinatally and – depending on the species – is of variable duration during the neonatal period. The physiological significance of this activation of testosterone production is not entirely clear. In the rat model, blockade of the neonatal androgen secretion by a GnRH antagonist provoked a delay of puberty and infertility in the adult animals (Kolho and Huhtaniemi 1989a; 1989b). Surprisingly, spermatogenesis remained unaffected and infertility resulted from the inability to inseminate the females during mating. However, the infertile status progressively reverted over time and following an observation period of 350 days, animals regained fertility.

Long-term studies were also conducted in two nonhuman primate models, the rhesus monkey and the common marmoset. In these studies, neonatal androgen secretion was either blocked by the administration of GnRH agonist or antagonist. This interference delayed pubertal onset and attenuated the testicular weight gain (Mann *et al.* 1993; 1998; McKinnell *et al.* 2001; Sharpe *et al.* 2000). Penile length and detachment of prepuce were also affected transiently but recovered by week 52 (Brown *et al.* 1999). Animals were followed until adulthood and various male reproductive parameters including fertility and mating behaviour (Lunn *et al.* 1994) were assessed. No untoward effects on testicular function and fertility could be detected (Lunn *et al.* 1997). Hence, on the basis of available data, it appears that the neonatal testosterone peak is not related to subsequent development of male reproductive functions, timing of puberty and fertility. The only effect of loss of neonatal testosterone production that could be unravelled in adulthood was a dysfunction of some specific aspects of the immune system (Mann and Fraser 1996).

5.4.2 Pubertal initiation of spermatogenesis

Induction of spermatogenesis can be achieved in immature nonhuman primates by the administration of very high doses of testosterone although the number of spermatozoa in the ejaculate remained rather low (Marshall *et al.* 1984). This finding is in agreement with the earlier clinical observations that in boys with Leydig cell tumors, spermatogenesis was observed in those testicular areas bearing the tumor cells and producing high amounts of testosterone (Weinbauer and Nieschlag 1996, for details). These data would indeed suggest a direct local effect of testosterone.

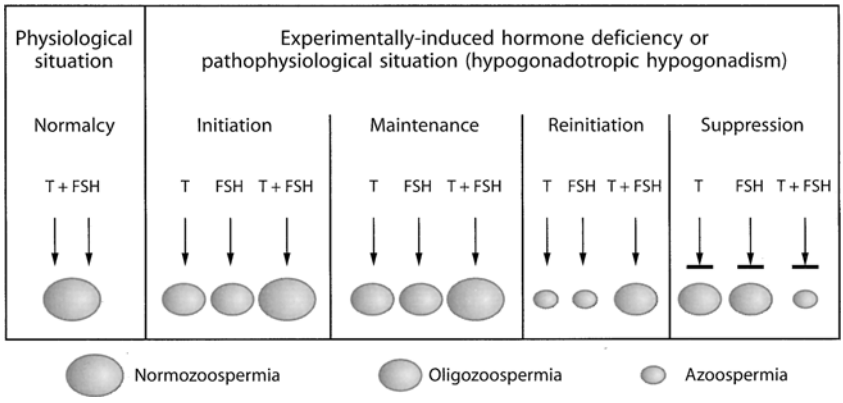


Fig. 5.3 Schematic representation of the role of follicle-stimulating hormone (FSH) and testosterone (T) alone or in combination for spermatogenesis in the primate. For normozoospermia (quantitatively normal spermatogenesis) both hormones are necessary and, conversely, the complete inhibition of spermatogenesis (azoospermia) requires suppression of both hormones. In clinical practice, both hormones are needed to re-establish fertility. In mice transgenic for human FSH or human FSH receptor, FSH action is not sufficient to induce the complete spermatogenic process. Because of the prolonged period of prepubertal quiescence in the primate, it is not clear whether FSH alone can initiate spermatogenesis. In the hpg mouse estradiol stimulates the complete spermatogenic process but this may involve concomitant FSH release and may not be applicable to primates.

More recently, analysis of testicular development in boys with activating mutations of the LH receptor has confirmed the concept that testosterone can initiate precocious puberty and maturation of the male gonad (Gromoll *et al.* 1998; Shenker *et al.* 1993).

Meanwhile it has become well established that testosterone is in fact essential to enable timely initiation of puberty. This knowledge stems from patients with inactivating mutations of the LH receptor. Aside from other effects such as reduced height and retarded bone maturation, these patients have comparatively small testes suggesting an impairment of germ cell development in association with reduced/absent local testicular testosterone production (Gromoll *et al.* 2000; Kremer *et al.* 1995).

Although these data clearly demonstrate the ability of testosterone to initiate the spermatogenic process (Fig. 5.3), they do not prove that testosterone is indispensable for the commencement of this process. "Fertile eunuchs" have atrophied Leydig cells but complete spermatogenesis (Behre *et al.* 2000). A patient with normal to slightly elevated gonadotropin levels along with a markedly reduced testosterone concentration but complete spermatogenesis has been described (de Roux *et al.* 1997). Hence it appears quite possible that spermatogenic induction can occur at least in the presence of substantially lowered testosterone levels.

Whereas there has been some debate previously as to whether testosterone can initiate the complete spermatogenic process in rats and mice, this issue is now better understood. In the hypogonadal (hpg) mouse lacking endogenous gonadotropin secretion, testosterone induced sperm formation and these sperm were fertile in vitro (Singh *et al.* 1995). In the rat the evidence is more indirect but also suggestive of a role for testosterone. Immunization of 18-day old rats against the LH receptor caused a 50% reduction of testicular sperm counts by 88 days of age (Graf *et al.* 1997).

Estradiol, in the adult male, directly interferes with gonadotropin production and secretion through negative feedback action followed by complete testicular involution and cessation of spermatogenesis. However, in the immature rat and mouse, treatment with estradiol provoked an increase of germ cell numbers (Ebling *et al.* 2000; Kula 1988). The study by Ebling *et al.* was conducted in hpg-mice and qualitatively normal spermatogenesis was induced by estradiol within 70 days. Since estradiol treatment also elevated FSH levels, it is possible, however, that the observed testicular effects of estradiol were indirect and at least partly mediated via FSH (Fig. 5.3).

It must be pointed out that during pubertal initiation, testosterone also stimulates growth hormone secretion and growth hormone-dependent growth factor levels. Whereas no evidence points to a role of GH in adult spermatogenesis (Sjogren *et al.* 1999), this is less clear for the developing testis. In GH-deficient and IGF-I/II-deficient animal models, testes – and body and organ size in general – are smaller. However, spermatogenesis is complete in these small testes, suggesting no direct involvement of GH and growth factors in initiation of spermatogenesis. For the GH-deficient dwarf rat it has been reported that spermatogenesis is also quantitatively normal (Bartlett *et al.* 1990b).

5.4.3 Adult spermatogenesis: maintenance and reinitiation

Much experimental work has been conducted in several animal species including nonhuman primates in order to clarify whether testosterone alone can maintain spermatogenesis. Suppression of LH/FSH secretion followed by concurrent and selective LH/testosterone replacement demonstrated unequivocally that testosterone alone could maintain qualitatively normal spermatogenesis, at least for the observation periods chosen. Studies employing selective immunization against LH or the LH receptor provided further support for a role of testosterone even in the presence of continued FSH secretion (Graf *et al.* 1997; Suresh *et al.* 1995). In a particular experimental setting, i.e. rats actively immunized against GnRH or depleted of Leydig cells, and supplemented with testosterone, even quantitatively normal spermatogenesis was maintained (Awoniyi *et al.* 1992; Sharpe *et al.* 1988a; 1988b).

In most instances, the experimental paradigm for animal studies differs largely from the clinical situation. While high doses of exogenous testosterone are used in animal experimentation, hCG is used for clinical therapy. The sustained use of hCG in animals, however, is obviated by the antigenic response to hCG and the development of neutralizing antibodies. Hence, in animals, high doses of testosterone must be given in order to achieve sufficient testicular androgen concentrations, whereas in patients Leydig cells and testosterone production are stimulated directly.

The clinical evidence derived from hypogonadotropic hypogonadal patients under endocrine therapy demonstrated that testosterone can maintain spermatogenesis but only to a qualitative extent. It must also be recognized that the successful reinitiation of spermatogenesis requires the addition of FSH activity (either via pulsatile GnRH or administration of hMG or urinary/recombinant human FSH) in most instances. In these studies, hCG is administered to patients, thus providing direct stimulation of Leydig cells and endogenous testosterone production (McLachlan 2000; Nieschlag *et al.* 1999). If patients continue only on testosterone substitution via hCG yielding normal peripheral androgen levels, the stimulatory effect on spermatogenesis is only maintained in a qualitative manner and sperm numbers decline over time (Depenbusch *et al.* 2002). This study and others (Meriggiola *et al.* 2002) confirm and extend earlier reports (Johnsen 1978; Vicari *et al.* 1992) and clearly show that testosterone alone can maintain spermatogenesis but only to a qualitatively normal extent. Vicari *et al.* (1992) also reported complete reinitiation of spermatogenesis by hCG alone in hypogonadotropic hypogonadal patients.

Thus, maintenance and reinitiation of spermatogenesis by testosterone/hCG in patients are possible (Nieschlag *et al.* 1999; Fig. 5.3). The certain diversity of results may result from the fact that hypogonadotropic hypogonadism is not a monocausal disease but may stem from various deficiencies (idiopathic hypogonadotropic hypogonadism, pre- and post-pubertal pituitary insufficiency, Kallmann syndrome). Also the preceding history of therapy and pretherapy testicular volume contribute to differential responsiveness of the patients (Liu *et al.* 2002). Comparing doses, reinitiation of spermatogenesis seems to require more testosterone – either higher doses or longer duration of exposure – than maintenance of spermatogenesis. This became particularly evident from studies using GnRH analogues and testosterone substation: concomitant supplementation with testosterone prevented the induction of azoospermia, whereas delayed substitution with the same dose of testosterone failed to do so (Weinbauer and Nieschlag 1996).

If very high doses of testosterone are used it is possible to restore spermatogenesis to a qualitatively normal extent in experimental models including the nonhuman primate. It was also shown in the Leydig cell-depleted rat model and the GnRH-immunized rat model, that high amounts of testosterone almost quantitatively

maintained or restored normal spermatogenesis (Awoniyi *et al.* 1992; Sharpe *et al.* 1988a; 1988b). It must be noted, however, that androgen receptors are present in many organs (Dankbar *et al.* 1995) and that the animals were exposed to exorbitantly high androgen levels in some of these studies. Hence the possibility cannot be ruled out entirely that the observed effects on spermatogenesis not only represent a selective and specific effect of testosterone on the testis but also a systemic action under pharmacological conditions.

5.5 Androgen action on spermatogenesis

5.5.1 Testicular androgen production, metabolism and transport

Testosterone is produced by the interstitial Leydig cells. The details and mechanisms of testosterone synthesis and secretion are presented in chapter 1. Testicular concentrations of testosterone can exceed those found in circulating blood up to 100-fold or beyond. It was thought initially that spermatogenesis requires high local amounts of testosterone. This view could not be corroborated and spermatogenesis in the rat can proceed in the presence of 5–10% of normal intratesticular androgen levels as described in the hallmark paper by Cunningham and Huckins (1979). Interestingly, it has also been observed that testosterone can inhibit certain populations of A-type spermatogonia in the rat model (Huang and Nieschlag 1986) and these observations have been corroborated in rat gonadal protection models (Meistrich and Shetty 2003). A rather obvious need for high local androgen concentrations results from the fact that sufficient peripheral testosterone levels and pulses must be provided by rapid secretion of testosterone from testis into blood. This may well require a very high local availability of testosterone.

It is commonly believed that testosterone and other steroids freely diffuse throughout the testis. A percutaneous testicular aspiration study in fertile men revealed that the testosterone concentration exceeded that of SHBG/ABP by about 200-fold (Jarow *et al.* 2001). Hence, a substantial surplus of testosterone exists within the male gonad. On the other hand, in genetically engineered mice lacking ganglioside synthetase, spermatogenesis was altered and Sertoli cells showed vacuolization (Takamiya *et al.* 1998). This enzyme deficiency prevented testosterone transport within the testis. It is conceivable that an active transport mechanism for androgens operates within the testis and plays a role in the regulation of the spermatogenic process.

Testosterone is metabolized to DHT by testicular 5α-reductase activity. It is unclear at present to what extent testicular 5α-reduction of testosterone is relevant for spermatogenesis. DHT can stimulate spermatogenesis even quantitatively in mice (Singh *et al.* 1995) and rat (Chen *et al.* 1994). The latter study suggested that DHT is more potent than testosterone for stimulation of spermatogenesis. On

the other hand, finasteride treatment did not alter spermatogenesis in volunteers (Kinniburgh *et al.* 2001; Overstreet *et al.* 1999). Also, administration of finasteride to immature and adult rats had no effect on testes weights and spermatogenesis (Rhoden *et al.* 2002).

Testosterone within the testis is aromatized to estradiol. Whether estradiol directly regulates spermatogenesis is unknown. The occurrence of testicular atrophy in estradiol receptor-deficient mice has been attributed to back-pressure effects of lack of fluid resorption in the efferent ducts (Hess *et al.* 1997). Aromatase activity is present in Sertoli cells but has also been found in germ cells (spermatocytes, spermatids and sperm) (Carreau *et al.* 2001). The use of aromatase inhibitors has not resulted in clear suppression of spermatogenesis (Turner *et al.* 2000) except in the studies by Shetty *et al.* (1997; 1998) in bonnet monkeys. This group reported impaired spermiogenesis and altered sperm chromatin condensation on the basis of flow cytometric analysis. Mice bearing lack of estrogen receptor expression or aromatase expression or overexpression of aromatase (Murata *et al.* 2002) display various degrees of spermatogenic disturbance. Clinically, isolated cases with estrogen receptor deficiency or aromatase deficiency did not provide clues as to whether spermatogenesis is causally affected by estradiol (O'Donnell *et al.* 2001b).

5.5.2 Testicular androgen concentrations and spermatogenesis

The precise quantitative relationship between local testosterone concentrations and spermatogenesis has been the subject of numerous studies and debates (Rommerts 1988; Sharpe *et al.* 1988a; 1988b). It has been suggested that under testosterone alone, approximately 30% of testicular androgen concentrations are needed to quantitatively support spermatogenesis in the rat. In this study testosterone was administered to Leydig cell-depleted rats, thus also demonstrating that testosterone is the only Leydig cell factor relevant for maintenance of spermatogenesis. In a specific experimental setting in which FSH secretion was retained, quantitative maintenance and restoration of spermatogenesis could be achieved with only 10% of normal testicular testosterone levels in the rat model (Rea *et al.* 1986a; 1986b). Generally, however, it has been difficult to establish a linear relationship between testosterone concentrations and the number of germ cells being produced. Several studies related to the issues of testicular androgen concentration and spermatogenesis are available for primates.

In nonhuman primate studies, experimental hypogonadotropism and hypogonadism were induced by administration of supraphysiological amounts of testosterone (Narula *et al.* 2002; Weinbauer *et al.* 2001a; 2001b) or treatment with a GnRH antagonist and testosterone substitution (Weinbauer *et al.* 1988). A clinical contraceptive study is also available in which volunteers were exposed to testosterone alone or testosterone plus DMPA (McLachlan *et al.* 2002b). Study durations were 15–26 weeks in nonhuman primates and 12 weeks in the clinical study. Animals

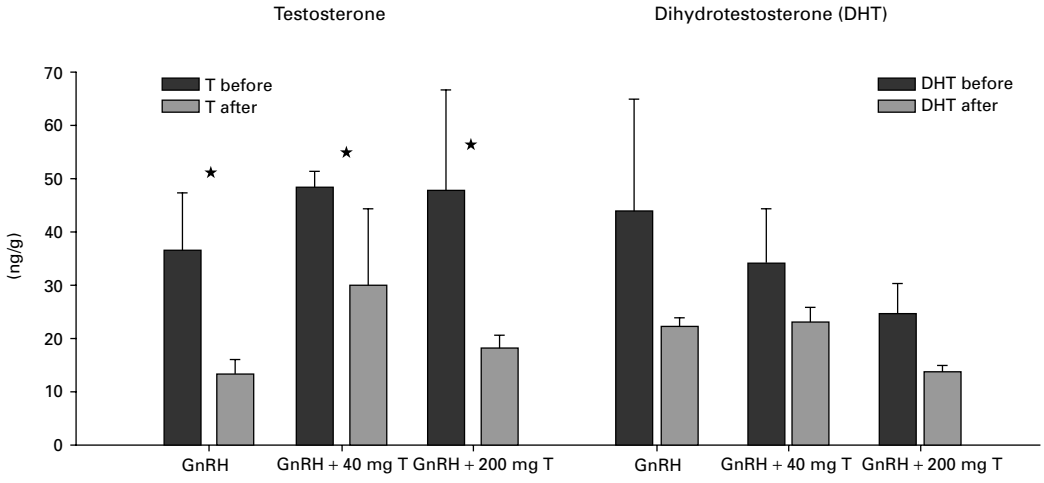


Fig. 5.4 Testicular concentrations of testosterone and DHT in biopsates of cynomolgus monkeys before and after 15 weeks of treatment with an GnRH antagonist (GnRH) alone or in combination with either 40 mg or 200 mg testosterone buciclate (T). Asterisk denotes significant differences compared to baseline (before). Note that DHT levels did not differ significantly before and after treatments although animals treated with GnRH antagonist alone were azoospermic and testosterone-supplemented animals were severely oligozoospermic. Data modified from Weinbauer *et al.* (1988). The observations on DHT have been confirmed in a recent study in gonadotropin-suppressed men (McLachlan *et al.* 2002b).

and subjects had markedly suppressed (bioactive) LH levels and were rendered severely oligozoospermic or azoospermic. All studies yielded a very similar finding, e.g. testicular levels of testosterone/androgen lacked any correlation to either testicular germ-cell numbers or numbers of sperm in the ejaculates. Equally surprising are those observations that the testicular levels of DHT did not differ significantly from control or baseline levels study (Fig. 5.4) (McLachlan *et al.* 2002b; Narula *et al.* 2002; Weinbauer and Nieschlag 1998; Weinbauer *et al.* 1988) nor did the levels of 5α-androstane-3α. In a shorter-term study of 16 and 25 days exposure to GnRH antagonist in cynomolgus monkeys (Zhengwei *et al.* 1998a; 1998b), no significant change of testicular testosterone was seen but a clear reduction of germ cell numbers.

Given the need for testosterone in spermatogenesis, it is unclear at present how severe spermatogenic suppression could be achieved in the above studies albeit having significant amounts of testicular testosterone and unchanged amounts of testicular DHT remaining. It is theoretically possible that sampling of testis tissue and the time elapsed until snap-freeze was a confounding factor (Maddocks and Sharpe 1989). However, if so, this confounding factor is surprisingly reproducible between different laboratories and experiments. It can also be considered that in those studies with testosterone administration, some testosterone diffused into the

testis although this explanation does not hold for the study using GnRH antagonist (Weinbauer *et al.* 1988; 1998). Since the primate testis has a lobular architecture with much connective tissue and several layers of myoid cells surrounding the seminiferous tubule, it might also be that androgens are trapped in the involuting gonad and are locally bound and retained but are biologically inactive.

Certain experimental paradigms of testicular damage exist for rodents in which "high" intratesticular testosterone exerts an inhibitory effect on spermatogonial development and repopulation of seminiferous tubules. 2,5-hexanedione damages Sertoli cells in rats and interrupts spermatogenesis at the spermatogonial level in the majority of tubules. However, suppression of testicular testosterone concentrations using GnRH agonist treatment was associated with spermatogonial activation and repopulation in a large proportion of damaged seminiferous tubules (Boekelheide and Schoenfeld 2001). Studies using gonadal protection approaches in rats also indicated that the suppression of Leydig cell function can advance spermatogenic recovery in the rat model (Meistrich and Shetty 2003). In the juvenile spermatogonial depletion (jsd) mouse in which only one wave of spermatogenesis takes place, followed by sterility, suppression of testicular testosterone initiated spermatogonial activation (Matsumiya *et al.* 1999). In this study administration of a GnRH antagonist further reduced testis size but clearly increased the number of differentiating seminiferous tubules (spermatogonia → spermatocytes).

5.5.3 Testicular androgen receptor and sites of androgen action

The testicular androgen receptor is only expressed in somatic cells (Fig. 5.5). Sertoli cells, Leydig cells and peritubular cells have been shown to express the androgen receptor (van Roijen *et al.* 1995). Whether androgen receptor is really present in some rat elongating spermatids has remained enigmatic (Vornberger *et al.* 1994). It is assumed that the stimulatory effects of testosterone upon spermatogenesis are indirect and mediated via somatic cells. Alternatively it has also been suggested that testosterone can act directly upon germ cells and is transported into these cells by androgen binding protein (ABP). Observations that rats made transgenic for ABP and expressing high testicular androgen levels, have disturbed spermatogenesis (Joseph *et al.* 1997a; 1997b; Larriba *et al.* 1995) would suggest active testosterone could be bound and inactivated by high ABP levels (Fig. 5.5).

Studies in nonhuman primates revealed that testosterone induced the appearance of α-smooth muscle actin in the peritubular cells of the testis (Schlatt *et al.* 1993). This observation strongly suggests that testosterone initiates the contractile function of these cells. These data suggest a very specific effect of testosterone on the differentiation of testicular primate cells. In these studies it was also observed that testosterone but not FSH induced an "adult-type" actin distribution in the Sertoli cells (Schlatt *et al.* 1995). Generally, testosterone appears to be a differentiation

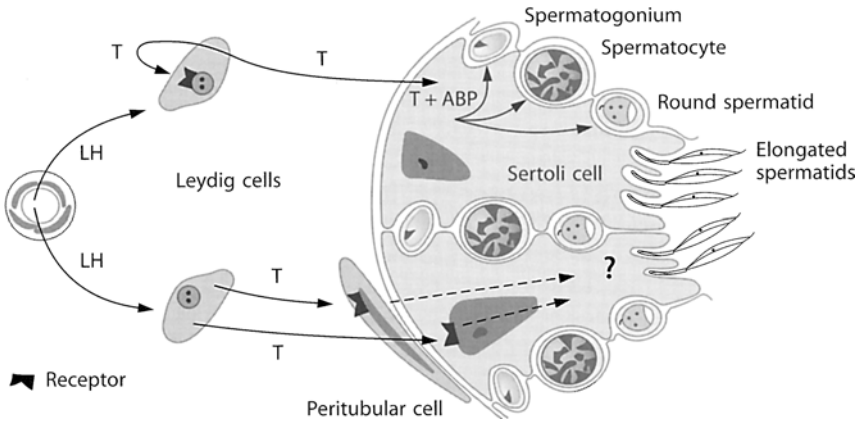


Fig. 5.5 Testicular production, distribution and action of testosterone (T). Testosterone is produced in Leydig cells and acts on specific receptors on Leydig cells, peritubular cells and Sertoli cells. Germ cells lack androgen receptors. Once inside the seminiferous tubule, testosterone can be bound to androgen binding protein (ABP) for further transport. The Testosterone/ABP complex has been reported to be internalized by germ cells. Within the seminiferous tubules testosterone is metabolised into DHT and estradiol. Whether these conversions of testosterone are essential for spermatogenesis is not entirely clear. Although it is reasonable to assume that testosterone induced the formation and secretion of essential prospermatogenic factors in peritubular cells and Sertoli cells, the nature of these factors is still to be discovered.

factor for somatic testicular cells. Differentiation of Leydig cells from fibroblast precursors is also under LH/androgen control (Teerds *et al.* 1989). In the rat model, testosterone is also involved in Sertoli cell-spermatid adhesion (McLachlan *et al.* 2002b for review).

The expression of the androgen receptor varies in relation to the spermatogenic stage of spermatogenesis. In the rat, highest expression and also highest levels of this androgenic steroid were observed in spermatogenic stages VII and VIII (Bremner *et al.* 1994). In these stages the spermatids finally elongate. Cytometric data for human testis suggest a peak of androgen protein expression immediately after the final stage of spermatid maturation (stage III) (Suarez-Quian *et al.* 1999). The expression of testicular androgen receptor is under the control of both testosterone and FSH.

5.6 Follicle-stimulating hormone (FSH) and spermatogenesis

5.6.1 FSH receptor and sites of FSH action

Remarkably, the expression of the FSH receptor appears to be restricted to Sertoli cells only. Previous reports on spermatogonial expression of FSH receptor have not been confirmed to date. In all likelihood, FSH also acts on spermatogenesis

indirectly via a single somatic cell type. Testicular FSH receptor expression in the rat is highest in stages XII-II (Heckert and Griswold 2002; Rannikko *et al.* 1996), e.g. when A-type spermatogonia develop further, first spermatocytes appear and when the second meiotic division is being completed. In the human testis, FSH receptor expression could not be clearly associated with a particular spermatogenic stage(s) but was unequivocally confined to Sertoli cells (Bockers *et al.* 1994; Vannier *et al.* 1996). Alternative splicing generates several FSH receptor transcripts and several FSH isoforms circulate but the relevance of this to spermatogenesis is not yet clear (Simoni *et al.* 2002; Ulloa-Aguirre *et al.* 2003). Despite substantial investigational efforts it has remained a mystery why FSH receptor expression is so cell-specific.

5.6.2 FSH dependence of spermatogenesis

Observations that testosterone alone in some studies could quantitatively maintain or reinitiate spermatogenesis in the adult rat model prompted the question of whether there is a need for FSH in spermatogenesis (Zirkin *et al.* 1994). This question received support by reports that spermatogenesis continued at least in qualitatively normal manner and fertility was retained in FSH-β-subunit deficient mice (Kumar *et al.* 1997). On the other hand, spermatogenesis and spermatid maturation are affected in FSH-receptor deficient mice (Krishnamurthy *et al.* 2000a; 2000b). Stereological analysis in these mice yielded a clear-cut reduction of numbers of Sertoi cells and germ cells (Wreford *et al.* 2001). Conversely, immunization against FSH or its receptor interfered with spermatogenesis in the rat (Graf *et al.* 1997) and inhibition of both FSH and LH induced a more pronounced inhibition of spermatogenesis than inhibition of LH alone (McLachlan *et al.* 2002a; 2002b). To date, a role for FSH in immature and adult rat spermatogenesis is unequivocally established (El Shennawy *et al.* 1998). How critical FSH is for spermatogenesis has also been clearly demonstrated in primate studies. Patients with selective inactivating mutations of the FSH-β-subunit exhibit hypogonadism and can be azoospermic (Lindstedt *et al.* 1998; Phillip *et al.* 1998). Immunization studies against FSH in rhesus monkeys and in bonnet monkey provoked marked testicular involution and even infertility (Moudgal *et al.* 1997a; 1997b; Nieschlag *et al.* 1999). In a hypophysectomized man bearing an activating mutation of the FSH receptor, spermatogenesis was sustained (Gromoll *et al.* 1996).

In the GnRH antagonist-treated nonhuman primate model, human FSH was able to qualitatively maintain and restore spermatogenesis (Weinbauer *et al.* 1991). Several studies also suggest that FSH may be influence the quality of sperm, i.e. chromatin condensation, in primate testis (Moudgal and Sairam 1998). Several clinical studies in volunteers for endocrine male contraception also point to a particular FSH dependence of human spermatogenesis (see Chapter 23). In general, incomplete suppression of FSH secretion prevented the achievement of azoospermia.

Unlike for LH, there seems to be a need to further increase the sensitivity of FSH assays in order to be able to assess whether FSH has been completely suppressed or not in clinical studies (Robertson *et al.* 2001). Corroborating findings for a essential role of FSH were also obtained in similar studies in cynomolgus monkeys (Narula *et al.* 2002; Weinbauer *et al.* 2001b). In the latter study, an escape of FSH but not bioactive LH secretion during testosterone-induced suppression was followed promptly by an increase of inhibin B levels, testis size and sperm number. In the former study, levels of bioactive FSH correlated with the induction of oligozoospermia and azoospermia.

Whether FSH alone can initiate complete spermatogenesis during pubertal development is not entirely clear. FSH unequivocally stimulated testicular cell numbers in the species studied. In the rhesus monkey, administration of human FSH preparations clearly increased spermatogonial and Sertoli cell numbers, and the development of some spermatocytes (Arslan *et al.* 1993; Ramaswamy *et al.* 1998; Schlatt *et al.* 1995). However, data on treating animals throughout a prolonged period of several weeks or months with species-specific FSH are lacking owing to insufficient amounts of appropriate preparations. In mice overexpressing either human FSH (Haywood *et al.* 2003) or human FSH receptor (Haywood *et al.* 2002) the spermatogenic process was initiated including the formation of postmeiotic cells and some degree of spermatid elongation. However, the complete spermatogenic process was not initiated.

5.7 Endocrine control of spermatogenesis

5.7.1 Synergistic and differential action of androgens and FSH on testicular functions

It can be stated safely that the combination of androgen and FSH consistently produced a better stimulatory effect on spermatogenesis than either factor alone. What might represent a functional distinction between testosterone and FSH is the fact that testosterone induces differentiation of somatic cells in the immature primate testis (Schlatt *et al.* 1993). This does not seem to be the case for FSH. Hence, developmentally, testosterone may have some primacy in providing somatic testis cell differentiation and maturation prior to FSH action. This view is supported by observations in rats, that elimination of Leydig cells by ethane dimethane sulphonate or prevention of androgen action by flutamide significantly reduced the stimulatory effects of FSH on spermatogenesis (Chandolia *et al.* 1991), i.e. FSH testicular action is improved by prior androgen exposure. Hence, androgens might be – in relative terms – the predominant differentiation factor and FSH the predominant proliferative factor (Schlatt *et al.* 1995).

In primates, testosterone and FSH act to stimulate spermatogonial multiplication (Figs 5.6 and 5.7). Testosterone was suggested to be responsible for providing

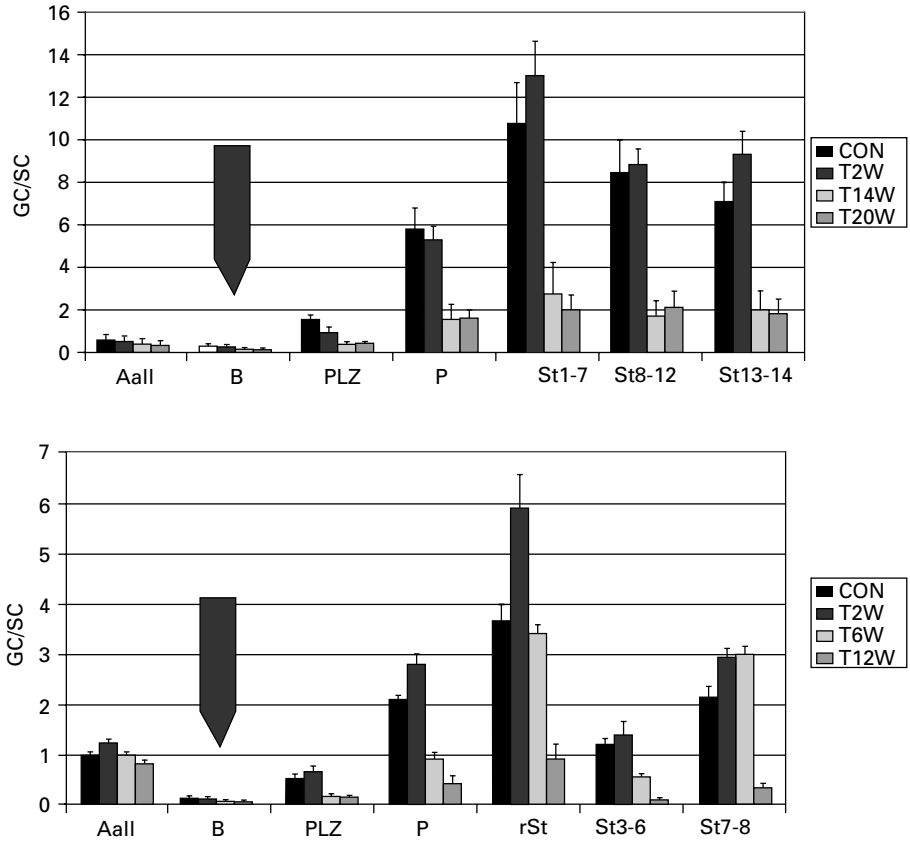


Fig. 5.6 Testicular germ cell numbers (expressed per Sertoli cell) in cynomolgus monkeys (upper panel) and healthy volunteers (lower panel) during steroid-induced suppression of gonadotropin secretion. Note the marked reduction of numbers of type-B spermatogonia. Numbers of type A-pale spermatogonia are also reduced but not those of type A-dark spermatogonia (not shown). Data taken from O'Donnell *et al.* (2001a) and McLachlan *et al.* (2002b).

adequate numbers of renewing A-type spermatogonia and FSH – on top of androgen action – ensures quantitatively normal production of differentiated B-type spermatogonia (Marshall *et al.* 1995). According to other studies in adult (O'Donnell *et al.* 2001b; van Alphen *et al.* 1988; Weinbauer *et al.* 1991) and immature nonhuman primates (Arslan *et al.* 1993; Schlatt *et al.* 1995) FSH also stimulated the number of renewing A-type spermatogonia. It is interesting to note that FSH also stimulates renewing A-type spermatogonial numbers and subsequent germ cell populations in normal and mature cynomolgus and rhesus monkeys (van Alphen *et al.* 1988), whereas this is not the case for hCG (Teerds *et al.* 1989a). In the immature monkey,

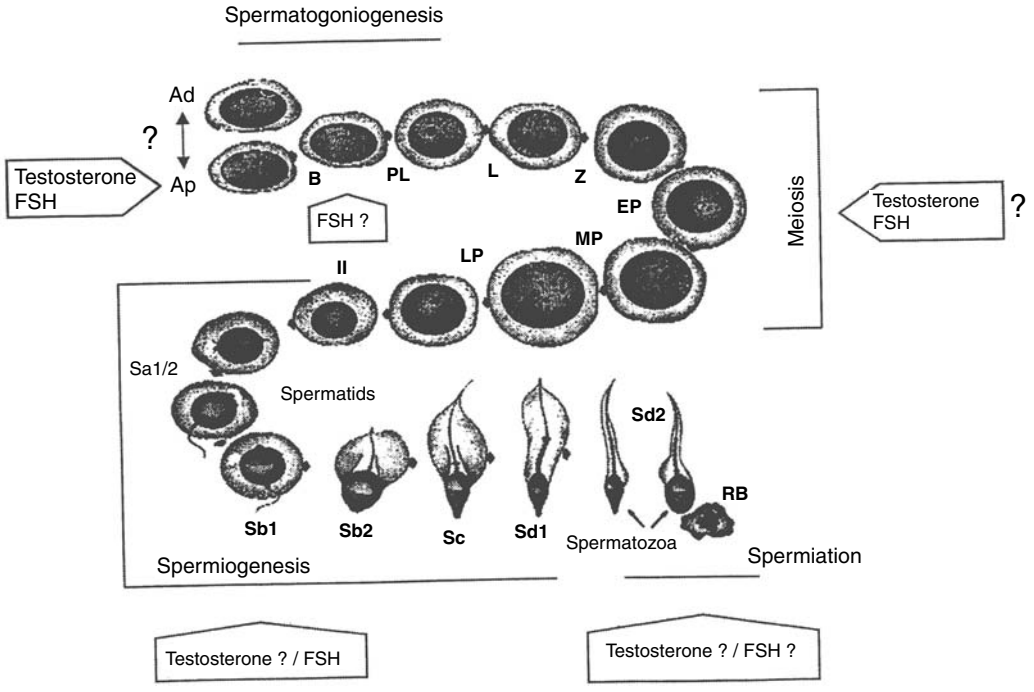


Fig. 5.7 Sites of action of testosterone and FSH on the spermatogenic process in primates. The precise interrelationships between type A-pale and type A-dark spermatogonia are not entirely clear. The majority of available data indicated that testosterone and FSH stimulate spermatogenesis via increasing numbers of type A-pale spermatogonia followed by in increase of subsequent germ cell populations. Meiotic transitions appear to be independent of testosterone/FSH action. FSH has been reported to play a role in chromatin condensation during spermiogenesis. Whether testosterone is needed for spermiogenesis has not been studied yet. Spermiation is affected by gonadotropin sufficiency but it is currently unclear whether this is related to diminished actions of testosterone or FSH or both.

testosterone also significantly increased the numbers of A_p-type spermatogonia (Schlatt *et al.* 1993).

Spermiogenesis regulation has been either linked to androgens (McLachlan *et al.* 2002a; 2002) or to FSH (Krishnamurthy *et al.* 2000a; 2000b; Moudgal and Sairam 1998) whereas spermiation seems to be dependant on both testosterone and FSH. The progression of spermatocytes into spermatids may be independent of LH/FSH as indicated by kinetic studies in gonadotropin-deficient cynomolgus monkeys using 5-bromodeoxyuridine incorporation (Aslam *et al.* 1999; Weinbauer *et al.* 1998).

Apart from stimulating cell numbers via cell multiplication, testosterone and FSH also promote germ cell survival. This was clearly demonstrated in the

gonadotropin-deficient rat (El Shennawy *et al.* 1998). Both testosterone and FSH were able to attenuate germ cell loss and were the most effective combination. Since germ cell loss in the hormone-deficient testes also involves apoptosis (Sinha Hikim *et al.* 1997), it can be speculated that both hormones interfere with apoptotic pathways.

5.8 Androgens and FSH: is there primacy for spermatogenesis?

Similar to testosterone, FSH is also able to initiate, maintain and reinitiate qualitatively normal spermatogenesis. This should not be surprising since both hormones act via the same intratubular cell, the Sertoli cell. The evidence is compelling for a synergistic and additive effect of testosterone plus FSH on spermatogenesis and germ cell development. These findings suggest that testosterone and FSH – at least to some extent – stimulate different factors and processes. If the mechanism of action were identical for testosterone and FSH, a competitive rather than an additive effect on spermatogenesis would be expected. Therefore, a primacy of adult spermatogenesis for testosterone or FSH does not exist, rather a true synergism prevails.

5.9 Clinical relevance of animal models for the study of androgen actions

A comprehensive set of data is available to indicate that the cynomolgus monkey model is highly representative for preclinical studies on the endocrine regulation of spermatogenesis. Unlike the rhesus monkey, bonnet monkey or Japanese macaque, this nonhuman primate species does not show annual variations of testicular activity. A series of experimental studies is available and has been reviewed above. The findings obtained clearly prove that the data collected in the cynomolgus monkey on the LH/testosterone and FSH regulation of spermatogenesis are fully representative for what would be seen in man. This relationship holds even true for quantitative aspects of spermatogenesis. Hence, at present, the cynomolgus monkey is recommended as preclinical model for the study of the relationship between testosterone and other reproductive hormones and human spermatogenesis.

The rat model provides an interesting feature regarding the effects of selective testosterone replacement. In the gonadotropin-suppressed rat testosterone paradoxically stimulates the levels of FSH. Studies showed that testosterone maintains and restores the levels of immunoactive and bioactive FSH by direct action on pituitary FSH expression and also release. This is in sharp contrast to gonadotropin-suppressed nonhuman primates and men, in whom testosterone does not alter FSH secretion at all. Hence the rat model has only limited suitability for studying the relative roles of LH/testosterone and FSH for spermatogenesis.

Targeted disruption of FSH or the FSH receptor was initially thought not to interfere with spermatogenesis in mice (Kumar *et al.* 1997). Whether this really indicates that FSH is dispensable for mouse spermatogenesis remains to be seen. This view is derived from the fact that FSH receptor is only expressed in Sertoli cells and, hence, such specific expression pattern would imply a physiological role. More recently, it has become clear that FSH action is also important for spermatogenesis in the mouse (Huhtaniemi 2000; Sairam and Krishnamurthy 2001, and discussion above).

Another interesting exception to the endocrine control of primate spermatogenesis is provided by the seasonal control of reproduction in the Djungarian hamster. This species is a short-day breed undergoing testicular involution when the LD light regimen is shifted from 16:8 to 8:16. In this species administration of LH to animals with regressed testes is unable to restore spermatogenesis, whereas FSH reinitiates the entire process including the production of fertile sperm (Lerchl *et al.* 1993; Niklowitz *et al.* 1997). It is not entirely clear whether this separation of LH and FSH sensitivity of the involuted testis is confined to this hamster species or applies more generally to seasonally breeding mammals. In the prairie dog (*Cynomys ludovicanus*) also FSH but not LH/testosterone induced germ cell activation when administered during the seasonal involution phase (Foreman 1998). Interestingly, in the seasonal bonnet monkey, merely blocking the nocturnal peak of testosterone secretion is sufficient to inhibit spermatogenesis, implying a surprising dependence of adult spermatogenesis on diurnal testosterone in a primate. In general, however, using seasonal experimental species as models for the endocrine control of human spermatogenesis does not seem advisable in view of the fact that a highly relevant non-seasonal primate model (cynomolgus monkey) is available.

An alternative route of stimulating testosterone production from Leydig cells has apparently developed in the common marmoset (*Callithrix jacchus*). In the intact and normal marmoset, LH receptor exon 10, although genomically present, is not expressed (Zhang *et al.* 1997). For the human LH receptor, this exon is necessary for the expression of receptor protein (Zhang *et al.* 1998). Interestingly, a clinical case lacking LH receptor exon 10 has been described (Gromoll *et al.* 2000). This boy had developed a male phenotype but presented with retarded pubertal development, small testes and delayed bone maturation, all indicative of androgen deficiency. Given the similarity to marmoset LH receptor status, this patient was successfully treated with hCG, indicating that exon 10 is involved in differential LH/hCG recognition. More recently it was found that marmoset pituitary expresses hCG (Gromoll *et al.* 2003) raising the possibility that marmoset Leydig cells are driven by hCG rather than LH. It would be interesting to know the dependence of marmoset spermatogenesis on intratesticular testosterone. In any case, it is obvious that the control of Leydig cell function and testosterone production by LH is entirely different in

humans and marmosets. Equally interesting, lack of LH receptor exon 10 expression was also found in Cebidae and other Callithrichidae, raising the question whether this particular LH receptor type II could be predominant in neotropical primate species.

5.10 Key messages

- Testosterone is able to qualitatively initiate, maintain and re-initiate spermatogenesis and formation of spermatozoa.
- FSH is able to qualitatively initiate, maintain and re-initiate spermatogenesis and formation of spermatozoa.
- Under physiological circumstances, only the combination of testosterone and FSH yields quantitatively normal germ cell numbers.
- Adult primate spermatogenesis is probably more dependant on FSH than on testosterone.
- Testosterone acts indirectly via somatic testicular cells on spermatogenesis. Whether ABP or non-genomic actions mediate direct testosterone effects on germ cells is unclear.
- The testicular effects of testosterone are mediated via DHT and estradiol. Whether estradiol is essential for spermatogenesis is still unclear.
- Testosterone and FSH cooperate during regulation of spermatogenesis but act via different mechanisms.
- Macaques currently represent the most appropriate preclinical model for the study of the role of testosterone in spermatogenesis.

5.11 REFERENCES

Alastalo TP, Lonnstrom M, Leppa S, Kaarniranta K, Pelto-Huikko M, Sistonen L, Parvinen M (1998) Stage-specific expression and cellular localization of the heat shock factor 2 isoforms in the rat seminiferous epithelium. Exp Cell Res 240:16–27

Arslan M, Weinbauer GF, Schlatt S, Shahab M, Nieschlag E (1993) FSH and testosterone, alone or in combination, initiate testicular growth and increase the number of spermatogonia and Sertoli cells in a juvenile non-human primate (*Macaca mulatta*). J Endocrinol 136:235–243

Aslam H, Rosiepen G, Krishnamurthy H, Arslan M, Clemen G, Nieschlag E, Weinbauer GF (1999) The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys. J Endocrinol 161:281–288

Assinder SJ, Rezvani A, Nicholson HD (2002) Oxytocin promotes spermiation and sperm transfer in the mouse. Int J Androl 25:19–27

Awoniyi CA, Zirkin BR, Chandrashekar V, Schlaff WD (1992) Exogenously administered testosterone maintains spermatogenesis quantitatively in adult rats actively immunized against gonadotropin-releasing hormone. Endocrinology 130:3283–3288

Bartlett JM, Charlton HM, Robinson IC, Nieschlag E (1990a) Pubertal development and testicular function in the male growth hormone-deficient rat. J Endocrinol 126:193–201

Bartlett JM, Weinbauer GF, Nieschlag E (1990b) Stability of spermatogenic synchronization achieved by depletion and restoration of vitamin A in rats. Biol Reprod 42:603–612

Behre HM, Nieschlag E, Meschede D, Partsch CJ (2000) Diseases of the hypothalamus and the pituitary gland. In: Andrology – Male reproductive health and dysfunction. Nieschlag E, Behre HM (eds) Springer, Berlin, pp 125–143

Bockers TM, Nieschlag E, Kreutz MR, Bergmann M (1994) Localization of follicle-stimulating hormone (FSH) immunoreactivity and hormone receptor mRNA in testicular tissue of infertile men. Cell Tissue Res 278:595–600

Boekelheide K, Schoenfeld HA (2001) Spermatogenesis by Sisyphus: proliferating stem germ cells fail to repopulate the testis after 'irreversible' injury. Adv Exp Med Biol 500:421–428

Boekelheide K, Fleming SL, Allio T, Embree-Ku ME, Hall SJ, Johnson KJ, Kwon EJ, Patel SR, Rasoulpour RJ, Schoenfeld HA, Thompson S (2003) 2,5-hexanedione-induced testicular injury. Annu Rev Pharmacol Toxicol 43:125–147

Bremner WJ, Millar MR, Sharpe RM, Saunders PT (1994) Immunohistochemical localization of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. Endocrinology 135:1227–1234

Brown GR, Nevison CM, Fraser HM, Dixson AF (1999) Manipulation of postnatal testosterone levels affects phallic and clitoral development in infant rhesus monkeys. Int J Androl 22: 119–128

Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Bilinska B, Benahmed M, Levallet J (2001) Aromatase expression in male germ cells. J Steroid Biochem Mol Biol 79: 203–208

Chandolia RK, Weinbauer GF, Fingscheidt U, Bartlett JM, Nieschlag E (1991) Effects of flutamide on testicular involution induced by an antagonist of gonadotrophin-releasing hormone and on stimulation of spermatogenesis by follicle-stimulating hormone in rats. J Reprod Fertil 93:313–323

Chen H, Chandrashekar V, Zirkin BR (1994) Can spermatogenesis be maintained quantitatively in intact adult rats with exogenously administered dihydrotestosterone? J Androl 15:132–138

Clermont Y (1969) The cycle of the seminiferous epithelium in man. Am J Anat 112:35–46

Clermont Y (1972) Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 52:198–236

Clermont Y, Leblond CP (1955) Spermatogenesis of man, monkey, ram and other mammals as shown by the "periodic acid-schiff" technique. Am J Anat 96:229–253

Clouthier DE, Avarbock MR, Maika SD, Hammer RE, Brinster RL (1996) Rat spermatogenesis in mouse testis. Nature 381:418–421

Cunningham GR, Huckins C (1979) Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. Endocrinology 105:177–186

Dankbar B, Brinkworth MH, Schlatt S, Weinbauer GF, Nieschlag E, Gromoll J (1995) Ubiquitous expression of the androgen receptor and testis-specific expression of the FSH receptor in the cynomolgus monkey (*Macaca fascicularis*) revealed by a ribonuclease protection assay. J Steroid Biochem Mol Biol 55:35–41

de Franca LR, Hess RA, Cooke PS, Russell LD (1995) Neonatal hypothyroidism causes delayed Sertoli cell maturation in rats treated with propylthiouracil: evidence that the Sertoli cell controls testis growth. Anat Rec 242:57–69

de Kretser DM, Phillips DJ (1998) Mechanisms of protein feedback on gonadotropin secretion. J Reprod Immunol 39:1–12

de Rooij DG, Grootegoed JA (1998) Spermatogonial stem cells. Curr Opin Cell Biol 10:694–701

de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E (1997) A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 337:1597–1602

Depenbusch M, von Eckardstein S, Simoni M, Nieschlag E (2002) Maintenance of spermatogenesis in hypogonadotropic hypogonadal men with human chorionic gonadotropin alone. Eur J Endocrinol 147:617–624

Dietrich T, Schulze W, Riemer M (1986) Classification of the germinal epithelium in Java monkeys (*Macaca cynomolgus*) using digital image processing. Urologe [A] 25:179–186

Ebling FJ, Brooks AN, Cronin AS, Ford H, Kerr JB (2000) Estrogenic induction of spermatogenesis in the hypogonadal mouse. Endocrinology 141:2861–2869

El Shennawy A, Gates RJ, Russell LD (1998) Hormonal regulation of spermatogenesis in the hypophysectomized rat: cell viability after hormonal replacement in adults after intermediate periods of hypophysectomy. J Androl 19:320–334; discussion 341–342

Ergun S, Harneit S, Paust HJ, Mukhopadhyay AK, Holstein AF (1998) Endothelin and endothelin receptors A and B in the human testis. Anat Embryol (Berl) 199:207–214

Fingscheidt U, Weinbauer GF, Fehm HL, Nieschlag E (1998) Regulation of gonadotrophin secretion by inhibin, testosterone and gonadotrophin-releasing hormone in pituitary cell cultures of male monkeys. J Endocrinol 159:103–110

Foreman D (1998) Effects of exogenous hormones on spermatogenesis in the male prairie dog (*Cynomys ludovicianus*). Anat Rec 250:45–61

Franca LR, Ogawa T, Avarbock MR, Brinster RL, Russell LD (1998) Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod 59:1371–1377

Graf KM, Dias JA, Griswold MD (1997) Decreased spermatogenesis as the result of an induced autoimmune reaction directed against the gonadotropin receptors in male rats. J Androl 18:174–185

Gromoll J, Simoni M, Nieschlag E (1996) An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab 81:1367–1370

Gromoll J, Partsch CJ, Simoni M, Nordhoff V, Sippell WG, Nieschlag E, Saxena BB (1998) A mutation in the first transmembrane domain of the lutropin receptor causes male precocious puberty. J Clin Endocrinol Metab 83:476–480

Gromoll J, Eiholzer U, Nieschlag E, Simoni M (2000) Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. J Clin Endocrinol Metab 85:2281–2286

Gromoll J, Wistuba J, Terwort N, Godmann M, Muller T, Simoni M (2003) A new subclass of the luteinizing hormone/chorionic gonadotropin receptor lacking exon 10 messenger RNA in the New World monkey (*Platyrrhini*) lineage. Biol Reprod 69:75–80

Guo Q, Kumar TR, Woodruff T, Hadsell LA, DeMayo FJ, Matzuk MM (1998) Overexpression of mouse follistatin causes reproductive defects in transgenic mice. Mol Endocrinol 12:96–106

Harris GC, Nicholson HD (1998) Stage-related differences in rat seminiferous tubule contractility in vitro and their response to oxytocin. J Endocrinol 157:251–257

Haywood M, Tymchenko N, Spaliviero J, Koch A, Jimenez M, Gromoll J, Simoni M, Nordhoff V, Handelsman DJ, Allan CM (2002) An activated human follicle-stimulating hormone (FSH) receptor stimulates FSH-like activity in gonadotropin-deficient transgenic mice. Mol Endocrinol 16:2582–2591

Haywood M, Spaliviero J, Jimemez M, King NJ, Handelsman DJ, Allan CM (2003) Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. Endocrinology 144:509–517

Heckert LL, Griswold MD (2002) The expression of the follicle-stimulating hormone receptor in spermatogenesis. Recent Prog Horm Res 57:129–148

Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS, Lubahn DB (1997) A role for oestrogens in the male reproductive system. Nature 390:509–512

Holstein AF, Maekawa M, Nagano T, Davidoff MS (1996) Myofibroblasts in the lamina propria of human semi-niferous tubules are dynamic structures of heterogeneous phenotype. Arch Histol Cytol 59:109–125

Huang HF, Nieschlag E (1986) Suppression of the intratesticular testosterone is associated with quantitative changes in spermatogonial populations in intact adult rats. Endocrinology 118:619–627

Huhtaniemi I (2000) The Parkes lecture. Mutations of gonadotrophin and gonadotrophin receptor genes: what do they teach us about reproductive physiology? J Reprod Fertil 119:173–186

Jarow JP, Chen H, Rosner TW, Trentacoste S, Zirkint BR (2001) Assessment of the androgen environment within the human testis: minimally invasive method to obtain intratesticular fluid. J Androl 22:640–645

Jegou B (1993) The Sertoli-germ cell communication network in mammals. Int Rev Cytol 147: 25–96

Johnsen SG (1978) Maintenance of spermatogenesis induced by HMG treatment by means of continuous HCG treatment in hypogonadotrophic men. Acta Endocrinol (Copenh) 89: 763–769

Johnson L, McKenzie KS, Snell JR (1996) Partial wave in human seminiferous tubules appears to be a random occurrence. Tissue Cell 28:127–136

Joseph DR, O'Brien DA, Sullivan PM, Becchis M, Tsuruta JK, Petrusz P (1997a) Overexpression of androgen-binding protein/sex hormone-binding globulin in male transgenic mice: tissue distribution and phenotypic disorders. Biol Reprod 56:21–32

Joseph DR, Power SG, Petrusz P (1997b) Expression and distribution of androgen-binding protein/sex hormone-binding globulin in the female rodent reproductive system. Biol Reprod 56:14–20

Kinniburgh D, Anderson RA, Baird DT (2001) Suppression of spermatogenesis with desogestrel and testosterone pellets is not enhanced by addition of finasteride. J Androl 22:88–95

Kolho KL, Huhtaniemi I (1989a) Neonatal treatment of male rats with a gonadotropin-releasing hormone antagonist results in altered function of the pituitary-testicular axis in adult age. Biol Reprod 41:1084–1090

Kolho KL, Huhtaniemi I (1989b) Suppression of pituitary-testis function in rats treated neonatally with a gonadotrophin-releasing hormone agonist and antagonist: acute and long-term effects. J Endocrinol 123:83–91

Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, van Reen M, Milgrom E, Ropers HH, Mariman E (1995) Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. Nat Genet 9:160–164

Krishnamurthy H, Danilovich N, Morales CR, Sairam MR (2000a) Qualitative and quantitative decline in spermatogenesis of the follicle-stimulating hormone receptor knockout (FORKO) mouse. Biol Reprod 62:1146–1159

Krishnamurthy H, Kumar KM, Joshi CV, Krishnamurthy HN, Moudgal RN, Sairam MR (2000b) Alterations in sperm characteristics of follicle-stimulating hormone (FSH)-immunized men are similar to those of FSH-deprived infertile male bonnet monkeys. J Androl 21:316–327

Krishnamurthy H, Kats R, Danilovich N, Javeshghani D, Sairam MR (2001) Intercellular communication between Sertoli cells and Leydig cells in the absence of follicle-stimulating hormone-receptor signaling. Biol Reprod 65:1201–1207

Kula K (1988) Induction of precocious maturation of spermatogenesis in infant rats by human menopausal gonadotropin and inhibition by simultaneous administration of gonadotropins and testosterone. Endocrinology 122:34–39

Kumar TR, Wang Y, Lu N, Matzuk MM (1997) Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 15:201–204

Larriba S, Esteban C, Toran N, Gerard A, Audi L, Gerard H, Reventos J (1995) Androgen binding protein is tissue-specifically expressed and biologically active in transgenic mice. J Steroid Biochem Mol Biol 53:573–578

Lerchl A, Sotiriadou S, Behre HM, Pierce J, Weinbauer GF, Kliesch S, Nieschlag E (1993) Restoration of spermatogenesis by follicle-stimulating hormone despite low intratesticular testosterone in photoinhibited hypogonadotropic Djungarian hamsters (*Phodopus sungorus*). Biol Reprod 49:1108–1116

Lindstedt G, Nystrom E, Matthews C, Ernest I, Janson PO, Chatterjee K (1998) Follitropin (FSH) deficiency in an infertile male due to FSH beta gene mutation. A syndrome of normal puberty and virilization but underdeveloped testicles with azoospermia, low FSH but high lutropin and normal serum testosterone concentrations. Clin Chem Lab Med 36:663–665

Liu PY, Gebski VJ, Turner L, Conway AJ, Wishart SM, Handelsman DJ (2002) Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. Hum Reprod 17:625–633

Lunn SF, Recio R, Morris K, Fraser HM (1994) Blockade of the neonatal rise in testosterone by a gonadotrophin-releasing hormone antagonist: effects on timing of puberty and sexual behaviour in the male marmoset monkey. J Endocrinol 141:439–447

Lunn SF, Cowen GM, Fraser HM (1997) Blockade of the neonatal increase in testosterone by a GnRH antagonist: the free androgen index, reproductive capacity and postmortem findings in the male marmoset monkey. J Endocrinol 154:125–131

Maddocks S, Sharpe RM (1989) Dynamics of testosterone secretion by the rat testis: implications for measurement of the intratesticular levels of testosterone. J Endocrinol 122: 323–329

Mann DR and Fraser HM (1996) The neonatal period: a critical interval in male primate development. J Endocrinol 149:191–197

Mann DR, Akinbami MA, Gould KG, Tanner JM, Wallen K (1993) Neonatal treatment of male monkeys with a gonadotropin-releasing hormone agonist alters differentiation of central nervous system centers that regulate sexual and skeletal development. J Clin Endocrinol Metab 76:1319–1324

Mann DR, Akinbami MA, Gould KG, Paul K, Wallen K (1998) Sexual maturation in male rhesus monkeys: importance of neonatal testosterone exposure and social rank. J Endocrinol 156: 493–501

Marshall GR, Wickings EJ, Nieschlag E (1984) Testosterone can initiate spermatogenesis in an immature nonhuman primate, Macaca fascicularis. Endocrinology 114:2228–2233

Marshall GR, Zorub DS, Plant TM (1995) Follicle-stimulating hormone amplifies the population of differentiated spermatogonia in the hypophysectomized testosterone-replaced adult rhesus monkey (*Macaca mulatta*). Endocrinology 136:3504–3511

Matsumiya K, Meistrich ML, Shetty G, Dohmae K, Tohda A, Okuyama A, Nishimune Y (1999) Stimulation of spermatogonial differentiation in juvenile spermatogonial depletion (jsd) mutant mice by gonadotropin-releasing hormone antagonist treatment. Endocrinology 140:4912–4915

McConnell DS, Wang Q, Sluss PM, Bolf N, Khoury RH, Schneyer AL, Midgley AR, Jr., Reame NE, Crowley WF, Jr., Padmanabhan V (1998) A two-site chemiluminescent assay for activin-free follistatin reveals that most follistatin circulating in men and normal cycling women is in an activin-bound state. J Clin Endocrinol Metab 83:851–858

McKinnell C, Saunders PT, Fraser HM, Kelnar CJ, Kivlin C, Morris KD, Sharpe RM (2001) Comparison of androgen receptor and oestrogen receptor beta immunoexpression in the testes of the common marmoset (Callithrix jacchus) from birth to adulthood: low androgen receptor immunoexpression in Sertoli cells during the neonatal increase in testosterone concentrations. Reproduction 122:419–429

McLachlan RI (2000) The endocrine control of spermatogenesis. Baillieres Best Pract Res Clin Endocrinol Metab 14:345–362

McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, Robertson DM (2002a) Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Horm Res 57:149–179

McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, de Kretser DM, Robertson DM (2002b) Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. J Clin Endocrinol Metab 87:546–556

Meistrich ML, Shetty G (2003) Suppression of testosterone stimulates recovery of spermatogenesis after cancer treatment. Int J Androl 26:141–146

Meistrich ML, van Beek MEAB (1993) Spermatogonial stem cells. In: Desjardins C, Ewing LL (eds) Cell and molecular biology of the testis. Oxford University Press, New York, pp. 266–295

Meriggiola MC, Costantino A, Bremner WJ, Morselli-Labate AM (2002) Higher testosterone dose impairs sperm suppression induced by a combined androgen-progestin regimen. J Androl 23:684–690

Millar MR, Sharpe RM, Weinbauer GF, Fraser HM, Saunders PT (2000) Marmoset spermatogenesis: organizational similarities to the human. Int J Androl 23:266–277

Moudgal NR, Jeyakumar M, Krishnamurthy HN, Sridhar S, Krishnamurthy H, Martin F (1997a) Development of male contraceptive vaccine–a perspective. Hum Reprod Update 3:335–346

Moudgal NR, Sairam MR, Krishnamurthy HN, Sridhar S, Krishnamurthy H, Khan H (1997b) Immunization of male bonnet monkeys (M. radiata) with a recombinant FSH receptor preparation affects testicular function and fertility. Endocrinology 138:3065–3068

Moudgal NR, Sairam MR (1998) Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates? Hum Reprod 13:916–919

Murata Y, Robertson KM, Jones ME, Simpson ER (2002) Effect of estrogen deficiency in the male: the ArKO mouse model. Mol Cell Endocrinol 193:7–12

Narula A, Gu YQ, O'Donnell L, Stanton PG, Robertson DM, McLachlan RI, Bremner WJ (2002) Variability in sperm suppression during testosterone administration to adult monkeys is related to follicle stimulating hormone suppression and not to intratesticular androgens. J Clin Endocrinol Metab 87:3399–3406

Nieschlag E, Simoni M, Gromoll J, Weinbauer GF (1999) Role of FSH in the regulation of spermatogenesis: clinical aspects. Clin Endocrinol (Oxf) 51:139–146

Niklowitz P, Lerchl A, Nieschlag E (1997) In vitro fertilizing capacity of sperm from FSH-treated photoinhibited Djungarian hamsters (Phodopus sungorus). J Endocrinol 154:475–481

O'Donnell L, Narula A, Balourdos G, Gu YQ, Wreford NG, Robertson DM, Bremner WJ, McLachlan RI (2001a) Impairment of spermatogonial development and spermiation after testosterone-induced gonadotropin suppression in adult monkeys (Macaca fascicularis). J Clin Endocrinol Metab 86:1814–1822

O'Donnell L, Robertson KM, Jones ME, Simpson ER (2001b) Estrogen and spermatogenesis. Endocr Rev 22:289–318

Overstreet JW, Fuh VL, Gould J, Howards SS, Lieber MM, Hellstrom W, Shapiro S, Carroll P, Corfman RS, Petrou S, Lewis R, Toth P, Shown T, Roy J, Jarow JP, Bonilla J, Jacobsen CA, Wang DZ, Kaufman KD (1999) Chronic treatment with finasteride daily does not affect spermatogenesis or semen production in young men. J Urol 162:1295–1300

Phillip M, Arbelle JE, Segev Y, Parvari R (1998) Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. N Engl J Med 338:1729–1732

Ramaswamy S, Pohl CR, McNeilly AS, Winters SJ, Plant TM (1998) The time course of follicle-stimulating hormone suppression by recombinant human inhibin A in the adult male rhesus monkey (Macaca mulatta). Endocrinology 139:3409–3415

Rannikko A, Penttila TL, Zhang FP, Toppari J, Parvinen M, Huhtaniemi I (1996) Stage-specific expression of the FSH receptor gene in the prepubertal and adult rat seminiferous epithelium. J Endocrinol 151:29–35

Rea MA, Marshall GR, Weinbauer GF, Nieschlag E (1986a) Testosterone maintains pituitary and serum FSH and spermatogenesis in gonadotrophin-releasing hormone antagonist-suppressed rats. J Endocrinol 108:101–107

Rea MA, Weinbauer GF, Marshall GR, Nieschlag E (1986b) Testosterone stimulates pituitary and serum FSH in GnRH antagonist- suppressed rats. Acta Endocrinol (Copenh) 113:487–492

Ren HP, Russell LD (1991) Clonal development of interconnected germ cells in the rat and its relationship to the segmental and subsegmental organization of spermatogenesis. Am J Anat 192:121–128

Rhoden EL, Gobbi D, Menti E, Rhoden C, Teloken C (2002) Effects of the chronic use of finasteride on testicular weight and spermatogenesis in Wistar rats. BJU Int 89:961–963

Robertson DM, Pruysers E, Stephenson T, Pettersson K, Morton S, McLachlan RI (2001) Sensitive LH and FSH assays for monitoring low serum levels in men undergoing steroidal contraception. Clin Endocrinol (Oxf) 55:331–339

Rommerts FF (1988) How much androgen is required for maintenance of spermatogenesis? J Endocrinol 116:7–9

Rosiepen G, Chapin RE, Weinbauer GF (1995) The duration of the cycle of the seminiferous epithelium is altered by administration of 2,5-hexanedione in the adult Sprague-Dawley rat. J Androl 16:127–135

Russell LD, Brinster RL (1996) Ultrastructural observations of spermatogenesis following transplantation of rat testis cells into mouse seminiferous tubules. J Androl 17:615–627

Sairam MR, Krishnamurthy H (2001) The role of follicle-stimulating hormone in spermatogenesis: lessons from knockout animal models. Arch Med Res 32:601–608

Santiemma V, Rossi F, Guerrini L, Markouizou A, Pasimeni G, Palleschi S, Fabbrini A (2001) Adrenomedullin inhibits the contraction of cultured rat testicular peritubular myoid cells induced by endothelin-1. Biol Reprod 64:619–624

Schlatt S, Weinbauer GF, Arslan M, Nieschlag E (1993) Appearance of alpha-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. J Androl 14:340–350

Schlatt S, Arslan M, Weinbauer GF, Behre HM, Nieschlag E (1995) Endocrine control of testicular somatic and premeiotic germ cell development in the immature testis of the primate *Macaca mulatta*. Eur J Endocrinol 133:235–247

Schulze W, Rehder U (1984) Organization and morphogenesis of the human seminiferous epithelium. Cell Tissue Res 237:395–407

Sharpe RM (1994) Regulation of spermatogenesis. In: Knobil E and Neill JD (eds) The physiology of reproduction. Raven Press, New York, pp 1363–1394

Sharpe RM, Donachie K, Cooper I (1988a) Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. J Endocrinol 117: 19–26

Sharpe RM, Fraser HM, Ratnasooriya WD (1988b) Assessment of the role of Leydig cell products other than testosterone in spermatogenesis and fertility in adult rats. Int J Androl 11:507–523

Sharpe RM, Walker M, Millar MR, Atanassova N, Morris K, McKinnell C, Saunders PT, Fraser HM (2000) Effect of neonatal gonadotropin-releasing hormone antagonist administration on sertoli cell number and testicular development in the marmoset: comparison with the rat. Biol Reprod 62:1685–1693

Shenker A, Laue L, Kosugi S, Merendino JJ, Jr., Minegishi T, Cutler GB, Jr. (1993) A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 365:652–654

Shetty G, Krishnamurthy H, Krishnamurthy HN, Bhatnagar S, Moudgal RN (1997) Effect of estrogen deprivation on the reproductive physiology of male and female primates. J Steroid Biochem Mol Biol 61:157–166

Shetty G, Krishnamurthy H, Krishnamurthy HN, Bhatnagar AS, Moudgal NR (1998) Effect of long-term treatment with aromatase inhibitor on testicular function of adult male bonnet monkeys (*M. radiata*). Steroids 63:414–420

Siiteri JE, Karl AF, Linder CC, Griswold MD (1992) Testicular synchrony: evaluation and analysis of different protocols. Biol Reprod 46:284–289

Simoni M, Nieschlag E, Gromoll J (2002) Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. Hum Reprod Update 8:413–421

Singh J, O'Neill C, Handelsman DJ (1995) Induction of spermatogenesis by androgens in gonadotropin-deficient (hpg) mice. Endocrinology 136:5311–5321

Sinha Hikim AP, Rajavashisth TB, Sinha Hikim I, Lue Y, Bonavera JJ, Leung A, Wang C, Swerdloff RS (1997) Significance of apoptosis in the temporal and stage-specific loss of germ cells in the adult rat after gonadotropin deprivation. Biol Reprod 57:1193–1201

Sjogren I, Ekvarn S, Zuhlke U, Vogel F, Bee W, Weinbauer GF, Nieschlag E (1999) Lack of effects of recombinant human GH on spermatogenesis in the adult cynomolgus monkey (*Macaca fascicularis*). Eur J Endocrinol 140:350–357

Smithwick EB, Young LG, Gould KG (1996) Duration of spermatogenesis and relative frequency of each stage in the seminiferous epithelial cycle of the chimpanzee. Tissue Cell 28:357–366

Suarez-Quian CA, Martinez-Garcia F, Nistal M, Regadera J (1999) Androgen receptor distribution in adult human testis. J Clin Endocrinol Metab 84:350–358

Suresh R, Medhamurthy R, Moudgal NR (1995) Comparative studies on the effects of specific immunoneutralization of endogenous FSH or LH on testicular germ cell transformations in the adult bonnet monkey (Macaca radiata). Am J Reprod Immunol 34:35–43

Takamiya K, Yamamoto A, Furukawa K, Zhao J, Fukumoto S, Yamashiro S, Okada M, Haraguchi M, Shin M, Kishikawa M, Shiku H, Aizawa S (1998) Complex gangliosides are essential in spermatogenesis of mice: possible roles in the transport of testosterone. Proc Natl Acad Sci USA 95:12147–12152

Teerds KJ, de Rooij DG, Rommerts FF, van den Hurk R, Wensing CJ (1989) Proliferation and differentiation of possible Leydig cell precursors after destruction of the existing Leydig cells with ethane dimethyl sulphonate: the role of LH/human chorionic gonadotrophin. J Endocrinol 122:689–696

Tripiciano A, Peluso C, Morena AR, Palombi F, Stefanini M, Ziparo E, Yanagisawa M, Filippini A (1999) Cyclic expression of endothelin-converting enzyme-1 mediates the functional regulation of seminiferous tubule contraction. J Cell Biol 145:1027–1038

Turner KJ, Morley M, Atanassova N, Swanston ID, Sharpe RM (2000) Effect of chronic administration of an aromatase inhibitor to adult male rats on pituitary and testicular function and fertility. J Endocrinol 164:225–238

Ulloa-Aguirre A, Timossi C, Barrios-de-Tomasi J, Maldonado A, Nayudu P (2003) Impact of carbohydrate heterogeneity in function of follicle-stimulating hormone: studies derived from in vitro and in vivo models. Biol Reprod 69:379–389

van Alphen MM, van de Kant HJ, de Rooij DG (1988) Follicle-stimulating hormone stimulates spermatogenesis in the adult monkey. Endocrinology 123:1449–1455

van Alphen MM, van de Kant HJ, de Rooij DG (1989) Protection from radiation-induced damage of spermatogenesis in the rhesus monkey (*Macaca mulatta*) by follicle-stimulating hormone. Cancer Res 49:533–536

van Roijen JH, Van Assen S, Van Der Kwast TH, de Rooij DG, Boersma WJ, Vreeburg JT, Weber RF (1995) Androgen receptor immunoexpression in the testes of subfertile men. J Androl 16:510–516

Vannier B, Loosfelt H, Meduri G, Pichon C, Milgrom E (1996) Anti-human FSH receptor monoclonal antibodies: immunochemical and immunocytochemical characterization of the receptor. Biochemistry 35:1358–1366

Veldhuis JD, Iranmanesh A, Samojlik E, Urban RJ (1997) Differential sex steroid negative feedback regulation of pulsatile follicle-stimulating hormone secretion in healthy older men: deconvolution analysis and steady-state sex-steroid hormone infusions in frequently sampled healthy older individuals. J Clin Endocrinol Metab 82:1248–1254

Vicari E, Mongioi A, Calogero AE, Moncada ML, Sidoti G, Polosa P, D'Agata R (1992) Therapy with human chorionic gonadotrophin alone induces spermatogenesis in men with isolated hypogonadotrophic hypogonadism-long-term follow-up. Int J Androl 15:320–329

Vornberger W, Prins G, Musto NA, Suarez-Quian CA (1994) Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134:2307–2316

Weinbauer GF, Nieschlag E (1996) The Leydig cell as a target for male contraception. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig cell. Cache River Press, Vienna, pp 629–662

Weinbauer GF, Nieschlag E (1998) The role of testosterone in spermatogenesis. In: Nieschlag E, Behre HM (eds) Testosterone – action, deficiency, substitution. Springer Verlag, Berlin, pp. 143–168

Weinbauer GF, Korte R (eds) 1999 Reproduction in nonhuman primates: a model system for human reproductive physiology and toxicology. Waxmann Publisher, Muenster/New York

Weinbauer GF, Gockeler E, Nieschlag E (1988) Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). J Clin Endocrinol Metab 67:284–290

Weinbauer GF, Behre HM, Fingscheidt U, Nieschlag E (1991) Human follicle-stimulating hormone exerts a stimulatory effect on spermatogenesis, testicular size, and serum inhibin levels in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). Endocrinology 129:1831–1839

Weinbauer GF, Schubert J, Yeung CH, Rosiepen G, Nieschlag E (1998) Gonadotrophin-releasing hormone antagonist arrests premeiotic germ cell proliferation but does not inhibit meiosis in the male monkey: a quantitative analysis using 5-bromodeoxyuridine and dual parameter flow cytometry. J Endocrinol 156:23–34

Weinbauer GF, Aslam H, Krishnamurthy H, Brinkworth MH, Einspanier A, Hodges JK (2001a) Quantitative analysis of spermatogenesis and apoptosis in the common marmoset (*Callithrix jacchus*) reveals high rates of spermatogonial turnover and high spermatogenic efficiency. Biol Reprod 64:120–126

Weinbauer GF, Schlatt S, Walter V, Nieschlag E (2001b) Testosterone-induced inhibition of spermatogenesis is more closely related to suppression of FSH than to testicular androgen levels in the cynomolgus monkey model (*Macaca fascicularis*). J Endocrinol 168:25–38

Wistuba J, Schrod A, Greve B, Hodges JK, Aslam H, Weinbauer GF, Luetjens CM (2003) Organization of seminiferous epithelium in primates: relationship to spermatogenic efficiency, phylogeny, and mating system. Biol Reprod 69:582–591

Wreford NG, Rajendra Kumar T, Matzuk MM, de Kretser DM (2001) Analysis of the testicular phenotype of the follicle-stimulating hormone beta-subunit knockout and the activin type II receptor knockout mice by stereological analysis. Endocrinology 142:2916–2920

Zannini C, Turchetti S, Guarch R, Buffa D, Pesce C (1999) Cell counting and three-dimensional reconstruction to identify a cellular wave in human spermatogenesis. Anal Quant Cytol Histol 21:358–362

Zhang FP, Rannikko AS, Manna PR, Fraser HM, Huhtaniemi IT (1997) Cloning and functional expression of the luteinizing hormone receptor complementary deoxyribonucleic acid from the marmoset monkey testis: absence of sequences encoding exon 10 in other species. Endocrinology 138:2481–2490

Zhang FP, Kero J, Huhtaniemi I (1998) The unique exon 10 of the human luteinizing hormone receptor is necessary for expression of the receptor protein at the plasma membrane in the human luteinizing hormone receptor, but deleterious when inserted into the human follicle-stimulating hormone receptor. Mol Cell Endocrinol 142:165–174

Zhengwei Y, Wreford NG, Royce P, de Kretser DM, McLachlan RI (1998a) Stereological evaluation of human spermatogenesis after suppression by testosterone treatment: heterogeneous pattern of spermatogenic impairment. J Clin Endocrinol Metab 83:1284–1291

Zhengwei Y, Wreford NG, Schlatt S, Weinbauer GF, Nieschlag E, McLachlan RI (1998b) Acute and specific impairment of spermatogonial development by GnRH antagonist-induced gonadotrophin withdrawal in the adult macaque (*Macaca fascicularis*). In J Reprod Fertil 112:139–147

Zirkin BR, Awoniyi C, Griswold MD, Russell LD, Sharpe R (1994) Is FSH required for adult spermatogenesis? J Androl 15:273-276

6

Androgens and hair: a biological paradox

V. A. Randall

Contents

6.1 Introduction

Hair growth plays significant roles in human social and sexual communication. People all over the world classify a person's state of health, sex, sexual maturity and age, often subconsciously, by assessing their scalp and body hair. The importance of hair is seen in many social customs in different cultures, such as shaving the head of Buddhist monks or no cutting of scalp hair by Sikhs. Body hair is also involved; for example, the widespread customs of daily shaving men's beards and women's axillary hair in Northern Europe and the USA. When this is considered, it is not

surprising that abnormalities of hair growth, either greater or less than "normal", even including common male pattern baldness, cause widespread psychological distress.

Androgens are the most obvious regulators of human hair growth. Although hair with a major protective role, such as the eyelashes, eyebrows and scalp hair, is produced by children in the absence of androgens, the formation of long pigmented hair on the axillae, pubis, face etc. needs androgens in both sexes. In contrast, androgens may also inhibit hair growth on the scalp, causing baldness. How one type of hormone can simultaneously cause these contradictory effects in the same tissue in different body sites within one person is an endocrinological paradox. The hair follicle has another exciting characteristic. It is the only tissue in the adult body which can regenerate, often producing a new hair with different features. This is how androgens can stimulate such major changes.

In the last 15 years, there has been a great deal of interest in the hair follicle promoted by the discovery that the antihypertensive drug, minoxidil, could sometimes stimulate hair growth. However, still relatively little is known about the precise functioning of this complex cell biological system at the biochemical level. Nevertheless, our increased comprehension of the mechanism of androgens in the follicle has enabled the treatment of hirsutism in women with antiandrogens, such as cyproterone acetate, and the 5α-reductase type 2 inhibitor, finasteride, developed to regulate prostate disorders, is now available in many countries for use in male pattern baldness. Greater understanding of hair follicle biology may also enable the development of further treatments in the future.

People have been intrigued by the changes in hair growth during a person's life for thousands of years. Various approaches have been used to establish the roles of androgens since Aristotle first recognised the connection between beard growth and the testes (reviewed Randall 2003). This chapter will cover our current knowledge of the structure and function of hair follicles, their responses to androgens, the mechanism of action of androgens in the follicle and current modes of control of androgen-potentiated disorders.

6.2 Structure and function of the hair follicle

6.2.1 The roles of human hair

Hairs cover almost all the body surface of human beings except for the soles of the feet, palms of the hands and the lips. They are fully keratinised tubes of dead epithelial cells where they project outside the skin. They taper to a point, but otherwise are extremely variable in length, thickness, colour and cross-sectional shape. These differences occur between individuals e.g. blonde, red or dark haired people and between specific body areas within one individual such as the long, thick scalp and adult male beard hairs and the short, fine ones on the back of the hand.

Changes also occur on the same parts of an individual at different stages of their life e.g. darker, thicker and longer beard hairs replace the fine, short, almost colourless hairs on a boy's face in adulthood.

The main functions of mammalian hair are insulation and camouflage. These are no longer necessary for the "naked ape," although vestiges of this remain in the seasonal patterns of our hair growth (Randall and Ebling 1991) and the erection of our body hairs when shivering with cold. Mammals often have specialised hairs as neuroreceptors e.g. whiskers and this remains slightly in human body hair with its good nerve supply. However, the main functions of human hair are protection and communication. Eyelashes and eyebrow hairs prevent substances entering the eyes and scalp hair may protect the scalp and back of the neck from sun damage during our upright posture. During puberty the development of axillary and pubic hair signals the beginning of sexual maturity in both sexes (Marshall and Tanner 1969; 1970; Winter and Faiman 1972; 1973) while the male beard, like the mane of the lion, readily distinguishes the sexes.

6.2.2 Structure of the hair follicle

Each hair is produced by a hair follicle. Hair follicles are cylindrical epithelial down-growths from the epidermis into the dermis and subcutaneous fat (Fig. 6.1). Each enlarges at its base into a hair bulb where it surrounds the tear-shaped, mesenchyme-derived dermal papilla. The dermal papilla, which contains specialised fibroblast-like cells embedded in an extracellular matrix and separated from the epithelial components by a basement membrane, regulates many aspects of hair growth (Jahoda and Reynolds 1996).

The hair is produced by epithelial cell division in the bulb; the keratinocytes move upwards, undergoing differentiation into the various layers of the follicle. The central portion forms the hair itself whose colour is produced by pigment donated by the follicular melanocytes. By the time it reaches the surface the cells are fully keratinised and dead. The hair is surrounded by two multi-layered epithelial sheaths: the inner root sheath, which helps it move through the skin and which disintegrates when level with the sebaceous gland, and the outer root sheath, which becomes continuous with the epidermis, completing the skin's protective barrier (Fig. 6.1).

6.2.3 The hair follicle growth cycle

Cell division continues until the hair reaches the appropriate length for its body site. The length of this period of hair growth, or *anagen*, can range from two years or more on the scalp (Kligman 1959) to only about two months on the finger (Saitoh and Sakamoto 1970). At the end of anagen, cell division stops and the lower follicle regresses, entering a transient stage known as *catagen* (Kligman 1959). The hair itself becomes fully keratinised with a swollen or "club" end and moves up in

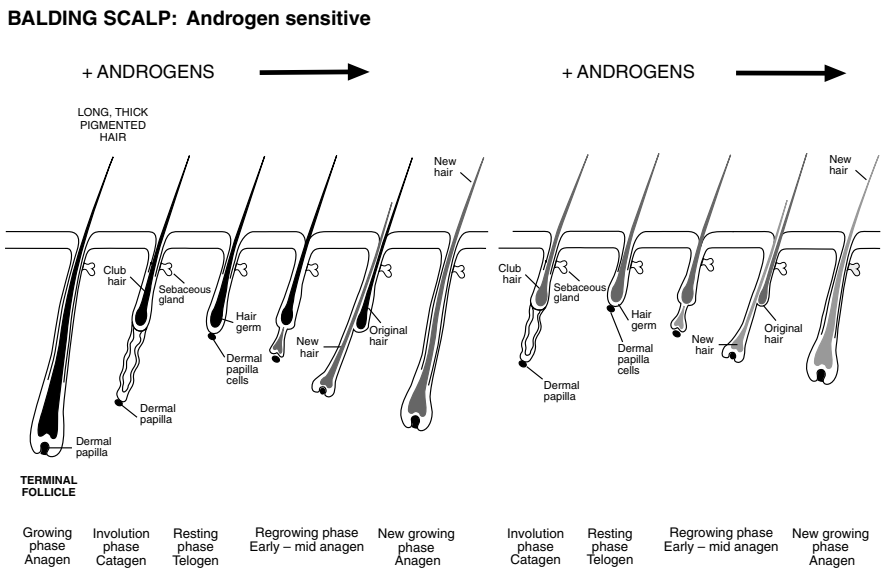


Fig. 6.1 Diagram of two hair follicle growth cycles where the new scalp follicle is smaller due to androgen inhibition

Hair follicles pass through regular cycles of growth (anagen), regression (catagen) and rest (telogen) during which the lower part of the follicle is regenerated. This enables the follicle to produce a different type of hair in response to hormonal stimuli to co-ordinate to changes in the body's development e.g. sexual maturity or seasonal climate changes. The regenerated follicle illustrated is smaller, protrudes less into the dermis and produces a smaller, less pigmented hair. Reproduced from Randall 2000b.

the skin, resting below the level of the sebaceous gland. The dermal papilla also regresses, losing the extracellular matrix and the cells become inactive. The dermal papilla cells rest below the *club hair* associated with epithelial cells (Fig. 6.1) and the follicle then enters a variable period of rest termed *telogen*. At the end of telogen the dermal papilla cells reactivate, epithelial cells recommence cell division and a lower follicle is regenerated growing back down into the dermis and producing a new hair (Fig. 6.1). The new hair grows up into the permanent part of the hair follicle alongside the old hair which is shed. The new hair may resemble the old one or may be larger, smaller and/or a different colour depending on the environment or stage of a mammal's maturity (Fig. 6.1). A further stage of *exogen* has recently been proposed involving an active, rather than passive, shedding of the old club hair (Stenn *et al.* 1998).

The origin of the epithelial cells which give rise to the new lower follicle is currently the subject of some debate. Epithelial stem cells were identified in the bulge region of the outer root sheath below the sebaceous gland (Cotsarelis *et al.* 1990), contrasting with the traditional view of stem cells in the epithelial germ, known as germinative

epithelial cells, supported by elegant cell co-culture experiments of the various follicular cell types (Jahoda and Reynolds 1996). The bulge contains stem cells with a wide potency which are able to replace cells of the epidermis and sebaceous glands as well as the hair follicle (Lavker *et al.* 2003; Taylor *et al.* 2000). It seems likely that both stem cell types are involved in the hair follicle, with the bulge cells as less specialised, higher order stem cells in line with the haemopoitiec system, possibly providing a source of cells ready to produce the germinative matrix cells for the anagen period of the next hair growth cycle (Pantelevev *et al.* 2001).

Although the hair follicle growth cycle has been well documented (Kligman 1959), the control mechanisms are complex and still not understood (Paus *et al.* 2000). It is clear that the early stages of anagen at least partially recapitulate the embryogenesis of the hair follicle to an unique extent in the adult. The processes of the hair growth cycle allow the follicle to replace the hair with a different type to correlate with changes in the environment or maturity of the individual. These changes are co-ordinated by the pineal-hypophysis-pituitary system (Ebling *et al.* 1991). Co-ordination to the environment is particularly important for some mammals, such as mountain hares, which need a longer, warmer and white coat in the snowy winter but a shorter, brown coat in the summer to increase their chances of survival (Flux 1970). Human beings in the temperate regions also exhibit seasonal changes in both scalp (Courtois *et al.* 1996; Orentreich 1969; Randall and Ebling 1991) and body hair (Randall and Ebling 1991). The main change in human hair growth is the production of adult patterns of body hair growth after puberty, like the male lion's mane, in response to androgens; some seasonal fluctuations in human body hair growth may also co-ordinate at least in part to those of androgens (discussed in Randall and Ebling 1991).

6.3 The paradoxical effects of androgens on human hair growth

6.3.1 Human hair growth before and after puberty

In utero the human body is covered with quite long, colourless *lanugo* hairs. These are shed before birth and at birth, or shortly after, babies normally exhibit pigmented, quite thick protective hairs on the eyebrows and eyelashes and variable amounts on the scalp; by the age of three or four the scalp hair is usually quite well developed, though it will not yet have reached its maximum length. These readily visible pigmented hairs are known as *terminal* hairs and are formed by large deep *terminal follicles* (Fig. 6.2). This emphasises that terminal hair growth on the scalp, eyelashes and eyebrows is not androgen-dependent. The rest of the body is often considered hairless but, except for the glabrous skin of the lips, palms and sole of the feet, is normally covered with fine, short almost colourless *vellus hairs* produced by small short *vellus follicles* (Fig. 6.2). The molecular mechanisms involved in the distribution and formation of the different types of follicles during embryogenesis are not

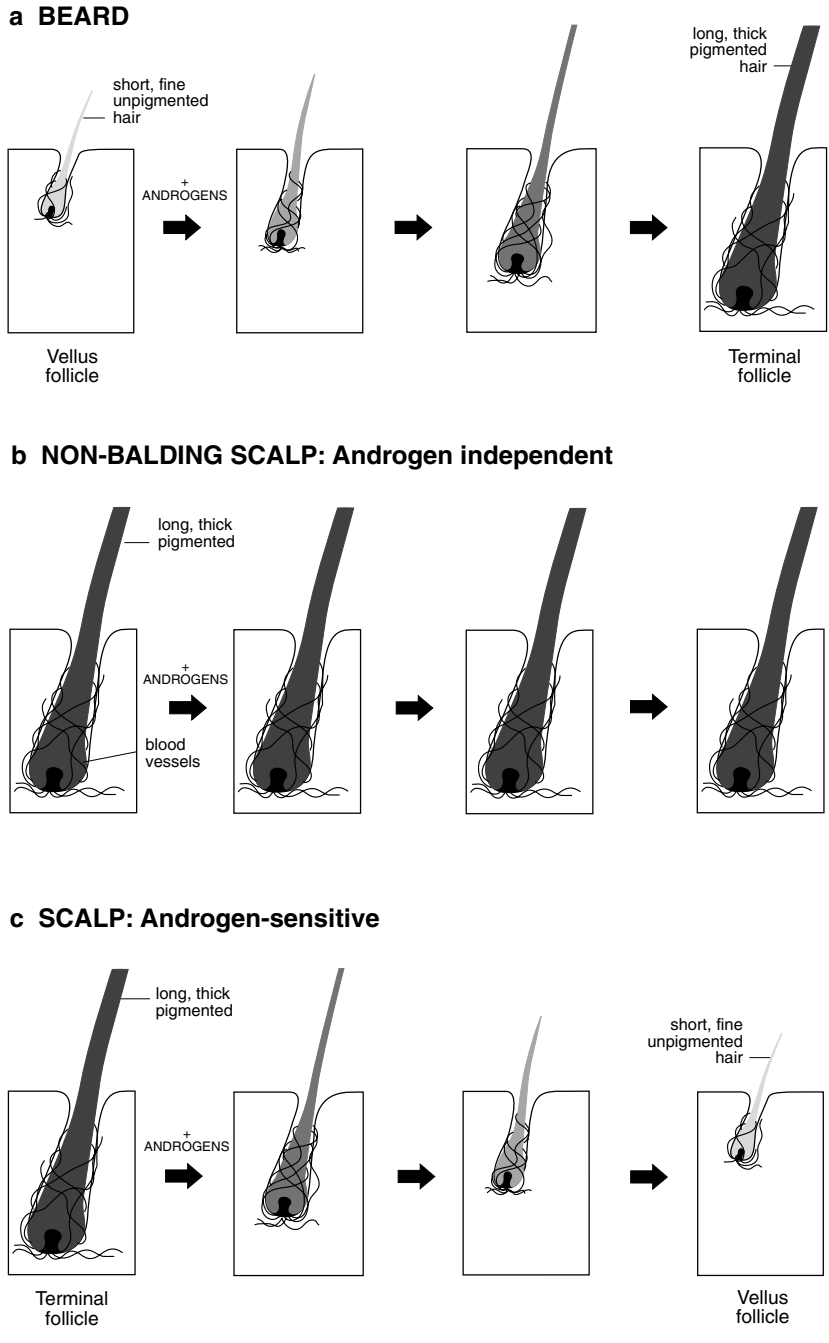


Fig. 6.2 Summary of various paradoxical effects of androgens on hair follicles
Androgens have no effects on some follicles (lower diagram), stimulate the gradual transformation of vellus follicles to terminal ones producing large pigmented hairs in many regions (upper diagram), while causing the reverse effect on the scalp in genetically-disposed individuals (middle diagram). The hair follicle undergoes several hair cycles (see Figure 6.1) between producing vellus and terminal hairs (modified from Randall 1996).

clear, but secreted signalling factors such as sonic hedgehog, Wnt and growth factors (e.g. the EGF and FGF families), nuclear factors including various homeobox genes and others such as *Hairless* and *Tabby* plus transmembrane molecules and extracellular matrix molecules have all been implicated (Wu-Kuo and Chuong 2000).

One of the first signs of puberty is the gradual appearance of a few larger and more pigmented *intermediate* hairs, firstly in the pubic region and later in the axillae. These are replaced by longer and darker hairs (Fig. 6.2) and the area spreads. In boys, similar changes occur gradually on the face starting above the mouth and on the central chin, eventually generally spreading over the lower part of the face and parts of the neck, readily distinguishing the adult male (Marshall and Tanner 1969; 1970). The adult man's pubic hair distribution also differs from the woman, extending in a diamond shape up to the navel in contrast to the woman's inverted triangle. Terminal hair on the chest and sometimes the back is also normally restricted to men, though both sexes may also develop intermediate terminal hairs on their arms and legs, with terminal hairs normally restricted to the lower limbs in women (Fig. 6.3). In all areas the responses are gradual, often taking many years. Beard weight increases dramatically during puberty but continues to rise until the mid-thirties (Hamilton 1958), while terminal hair growth on the chest and in the external ear canal may first be seen many years after puberty (Hamilton 1946).

The amount of body hair is very variable and differs both between families within one race and between races, with Caucasians generally exhibiting more than Asians (Hamilton 1958). This implies a genetically-determined response to circulating triggers. The responses of the follicles themselves also vary, with female hormone levels being sufficient to stimulate terminal hair growth in the pubis and axillae, but male hormones being required for other areas, such as the beard and chest. Beard hair growth also remains high, right into a man's seventies, while axillary growth is maximal in the mid-twenties and falls quite rapidly then in both sexes (Fig. 6.4) (Hamilton 1958). This is a paradoxically different response in the two areas to apparent stimulation by the same hormones.

During early puberty the frontal hair line is usually straight across the top of the forehead. With increasing age there is a progressive regression of the frontal hair line in a prescribed manner (described below 6.3.3.1) accompanied by progressive thinning of terminal hair on the vertex. This is characterised by a gradual inhibition of terminal follicles to smaller vellus follicles (Fig. 6.2) with the length of anagen decreasing and that of telogen increasing. This is another example of a much more dramatic biological paradox. How does one hormone stimulate hair growth in many areas such as the face, have no effect in others e.g. eyelashes, while inhibiting follicles on the scalp? These contrasts are presumably due to differential gene expression within follicles from the various body sites. The intrinsic response of individual follicles is retained when follicles are transplanted to other skin sites

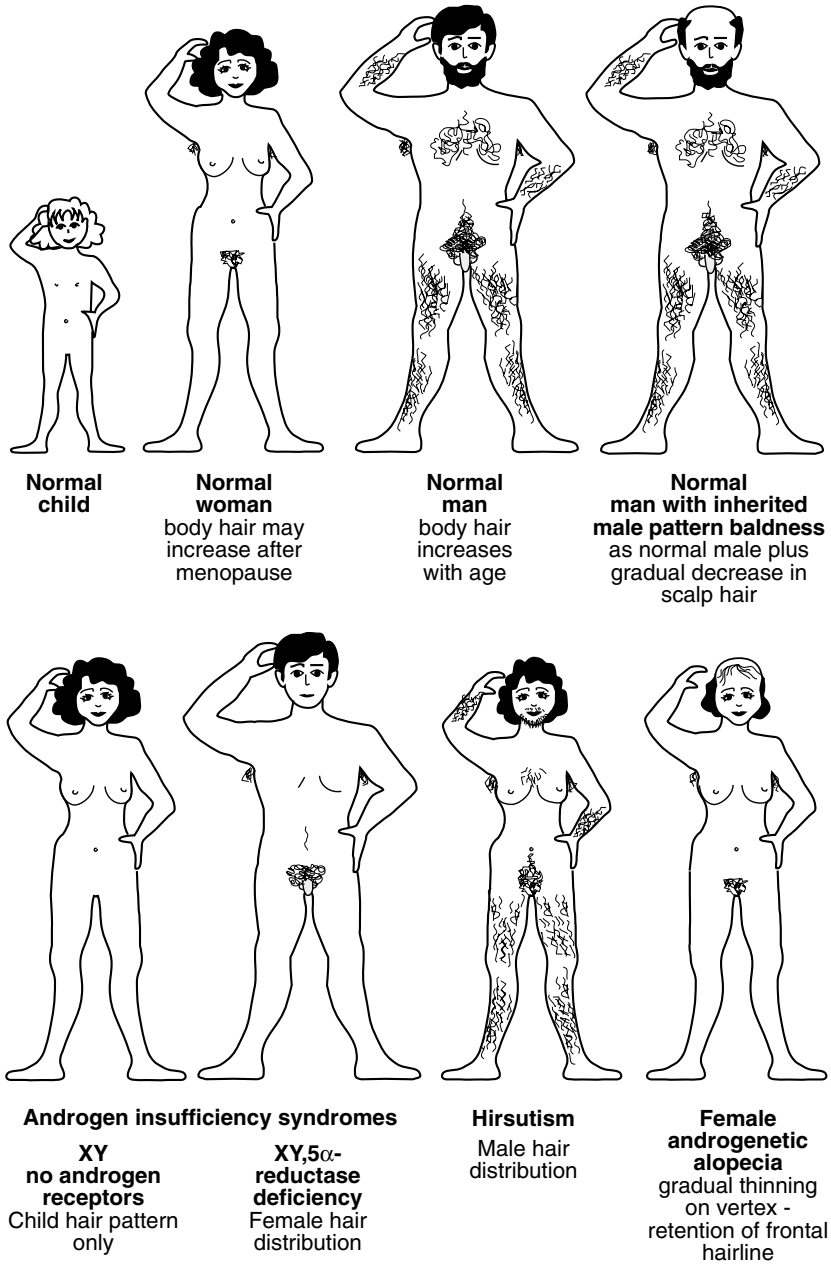


Fig. 6.3 Terminal hair distribution in people under differing endocrine conditions

Terminal hair with protective functions normally develops in children on the scalp, eyelashes and eyebrows. During, and after, puberty this is augmented by axillary and pubic hair in both sexes and beard, chest and greater body hair in men. In people with the appropriate genetic tendency, androgens may also stimulate hair loss from the scalp in a patterned manner causing androgenetic alopecia. None of this occurs without functional androgen receptors and only axillary and the lower pubic triangle hairs are formed in the absence of 5α-reductase type 2 (lower panel). Male pattern hair growth (hirsutism) may occur in women with circulating abnormalities of androgens or from idiopathic causes.

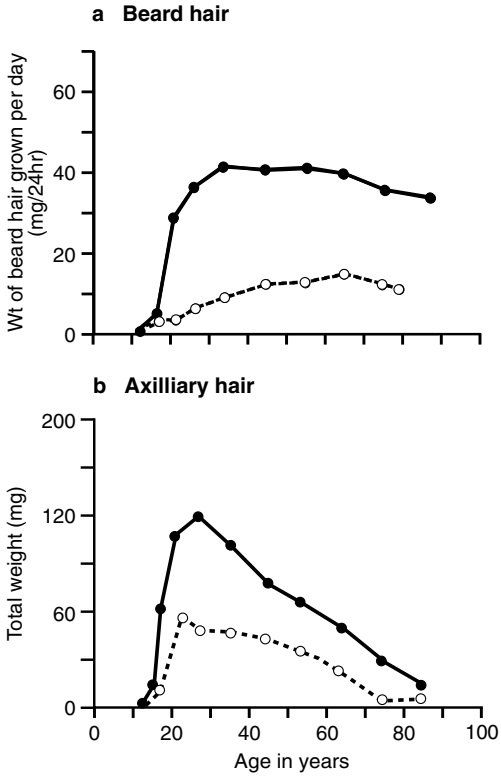


Fig. 6.4 Paradoxically different patterns of hair growth in two androgen-dependent areas: the beard and the axilla

Both beard and axillary hair growth is stimulated by androgens during puberty in Caucasian (solid lines) and Japanese (dotted lines) men. However, while beard growth is maintained at high levels into old age in both races, axillary hair growth is maximal at 30 and decreases regularly to prepubertal levels. Reproduced from Randall 2000b showing data redrawn from Hamilton 1958.

(Ebling and Johnson 1959); this is the basis of corrective hair follicle transplant surgery (Orentreich and Durr 1982).

6.3.2 Evidence for the role of androgens

Although androgens are the clearest regulators of human hair growth, unlike in most mammals (Ebling *et al.* 1991), various other circulating factors (reviewed in Randall 1994a) have an effect. These include adequate nutritional supplies, due to the follicles' high metabolic demands (Bradfield 1971), the hormones of pregnancy, which cause a prolonged anagen resulting in a synchronised shedding of a proportion of scalp hairs post-partum (Lynfield 1960), and lack of thyroid hormone which restricts hair growth (Jackson *et al.* 1972). Growth hormone is also necessary in combination with androgens for normal body hair development in

boys (Zachmann and Prader 1970). There is a range of evidence supporting the importance of androgens which fits in well with the concept of much terminal hair growth being a secondary sexual characteristic. Terminal hair appearance in puberty parallels the rise in circulating androgen levels and occurs later in boys than girls (Marshall and Tanner 1969; 1970; Winter and Faiman 1972; 1973). Testosterone also stimulates beard growth in eunuchs and elderly men (Chieffi 1949) and increased beard growth noted by an isolated endocrinologist is ascribed to his rising androgens on anticipating his girlfriend's arrival (Anonymous 1970)! An extensive study in the USA also showed that castration before puberty prevented beard and axillary hair growth and after puberty reduced them (Hamilton 1951a; 1958). Nevertheless, the strongest evidence for the essential nature of androgens is the lack of any body hair, even the female pubic and axillary pattern, or evidence of any male pattern baldness, in adult XY androgen insensitivity patients with absent or dysfunctional androgen receptors despite normal or raised circulating levels of androgens (see Chapter 3).

6.3.3 Androgen-dependent hair growth conditions

6.3.3.1 Androgenetic alopecia

A generalised loss of hair follicles from the scalp known as *senescent balding* has been reported in both sexes by the seventh or eighth decade (Courtois *et al.* 1995; Kligman 1988). This differs from the progressive baldness seen in *androgenetic alopecia*, also known as *male pattern baldness*, *male pattern alopecia, common baldness* or *androgen-dependent alopecia*. The connective tissue sheath left in the dermis when the follicle becomes miniaturised during androgenetic alopecia may become subject to chronic inflammation; this may prevent terminal hair regrowth in long-term baldness (Kligman 1988) although this is currently a matter of debate. Balding occurs in a precise pattern described by Hamilton (1951b), starting with regression of the frontal hairline in two wings and balding in the centre of the vertex. These areas gradually expand and coalesce, exposing large areas of scalp; generally the back and sides of the scalp retain terminal hair even in extreme cases (Fig. 6.3). Hamilton's scale was later modified by Norwood (1975) to include a wider range of patterns. The physiology and pathophysiology of androgenetic alopecia is reviewed more fully in Randall 2000a and 2001.

Male pattern baldness is androgen-dependent, since it does not occur in castrates, unless they are given testosterone (Hamilton 1942), nor in XY individuals with androgen insensitivity due to non-functional androgen receptors (Hiort this volume, Chapter 3). There is also a marked inherited tendency to develop it (Hamilton 1942), though the genetics are not yet established. Known dimorphic and polymorphic markers within the androgen receptor gene were recently investigated in Caucasian men (Ellis *et al.* 2001). The *Stu* I restriction fragment length polymorphism (RFLP) in exon 1 was present in 98% of 54 young balding men and 92% of 392 older balding men, but was also found in 77% of their older, non-balding

controls. When two triplet repeat polymorphisms were examined the distribution of neither short or long single triplet repeats of CAG or GAC differed significantly, but the incidence of short/short polymorphic CAG/GGC haplotypes were significantly higher (50% compared to 30%) in balding subjects and short/long were lower (7% rather than 22%) though no significance was stated in the paper. Interestingly, analysis of Spanish girls with precocious puberty i.e. appearance of pubic hair before 8 years of age showed the mean number of CAG repeats was shorter than controls (Ibanez *et al.* 2003). Shorter triplet repeat lengths have also been associated with another common androgen-dependent condition, prostate cancer (Stanford *et al.* 1997). Whether this has functional significance such as an increased androgen sensitivity or simply reflects linkage disequilibrium with a causative mutation is not clear. However, when the binding capacity for a range of steroids was compared between androgen receptors from balding and non-balding follicle dermal papilla cells no differences were detected (Hibberts *et al.* 1998).

The incidence of androgenetic alopecia in Caucasians is high with estimates varying widely but progression to stage type II being detected in 95% (Hamilton 1951b). Other races exhibit it to a lesser extent (Hamilton 1951b; Setty 1970) and it is also seen in other primates, being well studied in the stump-tailed macaque. This suggests a natural progression of a secondary sexual characteristic rather than the malfunction of a disease. Marked androgenetic alopecia would obviously highlight the surviving older man as a leader like the silver back of the chief male gorilla and the larger antlers of the mature deer stags. Others have speculated that the flushed bald skin would look aggressive to an opponent (Goodhart 1960) or mean there was less hair for the opposition to pull (Ebling 1985), giving the bald man important advantages. The lower incidence of androgenetic alopecia amongst men from African races (Setty 1970) suggests that any advantages did not outweigh the evolutionary survival advantages of the hairs' protection of the scalp from the hot tropical sun.

In the current youth-orientated culture of industrialised societies the association of increasing hair loss with age combined with the major role of hair in human communication means that androgenetic alopecia has strong negative connotations. It often causes psychological distress and reduction in the quality of life, even though it is not life-threatening or physically painful, in both men (Cash 1992; Franzoi *et al.* 1999; Girman *et al.* 1998; Maffei *et al.* 1990; Terry and Davis 1976; Wells *et al.* 1995) and women (Cash 1993; van der Dank *et al.* 1991). Other people perceive men with visible hair loss as older, less physically and socially attractive, weaker and duller. In parallel, people with androgenetic alopecia have a poor self-image, feel older and lacking in self-confidence, even those who seem accepting of their condition and have never sought treatment (Girman *et al.* 1998). Male pattern baldness primarily causes concern amongst those who develop marked loss before their forties and early balding has been linked to myocardial infarction (Lesko *et al.* 1993). Whether this indicates a dual end-organ sensitivity or reflects the psychological stress early

balding induces in the youth-orientated American culture is unknown. No relationship between the incidence of balding and prostatic carcinoma was detected in men between fifty and seventy (Demark-Wahnefried *et al.* 1997).

Androgenetic alopecia has also been described in women, but the pattern of expression is normally different. Women generally do not show the frontal recession, but retain the frontal hairline and exhibit thinning on the vertex which may lead to balding (Ludwig 1977) (Fig. 6.3). Post-menopausal women may exhibit the masculine pattern (Venning and Dawber 1988). The progression of balding in women is normally slow and a full endocrinological investigation is recommended if a rapid onset is seen (Dawber and Van Neste 1995). Although female pattern hair loss is seen frequently in association with hyperandrogenism, other women frequently have no other symptoms of androgen abnormality. Therefore, there is some debate about whether androgen is essential for this hair loss in women (Birch *et al.* 2002) though this is still generally assumed. If, as occurs in men, the changes develop due to the genetically influenced, specific follicular responses within the scalp follicles themselves, it is not surprising that circulating androgen abnormalities are often absent.

6.3.3.2 Hirsutism

Hirsutism is the development of male pattern body hair growth in women. This also causes marked psychological distress because the person erroneously feels that they are changing sex. The extent of body hair growth which causes a problem varies and depends on the amount of normal body hair amongst her race or sub-group. Normally hirsutism would include terminal hair on the face, chest or back. Ferriman and Gallwey (1961) introduced a scale for grading hirsutism which is widely used, especially to monitor hirsutism progression with, or without, treatment.

Hirsutism is often associated with an endocrine abnormality of the adrenal or ovary causing raised androgens and is frequently associated with polycystic ovarian (PCO) syndrome. Some women have no obvious underlying disorder and are termed "idiopathic". The proportion of these is larger in older papers as modern methods increase the range of abnormalities that can be detected e.g. low sex hormone binding globulin. The assumption that idiopathic hirsutism is due to a greater sensitivity of the follicles to normal androgens is given credence by hirsutism occurring asymmetrically on only one side of a woman (Jenkins and Ash 1973).

6.4 The mechanism of androgen action in the hair follicle

6.4.1 Hair growth in androgen insufficiency syndromes

As described in Chapters 1 and 2 of this book, androgens from the blood stream enter the cell and bind to specific, intracellular androgen receptors, usually in

the form of testosterone or its more potent metabolite, 5α-dihydrotestosterone. The hormone-receptor complex, generally in combination with transcriptional regulators then activates the appropriate gene transcription for that cell type.

Androgen insufficiency patients without functional androgen receptors demonstrate the essential requirement for androgen receptors within hair follicles for the development of the hair growth ascribed in 6.3.2 to androgens (Hiort, this volume Chapter 3). These individuals produce no body hair at puberty, even with high circulating androgen levels, nor do they go bald (Fig. 6.3).

Men with 5α-reductase deficiency also contribute to our understanding because they exhibit axillary and female pattern pubic hair, but very little beard growth; they are not reported to have male pattern baldness either (Griffin and Wilson 1989) (Fig. 6.3). A role for 5α-reductase in male pattern baldness is also supported by the ability of oral finasteride, a 5α-reductase type 2 inhibitor, to promote hair regrowth (Kaufman *et al.* 1998; Shapiro and Kaufman 2003). This suggests that the formation of terminal pubic and axillary hair can be mediated by testosterone itself, while that of the secondary sexual hair of men requires the presence of 5α-dihydrotestosterone. This demonstrates a third paradox in androgen effects on hair follicles. Why does the stimulation of increasing size in some follicles e.g. beard require 5α-dihydrotestosterone formation, while follicles in the axillary and pubic regions carry out the same changes in the absence of 5α-dihydrotestosterone? Since androgens are stimulating the same transformation, presumably via the same receptor, this is currently difficult to understand, although it is further evidence of the intrinsic differences within hair follicles. It suggests that some less well known aspects of androgen action are involved in hair follicles normally specific to men which requires 5α-dihydrotestosterone, such as interaction with a specific transcription factor. Interestingly, androgen-dependent sebum production by the sebaceous glands attached to hair follicles is also normal in 5α-reductase deficiency (Imperato-McGinley *et al.* 1993). The identification of two forms of 5α-reductase, type 1 and type 2, has made the situation more complex, but all individuals with 5α-reductase deficiency so far have been shown to be deficient in 5α-reductase type 2 (reviewed by Randall 1994b) which appears to be the important form for much androgen-dependent hair growth.

6.4.2 The current model for androgen action in the hair follicle

6.4.2.1 The role of the dermal papilla

The mesenchyme-derived dermal papilla plays a major role in determining the type of hair produced by a follicle as shown by an elegant series of experiments involving the rat whisker by Oliver, Jahoda, Reynolds and colleagues (reviewed by Jahoda and Reynolds 1996). Whisker dermal papillae transplanted into ear or glabrous skin stimulated the production of whisker follicles and hair growth could

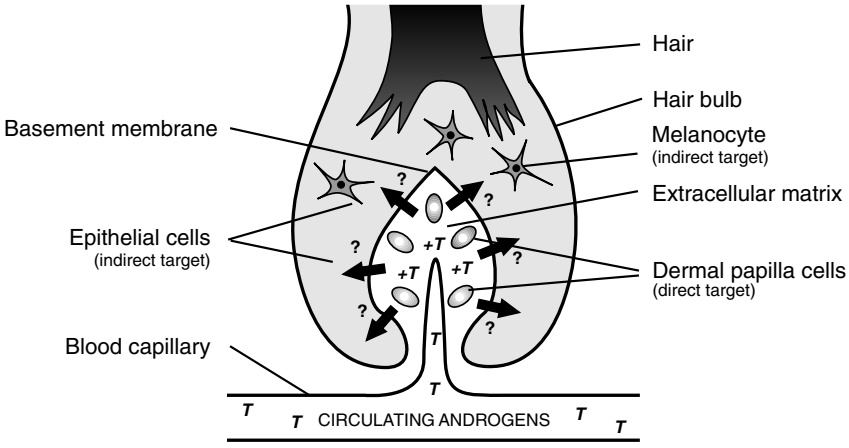


Fig. 6.5 The current model of androgen action in the hair follicle
Androgens from the blood enter the hair follicle via the dermal papilla's blood supply. They are bound by androgen receptors in the dermal papilla cells which then alter their production of regulatory paracrine factors; these then alter the activity of follicular keratinocytes and melanocytes. T = Testosterone; ? = unknown paracrine factors (modified from Randall 1994a).

also be stimulated by cultured dermal papilla cells reimplanted in vivo (Jahoda *et al.* 1984).

In many embryonic steroid-regulated tissues, including the prostate and the breast, steroids act via the mesenchyme (Cunha *et al.* 1987). Since hair follicles recapitulate the stages of embryogenesis during their growth cycles to reform a new lower hair follicle, they may behave like an embryonic tissue in the adult. Studies on testosterone metabolism in vitro by plucked hair follicles, which leave the dermal papilla behind in the skin, from different body sites did not reflect the requirements for 5α-reductase in vivo (reviewed in Randall *et al.* 1991; Randall 1994a), leading to the hypothesis that androgens would act on the other components of the hair follicle via the dermal papilla (Randall *et al.* 1991; Randall 1994a). In this hypothesis androgens would alter the ability of the dermal papilla cells to synthesise or release controlling factors which would affect follicular keratinocytes, melanocytes and connective tissue sheath cells and also probably the dermal endothelial cells to alter the follicles' blood supply in proportion to its change in size (Fig. 6.5). These factors could be growth factors and/or extracellular matrix proteins. This model would facilitate a mechanism for precise control of the follicle during the complex changes needed to increase or decrease the size of a follicle in response to androgens.

This hypothesis has now received a great deal of experimental support. Androgen receptors have been localised by immunohistochemistry in the dermal papilla and not the keratinocyte cells (Choudhry *et al.* 1992; Itami *et al.* 1995a). Cultured dermal papilla cells derived from androgen-sensitive follicles such as beard (Randall *et al.* 1992) and balding scalp (Hibberts *et al.* 1998) contain higher levels of specific, saturable androgen receptors than androgen-insensitive non-balding scalp in vitro; this has been confirmed by studies using RT-PCR (Ando *et al.* 1999). Most importantly, metabolism of testosterone by cultured dermal papilla cells also reflects hair growth in 5α-reductase deficiency patients with beard, but not pubic or non-balding scalp, cells forming 5α-dihydrotestosterone in vitro (Itami *et al.* 1990; Hamada *et al.* 1996; Thornton *et al.* 1993); similar results have been obtained examining gene expression of 5α-reductase type 2 by RT-PCR (Ando *et al.* 1999). All these results have led to wide acceptance of the hypothesis.

Recently the lower part of the connective tissue sheath, or dermal sheath, which surrounds the hair follicle and isolates it from the dermis has been shown to form a new dermal papilla and new human hair follicle development in another person of the opposite sex (Reynolds *et al.* 1999). Cultured dermal sheath cells from the beard hair follicles contain similar levels of androgen receptors to beard dermal papilla cells (Merrick *et al.* 2004) and balding scalp dermal sheath expresses the mRNA for 5α-reductase type 2 like the dermal papilla (Asada *et al.* 2001). Clearly the dermal sheath also plays an important role in the hair follicle. This may be as a reserve to replace the key inductive and controlling role of the dermal papilla cells if they are lost. Alternatively, or in addition, it seems highly probable that the dermal sheath cells may respond directly to androgens to facilitate the increase or decrease in size of the sheath or even the dermal papilla in the development of a new anagen follicle; this would enable the new hair follicle to be larger or smaller depending on the follicle's specific response to androgens. These results merit a modification of the model to include a direct action of androgens on the lower dermal sheath too.

6.4.2.2 Paracrine factors implicated in mesenchyme-epithelial interactions in the hair follicle

The production of growth factors by cultured dermal papilla cells derived from human and rat hair follicles has been investigated by several groups on the basis of the primary role of the dermal papilla, its potential probable role in androgen action and the retention of hair growth-promoting ability by cultured rat cells (discussed above). Cultured dermal papilla cells secrete both extracellular matrix factors (Messenger *et al.* 1991) and soluble, proteinaceous growth factors (Randall *et al.* 1991). Bioassays demonstrate that human dermal papilla cells secrete factors which stimulate the growth of other dermal papilla cells (Randall *et al.* 1991; Thornton *et al.* 1998), outer root sheath cells (Itami *et al.* 1995a), transformed epidermal keratinocytes (Hibberts and Randall 1996) and endothelial cells (Hibberts

et al. 1996c). Importantly, testosterone in vitro stimulated greater mitogenic capacity of beard cells to affect beard, but not scalp, dermal papilla cells (Thornton *et al.* 1998), outer root sheath cells (Itami *et al.* 1995a) and keratinocytes (Hibberts and Randall 1996). In contrast, testosterone decreased the mitogenic capacity of androgenetic alopecia dermal papilla cells from both men (Hibberts and Randall 1996) and stump-tailed macaques (Obana *et al.* 1997). As well as supporting the hypothesis for the mechanism of action, these results demonstrate that the paradoxical effects of androgen on hair follicles observed in vitro are reflected in vitro, strengthening the use of cultured dermal papilla cells as a model system for studying androgen action in vitro.

The main emphasis of research now lies in identifying specific factors whose production by dermal papilla cells is altered by androgens (reviewed Randall *et al.* 2001a). To date only insulin-like growth factor (IGF-1) has been identified as androgen-stimulated in vitro (Itami *et al.* 1995b), but stem cell factor (SCF), the ligand for the melanocyte receptor *c-kit*, is secreted in greater quantities by beard dermal papilla cells than non-balding scalp cells (Hibberts *et al.* 1996a) unlike vascular endothelial growth factor (Hibberts *et al.* 1996b). Other factors which have been implicated in the follicular dermal papilla include keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF), though many more have been located in the epidermis (reviewed by Philpott 2000). The expression of mRNA for the protease nexin-1 in dermal papilla cells is also altered by androgens (Sonada *et al.* 1999). This may play a role by altering the amount of extracellular matrix components produced (discussed Randall *et al.* 2001b) and therefore the size of the follicle and hair produced (Elliott *et al.* 1999). Recently, dermal papilla cell conditioned media from balding scalp follicles has been shown to inhibit the growth of both human and rodent whisker dermal papilla cells in vitro and delay mouse hair growth in vivo (Hamada and Randall 2003). This suggests the active secretion of an inhibitory factor or factors. A possible candidate is transforming growth factor-β1 (TGF-β1) which has been induced by androgens in balding dermal papilla cells with transfected androgen receptors (Inui *et al.* 2003). TGF-β also inhibits hair follicle growth in vitro (Philpott 2000) and a probable suppressor of TGF-β1 delayed catagen progression in mice in vivo (Tsuji *et al.* 2003). Further study of this area should increase our understanding of the complex hair follicle and lead to better treatments for hair follicle disorders.

6.5 The treatment of androgen-potentiated hair disorders

6.5.1 Androgenetic alopecia

Currently, the most effective treatment for male pattern baldness is the transplant of follicles from non-balding sites into the balding region, capitalising on the retention

of the different intrinsic responses to androgen discussed earlier. This has significant disadvantages; not only is it very invasive and heavily reliant on the skill of the operator for a good cosmetic result, but the alopecia continues to progress behind the transplanted area so that further transplants are often required.

Antiandrogen therapy is not a practical option for men due to the side-effects, but cyproterone acetate, in combination with estrogen to ensure contraception, has been used in women. It increased the percentage of hair follicles in anagen and may cause some regrowth, but is probably most effective in preventing further progression (Dawber and Van Neste 1995; Peereboom-Wynia *et al.* 1989). Since cyproterone acetate is unavailable in the USA, spirolactone and high-dose cimetidine have been used as alternative antiandrogens.

Minoxidil, a vasodilator used for hypertension, stimulated excessive hair growth as a side-effect. This provoked major interest in hair follicle biology because it demonstrated that vellus follicles could be stimulated to form terminal hairs. Topical application of minoxidil has been used in both male and female androgenetic alopecia. It stimulates regrowth in up to 30% with only about 10% obtaining complete regrowth, probably by acting as a potassium channel regulator; most success occurs with younger men and with the early stages of balding, i.e. Hamilton stage V or less (Dawber and Van Neste 1995). More recently, a stronger topical application of a 5% solution has been licensed for use in men (Olsen *et al.* 2002).

Finasteride, a 5α-reductase type 2 inhibitor, was developed to treat androgen-potentiated prostate disorders and is now available as an oral treatment for androgenetic alopecia in men in many countries at a lower dose of 1 mg per day. Clinical trials demonstrated significant effects on stimulating hair regrowth in men with mild to moderate hair loss (Kaufman *et al.* 1998; Shapiro and Kaufman 2003). Even if hair did not regrow, balding progression was frequently halted. Unfortunately, no effects of finasteride have been seen in post-menopausal women with androgenetic alopecia (Price *et al.* 2000); use in pre-menopausal women requires ensuring against contraception in case of potential feminisation of a male fetus.

Although a range of treatments are now available, they all need to be used continually because they are opposing a natural process which, if treatment is discontinued, retains all the components to continue to progress.

6.5.2 Hirsutism

Once a serious underlying pathology has been eliminated, a range of treatments is available for hirsutism (Azziz 2003). Cosmetic treatments such as bleaching, depilatory measures such as shaving, waxing, electrolysis or laser are common. Electrolysis with the aim of permanent removal by killing the dermal papilla and germinative epithelium/stem cells is the most established long-lasting treatment, but it is expensive, time consuming and may cause scarring; removal by laser

treatment is a more recently introduced alternative (Levy *et al.* 2001; Sanchez *et al.* 2002).

The most common endocrine treatment, outside the USA, is the antiandrogen, cyproterone acetate, given with estrogen if the woman is premenopausal; spirolactone or flutamide can be used as an alternative (Fruzzetti 1997; Lumachi and Rondinome 2003). Patients have to be well-motivated because hair growth on the face generally takes at least nine months before a noticeable effect occurs, although any acne will be cleared in a couple of months and effects on thigh hair growth will be seen in four to six months (Sawers *et al.* 1982). Facial responses are seen first on the sides of the face and last on the upper lip, in reverse order to the appearance of facial hair in men (personal observations). Finasteride has also been used for hirsutism with some success (Lacryc *et al.* 2003). This seems logical as 5α-reductase type 2 is necessary for male pattern body hair growth (see Section 6.4.1). Contraception is still required with all endocrine treatments due to the potential to affect the development of a male fetus. Metformin, insulin-sensitising therapy, aimed to alter the insulin resistance and hence the hyperandrogenism often associated with polycystic ovarian disease has been used clinically, but the evidence has yet to be rigorously tested (Harborne *et al.* 2003).

Overall, there have been major changes in the treatment of androgen-potentiated disorders over the last ten years. The ideal treatment of a uniformly effective, topical treatment which is inactivated on contact with the blood or is specific for hair follicles is not yet available. Further research on the biology of androgen action in the hair follicle may facilitate its development.

6.6 Key messages

- Androgens are the main regulator of human hair growth.
- Androgens have paradoxically different effects on hair follicles depending on their body site. They can stimulate the formation of large hairs e.g. beard, axilla, have no effect e.g. eyelashes or inhibit follicles on the scalp.
- All effects are gradual.
- Androgen-potentiated disorders of hair growth are common including hirsutism in women and androgenetic alopecia in both sexes.
- Androgen receptors are necessary for all androgen-dependent hair growth and 5α-reductase type 2 for most, but not for female patterns of axillary and pubic hair, even in men.
- The action of androgens on human hair follicles demonstrates several paradoxes: contrasting effects in different sites; major differences in the persistence of stimulatory effects depending on body region; a varying requirement for the formation of 5α-dihydrotestosterone even amongst follicles exhibiting increased growth. Since these are all site-related and retained on transplantation, these indicate intrinsic differences within follicles, presumably determined during embryonic development.

- The current model for androgen action in the hair follicle proposes that androgens act via the cells of the dermal papilla, altering their production of regulatory paracrine factors such as growth factors which then influence the activity of other follicular components, e.g. keratinocytes, melanocytes and endothelial cells. The dermal sheath may also play a role as a direct androgen target.
- Antiandrogens, generally cyproterone acetate, and a 5α-reductase type 2 inhibitor, finasteride, are being used to control androgenetic alopecia and hirsutism.
- Endocrine treatments may need several months to show their effects and will need to be used continually.
- Further understanding of the mechanism of androgens in the hair follicle is necessary to enable the development of better treatments, preferably working topically and specific to the hair follicle.

6.7 REFERENCES

Ando Y, Yamaguchi Y, Hamada K, Yoshikawa K, Itami S (1999) Expression of mRNA for androgen receptor, 5α-reductase and 17β-hydroxysteroid dehydrogenase in human dermal papilla cells. Br J Derm 141:840–845

Anonymous (1970) Effects of sexual activity on beard growth in men. Nature 226:869–870

Asada Y, Sonoda T, Ojiro M, Kurata S, Sato T, Ezaki T, Takayasu S (2001) 5α-reductase type 2 is constitutively expressed in the dermal papilla and connective tissue sheath of the hair follicle in vivo but not during culture in vitro. J Clin Endocr Metab 86:2875–2880

Azziz R (2003) The evaluation and management of hirsutism. Obstet Gynaecol 101:995–1007

Birch MP, Lalla SC, Messenger AG (2002) Female pattern hair loss. Clin Exp Derm 27:383–388

Bradfield RB (1971) Protein deprivation: comparative response of hair roots, serum protein and urinary nitrogen. Amer J Clin Nutrit 24:405–410

Cash TF (1992) The psychological effects of androgenetic alopecia in men. J Am Acad Derm 26:926–931

Cash TF (1993) Psychological effects of androgenetic alopecia on women: comparisons with balding men and with female control subjects. J Am Acad Derm 29:568–575

Chieffi M (1949) Effect of testosterone administration on the beard growth of elderly males. J Geront 4:200–204

Choudhry R, Hodgins MB, Van Der Kwast TH, Brinkman AO, Boersma, WJA (1992) Localisation of androgen receptors in human skin by immunohistochemistry: implications for the hormonal regulation of hair growth, sebaceous glands and sweat glands. J Endocr 133:467–475

Cotsarelis C, Sun T, Lavker R (1990) Label-retaining cells reside in the bulge area of the pilosebaceous unit; Implications for follicular stem cells, hair cycle and skin carcinogenesis. Cell 61:1329–1337

Courtois M, Loussouarn G, Hourseau C, Grollier JF (1995) Ageing and hair cycles. Br J Derm 132:86–93

Courtois M, Loussouarn G, Hourseau S, Grollier JF (1996) Periodicity in the growth and shedding of hair. Br J Derm 134:47–54

Cunha GR, Donjacour AA, Cook PS, Mee S, Bigby RH, Higgins S, Sugimura Y (1987) The endocrinology and developmental biology of the prostate. Endocr Rev 8:338–362

Dawber R, Van Neste D (1995) Hair and scalp disorders. Martin Dunitz, London

Demark-Wahnefried W, Lesko SM, Conaway MR, Robertson CN, Clark RV, Lobaugh B, Mathias BJ, Strigo TS, Paulsa DF (1997) Serum androgens: associations with prostate cancer risk and hair patterning. J Androl 18:495–500

Ebling FJG (1985) Age changes in cutaneous appendages. J Appl Cosmet 3:342–250

Ebling FJG, Johnson E (1959) Hair growth and its relation to vascular supply in rotated skin grafts and transposed flaps in the albino rat. J Embry Exp Morph 9:285–293

Ebling FJG, Hale PA, Randall VA (1991) Hormones and hair growth. In: Goldsmith LA (ed) Biochemistry and physiology of the skin, Second ed Clarenden Press, Oxford pp 660–696

Ellis JA, Stebbing M, Harrap SB (2001) Polymorphism of androgen receptor gene is associated with male pattern baldness. J Invest Derm 116:452–455

Elliot K, Stephenson TJ, Messenger AG (1999) Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses. J Invest Derm 113:873–877

Ferriman D, Gallwey JD (1961) Clinical assessment of body hair growth in women. J Clin Endocr Metab 21:1440–1447

Flux JEC (1970) Colour change of mountain hares (lepus timidus scoticus) in north-east Scotland. Zool 162:345–358

Franzoi SL, Anderson J, Frommelt S (1999) Individual difference in men's perceptions of and reactions to thinning hair. J Soc Psychol 130:209–218

Fruzetti F (1997) Treatment of hirsutism: antiandrogen and 5α-reductase inhibitor therapy. In: Azziz R, Nestler JE, Dewailly D (eds) Androgen excess disorders in women. Lippincott-Raven, Philadelphia pp 787–797

Girman CJ, Rhodes T, Lilly FRW, Guo SS, Siervogal RM, Patrick DL, Chumlea WC (1998) Effects of self-perceived hair loss in a community sample of men. Dermatology 197:223–229

Goodhart CB (1960) The evolutionary significance of human hair patterns and skin colouring. Adv Sci 17:53–58

Griffin JE, Wilson JD (1989) The resistance syndromes: 5α-reductase deficiency, testicular feminisation and related disorders. In: Baudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. Scriver CR, McGraw-Hill, New York pp 1919–1944

Hamada K, Randall VA (2003) Balding scalp dermal papilla cells secrete a soluble factor(s) which delays the onset of anagen in mice in vivo. J Invest Derm Symp Proc 8:139

Hamada K, Thornton MJ, Liang I, Messenger AG, Randall VA (1996) Pubic and axillary dermal papilla cells do not produce 5α-dihydrotestosterone in culture. J Invest Derm 106:1017–1022

Hamilton JB (1942) Male hormone stimulation is a prerequisite and an incitant in common baldness. Amer J Anat 71:451–480

Hamilton JB (1946) A secondary sexual character that develops in men but not in women upon aging of an organ present in both sexes. Anat Rec 94:466–467

Hamilton JB (1951a) Quantitative measurement of a secondary sex character and axillary hair. Ann N Y Acad Sci 53:585–599

Hamilton JB (1951b) Patterned loss of hair in man; types and incidence. Ann N Y Acad Sci 53:708–728

Hamilton JB (1958) Age, sex and genetic factors in the regulation of hair growth in man: a comparison of Caucasian & Japanese populations. In: Montagna W, Ellis RA (eds) The biology of hair growth. Academic Press, New York pp 399–433

Harborne L, Fleming R, Lyall H, Norman J, Sattar N (2003) Descriptive review of the evidence for the use of metformin in polycystic ovary syndrome. Lancet 361:1894–1901

Hibberts NA, Randall VA (1996) Testosterone inhibits the capacity of cultured balding scalp dermal papilla cells to produce keratinocyte mitogenic factors. In: Van Neste D, Randall VA (eds) Hair research for the next millennium. Elsevier Science, Amsterdam pp 303–306

Hibberts NA, Messenger AG, Randall VA (1996a) Dermal papilla cells derived from beard hair follicles secrete more stem cell factor (SCF) in culture than scalp cells or dermal fibroblasts. Biochem Biophys Res Commun 222:401–405

Hibberts NA, Sato K, Messenger AG, Randall VA (1996b) Dermal papilla cells from human hair follicles secrete factors (eg VEGF) mitogenic for endothelial cells. J Invest Derm 106:341

Hibberts NA, Howell AE, Randall VA (1998) Dermal papilla cells from human balding scalp hair follicles contain higher levels of androgen receptors than those from non-balding scalp. J Endocr 156:59–65

Ibanez L, Ong KK, Mongan N, Jaaskelainen J, Marcos MV, Hughes IA, De Zegher F, Dunger DB (2003) Androgen receptor gene CAG repeat polymorphism in the development of ovarian hyperandrogenism. J Clin Endocr Metab 88:3333–3338

Imperato-McGinley J, Gautier T, Cai L, Yee B, Epstein J, Pochi P (1993) The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. J Clin Endocr Metab 76:524–528

Inui S, Itami S, Pan HJ, Chang C (2000) Lack of androgen receptor transcriptional activity in human keratinocytes lack of AR activity in Ha CaT keratinocytes. J Derm Sci 23:87–99

Itami S, Kurata S, Takayasu S (1990) 5α-reductase activity in cultured human dermal papilla cells from beard compared with reticular dermal fibroblasts. J Invest Derm 94:150–152

Itami S, Kurata S, Sonada T, Takayasu S (1995a) Interactions between dermal papilla cells and follicular epithelial cells in vitro: effect of androgen. Br J Derm 132:527–532

Itami S, Kurata S, Takayasu S (1995b) Androgen induction of follicular epithelial cell growth is mediated via insulin-like growth factor I from dermal papilla cells. Biochem Biophys Res Commun 212:988–994

Jackson D, Church RE, Ebling FJG (1972) Hair diameter in female baldness. Br J Derm 87:361–367

Jahoda CAB, Reynolds AJ (1996) Dermal-epidermal interactions; adult follicle-derived cell populations and hair growth. In: Whiting DA (ed) Derm clinics 14. Update on hair disorders. WB Saunders, Philadelphia pp 573–583

Jahoda CAB, Horne KA, Oliver RF (1984) Induction of hair growth by implantation of cultured dermal papilla cells. Nature 311:560–562

Jenkins JS, Ash S (1973) The metabolism of testosterone by human skin in disorders of hair growth. J Endocr 59:345–351

Kaufman KD, Olsen EA, Whiting D, Savi R, De Villez R, Bergfeld W and the Finasteride Male Pattern Hair Loss Study Group (1998) Finasteride in the treatment of men with androgenetic alopecia. J Am Acad Derm 39:578–589

Kligman AM (1959) The human hair cycle. J Invest Derm 33:307–316

Kligman AM (1988) The comparative histopathology of male-pattern baldness and senescent baldness. Clin Derm 6:108–118

Lakryc EM, Motta EL, Soares JM Jr., Haider MA, de Lima GR, Baracat EC (2003) The benefits of finasteride for hirsute women with polycystic ovary syndrome or idiopathic hirsutism. Gynaecol Endocr 17:57–63

Lavker RM, Sun TT, Oshima H, Barrandon Y, Akiyama M, Ferraris C, Chevalier G, Favier B, Jahoda CAB, Dhouailly D, Panteleyer AA, Christiano AM (2003) Hair follicle stem cells. J Invest Derm Symp Proc 8:28–38

Lesko SM, Rosenberg L, Shapiro S (1993) A case-control study of baldness in relation to myocardial infarction in men. J Amer Med Ass 269:998–1003

Levy JL, Trelles MA, de Ramecourt A (2001) Epilation with a long-pulse 1064 nm Nd: YAG laser in facial hirsutism. J Cosmet Laser Ther 3:175–179

Ludwig E (1977) Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Derm 97:247–254

Lumachi F, Rondinone R (2003) Use of cyproterone acetate, finasteride, and spirolactone to treat idiopathic hirsutism. Fertil Steril 79:942–946

Lynfield YL (1960) Effect of pregnancy on the human hair cycle. J Invest Derm 35:323–327

Maffei C, Fossati A, Reialdi F, Ruia E (1990) Personality disorders and psychopathologic symptons in patients with androgenetic alopecia. Arch Derm 130:868–872

Marshall WA, Tanner JM (1969) Variations in pattern of pubertal change in girls. Arch Dis Child 44:291–303

Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23

Merrick AE, Randall VA, Messenger AG, Thornton MJ (2004) Beard dermal sheath cells contain androgen receptors: implications for future inductions of human hair follicles. J Invest Derm (submitted)

Messenger AG, Elliott K, Temple A, Randall VA (1991) Expression of basement membrane proteins and interstitial collagens in dermal papillae of human hair follicles. J Invest Derm 96:93–97

Norwood OT (1975) Male pattern baldness, classification and incidence. South Med J 68:1359–1365

Obana N, Chang C, Uno H (1997) Inhibition of hair growth by testosterone in the presence of dermal papilla cells from the frontal bald scalp of the post-pubertal stump-tailed macaque. Endocrinology 138:356–361

Olsen EA, Dunlap FE, Funicella T, Koperski JA, Swinehart JM, Tschen EH, Trancik RJ (2002) A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. J Am Acad Derm 47:377–85

Orentreich N (1969) Scalp hair replacement in man. In: Montagna W, Dobson RL (eds) Adv Biol Skin Vol 9. Hair growth. Pergamon Press, Oxford pp 99–108

Orentreich N, Durr NP (1982) Biology of scalp hair growth. Clin Plas Surg 9:197–205

Pantelevev AA, Jahoda CAB, Christiano AM (2001) Hair follicle predetermination. J Cell Sci 114:3419–3431

Paus R, Müller-Röver S, McKay I (2000) Control of the hair follicle growth cycle. In: Camacho FM, Randall VA, Price VH (eds) Hair and its disorders: Biology, pathology and management. Martin Dunitz, London pp 83–94

Peereboom-Wynia JDR, Van Der Willigen AH, Stolz E, Van Joost TH (1989) The effect of cyproterone acetate on hair roots and hair shaft diameter in androgenetic alopecia in females In: Van Neste D, Lachapelle JM, Antoine JL (eds) Trends in human hair growth and alopecia research. Kluwer, Dordrecht pp 207–213

Philpott MP (2000) The roles of growth factors in hair follicles: investigations using cultured hair follicles. In: Camacho F, Randall VA, Price VH (eds) Hair and its disorders: Biology, research and management, Martin Dunitz, London pp 103–113

Price VH, Roberts JL, Hordinsky M, Olsen EA, Savin R, Bergfeld W, Fiedler V, Lucky A, Whiting DA, Pappas F, Culbertson J, Kotey P, Meehan A, Waldstreicher J (2000) Lack of efficacy of finasteride in postmenopausal women with androgenetic alopecia. J Am Acad Derm 43:768–76

Randall VA (1994a) Androgens and human hair growth. Clin Endocr 40:439–457

Randall VA (1994b) The role of 5α-reductase in health and disease. In: Sheppard M, Stewart P (eds) Hormones, enzymes and receptors. Baillière's clinical endocrinology and metabolism 8:vol 2:405–431

Randall VA (2000a) The biology of androgenetic alopecia. In: Camacho F, Randall VA, Price VH (eds) Hair and its disorders: Biology, research and management, Martin Dunitz, London pp 123–136

Randall VA (2000b) Androgens: the main regulator of human hair growth. In: Camacho F, Randall VA, Price VH (eds) Hair and its disorders: Biology, research and management, Martin Dunitz, London pp 69–82

Randall VA (2003) Biological effects of androgens on the hair follicle: experimental approaches. In: Van Neste D (ed) Hair science and technology, Brussels pp 75–92

Randall VA (2004) Physiology and pathophysiology of androgenetic alopecia. In: Degroot LJ, Jameson JL (eds). Endocrinology 5th ed WB Saunders Co Philadelphia (in press)

Randall VA, Ebling FJG (1991) Seasonal changes in human hair growth. Br J Derm 124: 146–151

Randall VA, Hibberts NA, Thornton MJ, Merrick AE, Hamada K, Kato S, Jenner TJ, De Oliveira I, Messenger AG (2001a) Do androgens influence hair growth by altering the paracrine factors secreted by dermal papilla cells? Eur J Derm 11:315–320

Randall VA, Hibberts NA, Thornton MJ, Hamada K, Merrick AE, Kato S, Jenner TJ, De Oliveria I, Messenger AG (2001b) The hair follicle: a paradoxical androgen target organ. Horm Res 54:243–250

Randall VA, Sundberg JP, Philpott MP (2003) Animal and in vitro models for the study of hair follicles. J Invest Derm Symp Proc 8:39–45

Randall VA, Thornton MJ, Hamada K, Redfern CPF, Nutbrown M, Ebling FJG, Messenger AG (1991) Androgens and the hair follicle: cultured human dermal papilla cells as a model system. Ann N Y Acad Sci 642:355–375

Randall VA, Thornton MJ, Messenger AG (1992) Cultured dermal papilla cells from androgen-dependent human follicles (e.g. beard) contain more androgen receptors than those from non-balding areas. J Endocr 133:141–147

Reynolds AJ, Lawrence C, Cserhalmi-Friedman PB, Christiano AM, Jahoda CAB (1999) Transgender induction of hair follicles. Nature 402:33–34

Saitoh M, Sakamoto M (1970) Human hair cycle. J Invest Derm 54:65–81

Sanchez LA, Perez M, Azziz R (2002) Laser hair reduction in the hirsute patient: a critical assessment. Hum Reprod Update 8:169–181

Sawers RA, Randall VA, Iqbal MJ (1982) Studies on the clinical and endocrine aspects of antiandrogens. In: Jeffcoate SL (ed) Androgens and antiandrogen therapy. Current topics in endocrinology. John Wiley, Chichester pp 145–168

Setty LR (1970) Hair patterns of the scalp of white and negro males. Amer J Phys Anthrop 33:49–55

Shapiro J, Kaufman KD (2003) Use of finasteride in the treatment of men with androgenetic alopecia (male pattern hair loss). J Invest Derm Symp Proc 8:20–23

Sonada T, Asada Y, Kurata S, Takayasu S (1999) The mRNA for protease nexin-1 is expressed in human dermal papilla cells and its level is affected by androgen. J Invest Derm 113:308–313

Stanford JL, Just JJ, Gibbs M, Wicklund KG, Neal CL, Blumenstein BA, Ostrander EA (1997) Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 57:1194–1198

Stenn K, Parimoo S, Prouty S (1998) Growth of the hair follicle: a cycling and regenerating biological system. In: Chuong CM (ed) Molecular basis of epithelial appendage morphogenesis. Laudes, Austin, Texas

Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM (2000) Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102:451–461

Terry RL, Davis JS (1976) Components of facial attractiveness. Percept Motor Skills 42:918–923

Thornton MJ, Laing I, Hamada K, Messenger AG, Randall VA (1993) Differences in testosterone metabolism by beard and scalp hair follicle dermal papilla cells. Clin Endocr 39:633–639

Thornton MJ, Hamada K, Messenger AG, Randall VA (1998) Beard, but not scalp, dermal papilla cells secrete autocrine growth factors in response to testosterone in vitro. J Invest Derm 111:727–732

Tsuji Y, Denda S, Soma T, Raferty L, Momoi T, Hibino T (2003) A potential suppressor of TGF-β delays catagen progression in hair follicles. J Invest Derm Symp Proc 8:65–68

Van der Dank J, Passchier J, Knegt-Junk C, Wegen-Keijser MH, Nieboer C, Stolz E, Verhage F (1991) Psychological characteristics of women with androgenetic alopecia: a controlled study. Br J Derm 125:248–252

Venning VA, Dawber R (1988) Patterned androgenic alopecia. J Amer Acad Derm 18:1073–1077

Wells PA, Willmoth T, Russel RJH (1995) Does fortune favour the bald? Psychological correlates of hair loss in males. Br J Psychol 86:337–344

Winter JSD, Faiman C (1972) Pituitary-gonadal relations in male children and adolescents. Paed Res 6:125–135

Winter JSD, Faiman C (1973) Pituitary-gonadal relations in female children and adolescents. Paed Res 7:948–953

Wu-Kuo T, Chuong C-M (2000) Developmental biology of hair follicles and other skin appendages. In: Camacho FM, Randall VA, Price VH (eds) Hair and its disorders: Biology, pathology and management. Martin Dunitz, London pp 17–37

Zachmann M, Prader A (1970) Anabolic and androgenetic effect of testosterone in sexually immature boys and its dependency on GH. J Clin Endocr Metab 30:85–95

Zachmann M, Aynsley-Green A, Prader A (1976) Interrelations of the effects of growth hormone and testosterone in hypopituitarism. In: Pecile A, Muller EE (eds) Growth hormone and related peptides. Excerpta Medica, Amsterdam and Oxford pp 286–296

7

Androgens and bone metabolism

M. Zitzmann and E. Nieschlag

Contents

7.1 Introduction

Osteoporosis and fractures represent a major public health problem, not only in women but also in men. It has been estimated that at the age of 50 years, men have a risk of approximately 12–15% of suffering an osteoporotic fracture in later life, most commonly of the vertebra, hip or forearm (Melton and Chrischilles 1992; Nguyen *et al.* 1996). At the age of 60 years, the risk for a non-traumatic fracture rises to 25% (Nguyen *et al.* 1996). In the United States, about 150,000 hip fractures occur in men each year (Poor *et al.* 1995). Because of their higher peak bone mass, men present with hip, vertebral body, or forearm fractures about 10 years later than women. Hip fractures in men result in a 30% mortality rate at one year after fracture versus a rate of 17% in women (Campion and Maricic 2003). Hypogonadism, i.e. androgen deficiency, has been identified as an independent risk factor for such

incidences (Jackson *et al.* 1992). The role of androgens in bone metabolism and the effects of androgen therapy on bone tissue in hypogonadal men will be examined in this chapter.

7.2 Mechanisms of androgen action in bone tissue

7.2.1 In vitro studies

Growth and resorption of bone tissue are mediated by osteoblasts and osteoclasts, respectively. Both types of cells exert mutual influence on each other and equilibrium between the activity of both cell lines maintains net bone mass during constant renewal and turnover, while decreased osteoblast activity as well as enhanced osteoclast activity will result in loss of bone mass. Androgen receptors have been located on normal human osteoblasts (Colvard *et al.* 1989) and both aromatizable and non-aromatizable androgens can stimulate human osteoblast proliferation in vitro (Kasperk *et al.* 1997), a process that requires adequate storage of vitamin D (Somjen *et al.* 1989).

Bone deformation strain represents a stimulus for osteoblastic activity. Androgens modify the effects induced by the mechanoperception of human osteoblastic cells by affecting adhesion molecule expression, i.e. fibronectin and the fibronectin receptor. These substances facilitate the adhesion of bone cells to the extracellular matrix, which represents a crucial requirement for osteoblastic development and function (Liegibel *et al.* 2002). In addition, osteoprotegerin secretion, which is unaffected by mechanical strain alone, is doubled when this stimulus occurs in the presence of androgens. Osteoprotegerin represents a decoy receptor for RANKL (receptor activator of nuclear factor-kappaB ligand). RANKL is secreted by osteoblasts; it induces osteoclastogenesis and stimulates osteoclast differentation (Khosla 2001). Thus, osteoprotegerin inhibits bone resorptive effects induced by RANKL (Liegibel *et al.* 2002). Accordingly, testosterone levels are positively associated with osteoprotegerin concentrations in cross-sectional approaches in healthy men (Szulc *et al.* 2001). In contrast, dihydrotestosterone has been reported to decrease osteoprotegerin mRNA expression in a fetal human osteoblastic cell line (Hofbauer *et al.* 2002). Testosterone also directly inhibits shedding of RANKL by osteoblasts (Huber *et al.* 2001); similarly, androgen receptor knock-out mice exhibit an upregulation of RANKL production (Kawano *et al.* 2003).

Local action of cytokines such as interleukins 1 and 6 (IL-1 and IL-6) plays an important role in bone metabolism. Both substances induce bone resorption by promotion of osteoclast activation and diffentiation. Androgens inhibit expression of the IL-6 gene in marrow-derived stromal cells, an effect requiring the androgen receptor (Bellido *et al.* 1995, Hofbauer *et al.* 1999). Similar effects were observed concerning IL-1 expression (Pilbeam and Raisz 1990). Latter effect seems

to be age-dependent and is mitigated in cell cultures from older mice (Wang *et al.* 1999).

Parathyroid hormone (PTH) induces osteoclast formation and differentiation. Androgens have a direct inhibiting effect on this process via osteoclasts, which express androgen receptors: these cells are also blocked from PTH effects by androgens when no conversion to estrogens occurs (Chen *et al.* 2001). In addition, PTH-stimulated accumulation of cAMP in osteoblasts can also be mitigated by androgens (Fukayama and Tashijian 1989).

The direct impact of androgens on osteoclasts has not been fully resolved. A direct effect on resorption activities of osteoclasts has been reported: an inhibition of osteoclastic functions was seen in response to testosterone and dihydrotestosterone; this could be blocked by the androgen receptor antagonist flutamide. Interestingly, also β-DHT, a stereoisomer of α-DHT, that is inactive in other androgen receptor-dependent systems, can achieve androgen effects, which supports the hypothesis that the osteoclast androgen receptor has unusual ligand-binding properties (Pederson *et al.* 1999). In contrast, resorption pits produced by cultured primary osteoclasts from androgen receptor knock-out mice seemed normal both in terms of numbers and area when compared with osteoclasts from wild-type (WT)-littermates (Kawano *et al.* 2003).

It is possible that some of these effects are due to non-genotropic action of activated androgen receptors. There are indications that a non-specific activation of the ligand binding domain of the androgen receptor both by androgenic and estrogenic compounds can induce anti-apoptotic effects in osteoblasts and increase apoptosis in osteoclasts. This effect seems to be dissociated from transcriptional activity (Kousteni *et al.* 2001; 2003). Corresponding effects were seen in a mouse model (see 7.2.2).

Thus, androgens decrease the number of bone remodeling cycles by modifying the genesis of osteoclasts and osteoblasts from their respective progenitor cells. In addition, androgens also exert effects on the lifespan of mature bone cells: they exert pro-apoptotic effects on osteoclasts and anti-apoptotic effects on osteoblasts and osteocytes. Testosterone also modulates effects induced by other hormones and cytokines involved in bone metabolism. For a summary see Fig. 7.1.

7.2.2 Animal studies

Complementary to in vitro investigations, studies in animals can address questions concerning bone tissue related to systemic withdrawal and re-administration of aromatizable and non-aromatizable androgens, especially in regard to bone histomorphometry, where study designs are not feasible in humans.

The simplest approach to assess sex steroid effects in male animals is orchiectomy. The majority of such trials has been performed in rats, in which androgen

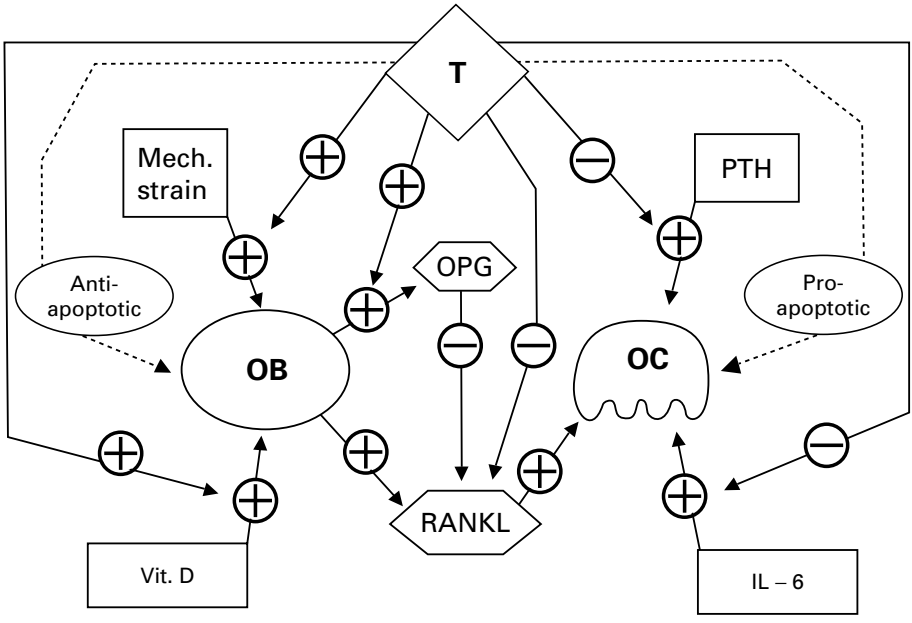


Fig. 7.1 Role of testosterone (T) in bone metabolism. The nature of exerted influence is indicated by plus (positive) or minus (negative) signs on the arrows. Abbreviations: OB: osteoblast, OC: osteoclast, OPG: osteoprotegerin, RANKL: receptor activator of nuclear kappaB-ligand, IL-6: interleukin 6, Vit. D: vitamin D, mech. strain: mechanical strain, PTH: parathyroid hormone. The broken lines symbolize putative non-genomic action.

effects depend both on age and the type of bone examined. In younger male rats, orchiectomy results in reduction of calcium content of long bones and increment of tibial osteoclasts (Saville 1969; Schoutens *et al.* 1984). Neither the cross-sectional bone area, nor its medullary or cortical compartment is altered by castration of young rats, but the periosteal cortical bone formation rate is reduced (Gunness and Orwoll 1995; Turner *et al.* 1989). In trabecular bone, osteoclast density increases after orchiectomy, resulting in loss of bone tissue. (Gunness and Orwoll 1995; Turner *et al.* 1989; Wakley *et al.* 1991). Corresponding effects were also seen in young orchiectomized beagle dogs: The mean trabecular thickness and the fraction of labeled osteoid surface decreased significantly three months after orchidectomy, but other histomorphometric parameters were unchanged. In the period 7–12 months after orchidectomy, bone volume, mean trabecular thickness, and the fraction of labeled trabecular surface decreased significantly compared with the pre-orchidectomy values (Fukuda and Jida 2000).

In older rats, orchiectomy results in reduction of the calcium content in femur and tibia, which is due to a loss of cortical thickness without affecting of bone density (Vanderschueren *et al.* 1992; Verhas *et al.* 1982). Nevertheless, trabecular

bone mass is reduced in these animals (Vanderschueren *et al.* 1992; Wink and Felts 1980). Such was also seen in the cancellous bone area of the proximal tibia of castrated male rats (Erben *et al.* 2000). The effect was related to an increase of tissue turnover facilitated by invasion of osteoclasts to the trabecular surface. All these effects on the skeleton of rats can be prevented by administration of aromatizable or non-aromatizable androgens (Vanderschueren *et al.* 1992). This was also observed in orchiectomized, testosterone-substituted estrogen-receptor-α knock-out mice (Vandenput *et al.* 2001) or castrated, androgen-supplemented and estrogen-receptor blocked rats (Vandenput *et al.* 2002), suggesting an androgen-receptor mediated effect. There are indications that the modulating influence of androgens on apoptosis of bone-morphogenetic cells is, at least partially and in mice, non-genomic (Kousteni *et al.* 2002). In contrast, there exists a significant influence of the CAG repeat polymorphism of the androgen receptor gene, which is associated with the transcriptional activity of androgen target genes, on bone density and bone turnover in humans (see Chapters 2 and 3).

In androgen-receptor knock-out (ARKO) mice, bone density of both femur and tibia was reduced in comparison to wild-type (WT) litter-mates. In a controlled model using orchiectomy in both ARKO and WT mice, bone density could be fully restored by testosterone substitution in the WT mice. A significant increment of bone density during testosterone substitution was also seen in the orchiectomized ARKO mice, but this was still markedly blunted in comparison to WT mice. This suggests a partial effect of aromatization mediated by the estrogen receptor and, simultaneously, that for full restoration of bone tissue, the androgen receptor is necessary. When the non-aromatizable androgen dihydrotestosterone was given to orchiectomized ARKO and WT mice, no effect on bone tissue was seen in the knock-out animals, while the WT mice exhibited a significant increment of bone density; the latter effect was, nevertheless, blunted in comparison to effects of aromatizable testosterone in orchiectomized WT mice, again suggesting the crucial role of both the androgen and estrogen receptor and, hence, both sex steroids, for complete beneficial effects on bone metabolism (Kawano *et al.* 2003) (also see 7.2.5).

7.2.3 Androgens and their relation to calcium regulatory hormones and IGF-1

Several older reports concerned direct androgen effects on calcium regulatory hormones. It has been claimed that calcitonin concentrations are lowered in hypogonadal men and levels can be increased by testosterone administration (Foresta *et al.* 1983; 1985). In rats, androgens seem to enhance hypocalcemia induced by calcitonin (Ogata *et al.* 1970). Concerning parathyroid hormone (PTH) concentrations, an increment under testosterone substitution therapy of hypogonadal men has been reported (Katznelson *et al.* 1996; Wang *et al.* 1996; 2001). The skeletal responsiveness to PTH seems to be increased in hypogonadism, as an experimental setting

in men receiving a GnRH-agonist demonstrates (Leder *et al.* 2001). This effect is likely to be indirect and caused by withdrawal of androgenic influence on osteoclast-responsiveness to PTH. In regard to vitamin D, which is under direct influence of PTH, no consistent findings concerning androgenic influence have been reported. Long-term physiological testosterone replacement did not alter 1,25-(OH)2 vitamin D levels in hypogonadal men (Finkelstein *et al.* 1989; Tenover 1992) while this was observed on a short-term basis in a small uncontrolled study (Hagenfeldt *et al.* 1992). Altogether, the modulation androgens exert on the effects of calcium regulatory hormones on bone morphogenetic cells seems to be relevant, while direct androgenic influence on the concentrations of these hormones do not seem to play an important role.

Some effects of testosterone on bone tissue may be facilitated indirectly by growth hormone (GH) and, consequently, insulin-like growth factor type 1 (IGF-1) levels. These hormones have an intrinsic effect on bone tissue, increasing bone mass and density (Baum *et al.* 1996; Grinspoon *et al.* 1995; Monson 2003). Administration of androgens to hypogonadal men enhances GH secretion, mediated at the hypothalamic level primarily by promoting GHRH shedding (Bondenelli *et al.* 2003). Elevation of testosterone levels increases exercise- or GHRH-stimulated GH pulsatility in hypo- and eugonadal men; consecutively, these effects were also observed for IGF-1 concentrations (Fryburg *et al.* 1997). This appears to be an important issue for assessing the role of the somatotropic axis is important when androgen effects on bone metabolism are investigated.

7.2.4 Androgens and bone turnover in men

The opposing activities of osteoblasts forming new bone and osteoclasts resorbing old bone create the continuous process of bone remodeling. While these actions are antagonistic, both types of cells exert mutual influence of both positive and negative nature on each other via paracrine cross-talk to maintain an equilibrium. Quantitative bone histomorphometry of biopsies to assess parameters of this homeostasis are useful in animal studies but are of restricted applicability in patients. In this case, chemical markers of osteoblast- and osteoclast activities, hence bone turnover, can be assessed in blood and urine. Osteoblast activity is reflected by concentrations of type 1 procollagen extension peptides (carboxy-terminal: P1CP or amino-terminal: P1NP) and other non-collagenous proteins secreted by osteoblasts, such as osteocalcin and bone specific alkaline phosphatase (BSAP). Also osteoprotegerin (OPG), as a decoy receptor for RANKL (see 7.2.2) can serve as marker of osteoblast action. Bone resorption, hence osteoclast activity, can be estimated by urinary excretion of degradation products of type I collagen, such as pyridinium cross-links (deoxypyridinoline, DPD) and collagen type I cross-linked N-telopeptide (NTX) (Riggs *et al.* 2002).

Small uncontrolled studies in hypogonadal men suggest an elevation of bone turnover: both markers of osteoblastic activity (osteocalcin and BSAP) and osteoclastic action (urinary hydroxyproline excretion) were found to be elevated in such patients (Goldray *et al.* 1993; Jackson *et al.* 1987; Stepan *et al.* 1989). Correspondingly, in healthy younger men, testosterone levels are negatively associated both with serum BSAP and urinary DPD concentrations. As the CAG repeat polymorphism of the androgen receptor gene is involved in this association (longer repeats mitigating androgen effects are positively related to BSAP and DPD levels), effects are most likely directly linked to androgens and their receptor (Zitzmann *et al.* 2001) (see 3.4.5). It can be speculated that the lower androgen levels allow for higher bone resorption activity. In counter-regulation, bone formation would be upregulated. In case of hypogonadism, this still would result in an dysequilibrium pointing towards bone resorption.

Several studies have addressed the effects of testosterone administration to hypogonadal men in regard to markers of bone turnover. In a large, uncontrolled study in over 200 hypogonadal men receiving testosterone gel for substitution, it was demonstrated bone that osteocalcin and PINP concentrations increased transiently upon elevation of androgen concentrations, returning to baseline after the initial 90 days of treatment, while NTX as bone resorption marker decreased dose-dependently (Wang *et al.* 2001). This confirms earlier reports (Katznelson *et al.* 1996; Wang *et al.* 1996).

While the study involving the androgen receptor polymorphism points to a direct androgen effect, it cannot be concluded from the interventional trials whether the effects of testosterone are caused directly or by its aromatization-metabolite estradiol (also see 7.2.5); this has been adressed by two short-term studies with a sophisticated design. One trial involved 59 healthy men aged 68 $\pm$ 6 years whose endogenous sex steroid production was suppressed by a long-acting GnRH agonist and an aromatase inhibitor; subjects were then randomized to four groups receiving transdermal substitution of either testosterone and estradiol, testosterone alone, estradiol alone or placebo. The respective treatment times lasted, however, only three weeks (Falahati-Nini *et al.* 2000). Another group used a similar approach which lasted 12 weeks: 70 younger healthy men aged 20 to 44 years were treated with a long-acting GnRH agonist and randomized to three groups receiving either no substitution or transdermal testosterone with or without aromatase inhibitor (Leder *et al.* 2003). Since the first approach is shorter but involves an estrogen-alone group, there is some dispute between the authors which design yields the most useful answers (Khosla and Riggs vs. Leder and Finkelstein 2003). The results are more or less uniform in regard to bone resorption markers: The shorter study sees a marked increase of urinary DPD and NTX excretion in the placebo-treated hypogonadal group in comparison to baseline. Treatment with testosterone alone showed a trend

to reduce these effects, while treatment with estradiol alone inhibits bone resorption to a stronger extent, but not fully. In the group receiving both sex steroids, no difference to baseline values was observed (Falahati-Nini *et al.* 2000). Corresponding results were seen in the longer study involving younger men, although testosterone effects on DPD excretion were significant (Leder *et al.* 2003). This demonstrates that androgens and estrogens play an independent and fundamental role in inhibition of bone resorption.

In regard to bone formation markers, results differ: the shorter study found that serum osteocalcin and P1NP declined at three weeks of estrogen and testosterone deficiency. Estrogen alone was able to prevent the decrease in both formation markers, whereas testosterone alone (in the presence of an aromatase inhibitor) prevented the decrease in osteocalcin, but not in P1NP. Continued treatment with both sex steroids resulted in no change in the formation markers. It was concluded that both estrogen and testosterone are important in the maintenance of mature osteoblastic function. It remains speculative whether differences between both markers are due to the fact that osteocalcin isa relatively late marker in osteoblast differentiation, possibly inhibiting apoptosis, and due to the fact that P1NP is produced throughout osteoblast differentiation (Falahati-Nini *et al.* 2000). In contrast, in the second, longer study, the untreated hypogonadal men exhibited an increase of the bone formation markers osteocalcin, P1CP and P1NP which was counteracted by testosterone alone and, to a stronger extent, by both sex steroids. It remains unresolved whether these contrasts are due to differences in the study populations (younger vs. older men) or caused by observations at different time-points, suggesting that the shorter study was better able to separate direct effects of gonadal steroid deprivation (such as increased osteoblast apoptosis) from indirect ones (resorption-coupled increase in osteoblast activity) (Khosla and Riggs vs. Leder and Finkelstein 2003). The results of the longer study are in agreement with observations of increased markers of bone formation in clinical long-term hypogonadism. Also weaker androgen effects due to longer CAG repeats in the androgen receptor gene (see above) may reflect both increased bone formation and resorption.

In conclusion, an independent role of androgens in protecting bone mass, both by promoting bone formation and attenuating bone resorption has been demonstrated in humans. Nevertheless, the role of its metabolite estradiol is pivotal in bone metabolism (see 7.2.5).

7.2.5 The role of estradiol as testosterone metabolite in bone metabolism

Aromatization of testosterone to estradiol is a pivotal event concerning effects of sex steroids on bone metabolism. Estrogen receptors (ER) have been localized in human osteoblasts (Eriksen *et al.* 1988; Komm *et al.* 1988), osteoclasts (Oursler *et al.* 1991; 1994) and osteocytes (Braidman *et al.* 2000; Tomkinson *et al.* 1998). The

ERα- variant is predominant, but also the ERß-type and heterodimers have been described (Kuiper *et al.* 1996; Petterson *et al.* 1997). Respective knock-out mouse models (αERKO, ßERKO and double-ERKO) have demonstrated decreased bone-growth, especially for those animals lacking the ERα (Couse *et al.* 1999; Vidal *et al.* 2000). Correspondingly, human males with mutations in the ERα or aromatase genes do not achieve normal bone density, despite normal or increased levels of serum testosterone (Bilezikian *et al.* 1998; Carani *et al.* 1997; Grumbach 2000; Morishima *et al.* 1995; Smith *et al.* 1994). Moreover, as the continual rise in serum estradiol levels during puberty probably causes epiphyseal closure in both sexes, young adult males who are unable to respond to estradiol or who are deficient of this hormone (Grumbach 2000; Smith *et al.* 1994) have open epiphyses, whereas men with testicular feminization due to mutations of AR achieve epiphyseal closure. Nevertheless, due to the missing intrinsic androgen effects, these patients also exhibit decreased bone density (Bertelloni *et al.* 1998; Marcus *et al.* 2000; Zachman *et al.* 1986). As a consequence, estradiol substitution in aromatase-deficient men can normalize bone density (Bilezikian *et al.* 1998; Carani *et al.* 1997; Rochira *et al.* 2000; 2002).

Moreover, ER polymorphisms that permit normal, but nevertheless significantly graduated effects of estradiol, also cause variations in bone density, both in women and men (Albagha *et al.* 2001; Becherini *et al.* 2000; Ho *et al.* 2000; Langdahl *et al.* 2000; Patel *et al.* 2000; Sowers *et al.* 1999; van Pottelbergh *et al.* 2003). The menopausal bone loss in women which can be inhibited by estrogen substitution is quite in agreement with these reports (Riggs *et al.* 2002). In conjunction with observations of estradiol-related effects on bone metabolism (see above), these animal studies and clinical reports provide compelling evidence that estrogens have a major role in bone metabolism.

7.2.6 Relation of androgens to bone tissue in healthy men

Bone density is determined both by peak bone mass achieved during skeletal development and the subsequent amount of maintenance and resorption of bone tissue. Androgens affect both processes and thus are a pivotal determinant of bone mass in men. Trabecular and cortical bone density increase dramatically during puberty, both in girls and boys (Krabbe *et al.* 1984), but peak cortical bone density is about 25% higher in healthy men compared to women, an observation which has been linked to higher testosterone levels present in males (Riggs *et al.* 2002). Bone density is maintained at a relatively stable level in younger men, then starts to decline slowly at the age of 30 to 35 years in healthy men (Fig. 7.2, Scopacasa *et al.* 2002; Zitzmann *et al.* 2002). The age-related bone loss is putatively associated with declining testosterone levels, a process partly leading to late-onset hypogonadism, but is not uniformly present. Thus, reports on sex steroid levels within the normal

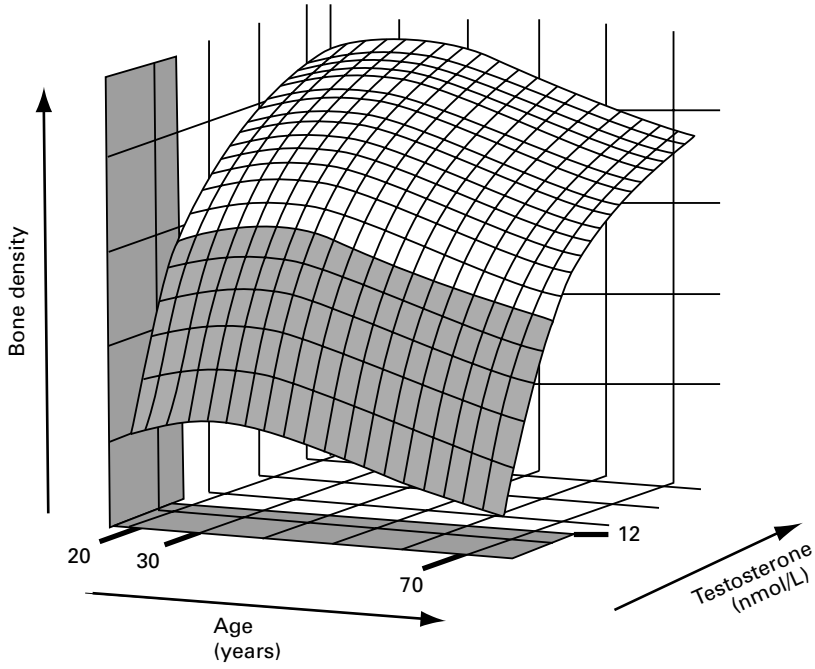


Fig. 7.2 Model of bone density in relation to age and testosterone levels. Data from 156 newly diagnosed untreated hypogonadal men (62 men with primary and 94 men with secondary hypogonadism) and 224 healthy controls aged 18 to 91 years. Bone density was assessed by phalangeal ultrasound. The surface was created according to non-linear regression models (third-degree association of age to bone density and logarithmic association of testosterone levels to bone density). The hypogonadal range is indicated in dark grey.

range and bone density vary concerning the importance of estradiol or testosterone concentrations within the normal range. Nevertheless, significant contributions of both sex steroids to bone density of older men have been frequently reported (Cetin *et al.* 2001; Greendale *et al.* 1997; Khosla *et al.* 2001; Rudman *et al.* 1994; van den Beld *et al.* 2000; van Pottelbergh *et al.* 2003). It can be assumed that age-related processes which are at best indirectly related to testosterone levels (e.g. inactivity, reduced muscle mass, increasing PTH concentrations) (Riggs *et al.* 2002) as well as androgen concentrations themselves exert influence on bone density (Zitzmann *et al.* 2002). While the latter gain importance when the threshold between eu- and hypogonadism is considered, differences in androgenic influence are much less overt when fluctuations within the normal range are investigated (Fig. 7.2). Variations of bone density in eugonadal men in relation to androgenic activity are, within an environment of more or less saturated androgen receptors, rather influenced by the CAG repeat polymorphism of the androgen receptor gene than by testosterone levels themselves. Men with longer CAG repeats, which are associated

with decreased androgen effects, exhibit lower bone mass and also a more rapid age-related decline of bone density than healthy men with shorter repeats (Zitzmann *et al.* 2001, also see Chapter 3).

While studies on bone density in eugonadal men are of some value in elucidating the relationship between androgens and bone tissue, they have no clinical consequence, quite in contrast to observations in hypogonadal men and the effects of androgen treatments on bone density in such patients, which will be discussed below.

7.3 Clinical aspects of bone density in relation to disorders of androgen action

7.3.1 Bone density in men with disorders of androgen action

A clinical model of androgen effects on bone tissue is represented by the cohort of men undergoing therapeutic orchiectomy for the treatment of prostate cancer or sexual delinquency. In 12 men of the latter group, bone mineral density of the lumbar spine decreased after bilateral orchiectomy (Stepan *et al.* 1989). Corresponding effects were seen in men treated with surgical or chemical castration for prostate cancer; as a consequence, osteoporotic fractures were significantly increased in comparison to controls (14% vs. 1%) (Daniell 1997; 2000). This has been recently confirmed by a study involving 429 men who underwent bilateral orchiectomy for treatment of prostate cancer. Fractures were ascertained from medical records and compared with expected numbers based on local incidence rates; this demonstrated a three-fold increase of fractures accounted for by moderate trauma of the hip, spine and distal forearm, locations traditionally linked with osteoporosis (Melton *et al.* 2003). The administration of long-acting GnRH agonists, which has been used for treatment of benign prostate hyperplasia has similar effects (Goldray *et al.* 1993). Inhibition of 5α-reductase by finasteride for benign prostate hyperplasia led to significantly decreased levels of dihydrotestosterone, but did not result in any changes of bone metabolism or density (Tollin *et al.* 1996). Also the inhibition of both 5α-reductase isoforms, which is not effected by finasteride but the new substance dutasteride, has no consequence on bone tissue (but an elevated incidence of impotence, decreased libido, ejaculation disorders and gynaecomastia were observed in large, randomized, double-blind clinical trials involving 5655 men) (Andriole and Kirby 2003). This points out that testosterone and its metabolite estradiol are sufficient for maintenance of bone tissue, but not the 5α-reduction metabolite dihydrotestosterone.

In patients with androgen insensitivity, the effects observed in ARKO mice (see above) are confirmed. In patients with this rare condition, cortical bone mineral density is markedly lower than in normal male controls but similar to that of

age-matched women (Bertelloni *et al.* 1998; Marcus *et al.* 2000; Zachman *et al.* 1986).

The majority of men with defective androgen action, however, present with other diagnoses: with primary hypogonadism due to Klinefelter syndrome or a condition after testicular tumors and men with secondary hypogonadism due to Kallmann syndrome, idiopathic hypogonadotropic hypogonadism, pituitary disorders of various kinds or late-onset hypogonadism (Behre *et al.* 2000). A marked decrease in bone density in comparison to controls is seen all these patient groups, but especially in those men with secondary hypogonadism as demonstrated by a large study involving 156 newly diagnosed untreated hypogonadal men (62 men with primary and 94 men with secondary hypogonadism) and 224 healthy controls aged 18 to 91 years. This is due to those men within a group of secondary hypogonadal patients with a congenital disorder of gonadotropin secretion causing impaired bone maturation during puberty (Zitzmann *et al.* 2002). An earlier report in a smaller cohort showed similar results (Behre *et al.* 1997). Bone density declines with decreasing total testosterone concentrations in a non-linear fashion, with a four to five-fold larger reduction for each nanomole-per-liter decrement in total testosterone in the hypogonadal range (<12 nmol/L) compared with the eugonadal range (Zitzmann *et al.* 2002). This is expressed in the non-linear association model demonstrated by Fig. 7.2 and suggests that hypogonadism does not present a uniform condition, but that androgen effects on bone tissue are still distinctly measurable depending on the androgen concentration, albeit below the eugonadal range. This is corroborated by findings of treatment effects which strongly depend on initial testosterone concentrations (see below).

7.3.2 Effects of androgen substitution on bone tissue

The effects of androgen replacement on bone mass have been addressed by several studies. An early report on results in a mixed group of 36 hypogonadal men demonstrated a significant increase of spinal bone density assessed by radiological methods dual-energy X-ray absorptiometry (DXA) and quantitative computer tomography (QCT) during 12 to 18 months of therapy. Corresponding results were seen in 37 men with primary and in 35 men with secondary hypogonadism. Bone density of the spine as determined by QCT increased particularly in those patients who had lower bone density at the start of the study and those who had not received gonadal steroid therapy. A more detailed approach in 32 of these patients demonstrated that this increase was due to gain of both trabecular and cortical bone tissue, while the vertebral body area did not increase (Leifke *et al.* 1998). In a prospective multicenter trial using different transdermal testosterone preparations in 227 men with hypogonadism of heterogeneous origin (about 60% of these men with prior testosterone treatment), significant increases both in spinal and hip bone density as detemined by DXA were seen (Wang *et al.* 2001). The importance of baseline androgen

concentrations on the outcome of testosterone replacement in respect to increment of bone density is stressed by a study in 108 men older than 65 years receiving either placebo or transdermal testosterone. Androgen concentrations were not a selection criterion and significant changes in terms of higher bone density as assessed by DXA occurred only in the hypogonadal men. Bone density of the eugonadal men was not influenced by testosterone administration, quite in agreement with models of non-linear testosterone effects on bone tissue (see above, Fig. 7.2) (Snyder *et al.* 1999). Corresponding results are also reported from a similar study in 44 men older than 65 years in a placebo-controlled study (Kenny *et al.* 2001).

The above-mentioned methods to assess bone density apply radiation and are costly, thus limiting their widespread and repeated use. The feasibility of inexpensive nonradiation methods applied by portable devices using quantitative ultrasound (QUS) to measure transmission speed in bone tissue has also been demonstrated. The feasibility of using phalangeal QUS in comparison to radiological methods for the assessment of fracture risk and monitoring changes in bone density during hormone substitution therapy or bisphosphonate medication was demonstrated in a large study involving more than 10,000 women (Wüster *et al.* 2000).

Concerning men, a detailed cross-sectional approach involving 521 men (226 healthy controls, 156 hypogonadal men and 141 men receiving testosterone substitution for at least two years) compared this method to QCT of the lumbar spine and showed differences between the respective patient groups (Zitzmann *et al.* 2002). In comparison to QCT, patients with a lumbar content of hydroxylapatite of $<100\ mg/cm^3$ were reliably identified by phalangeal QUS (cutoff level 1965 m/sec which is a T score of -3.5 based on eugonadal subjects). The receiver operating characteristics showed an area under the curve of 0.94 (sensitivity 94%, specificity 92%, $p < 0.0001$). Since the association was non-linear, QCT values within the normal range could not be predicted by pQUS. A treatment effect was visible over the complete age range of 20 to 70 years of substituted patients. In comparison to the eugonadal men, substituted individuals had an overall significantly lower bone density ($p < 0.004$), which was caused by differences in the age groups <50 years, but patients receiving substitution therapy had a significantly higher bone density in comparison to hypogonadal patients in all age groups ($p < 0.0001$). Significant differences of bone density were seen in all patient subgroups comparing untreated hypogonadal men to substituted individuals of the respective diagnosis (non-Klinefelter patients with primary hypogonadism, Klinefelter patients, idiopathic hypogonadotropic hypogonadal men, subjects with postpubertal onset of secondary hypogonadism or late-onset hypogonadism, respectively, with $p < 0.001$ for every subgroup). Age, treatment modality and duration of treatment did not have a significant influence on the effect of substitution ($p = 0.13$, $p = 0.96$, and $p = 0.24$, respectively), but levels of substituted testosterone showed a trend toward a positive association with bone density ($p = 0.07$) (Zitzmann *et al.* 2002).

In a longitudinal section of this study involving 54 men, a general improvement of bone density occurred ($p < 0.0001$). Those patients with initially lower bone density gained significantly more bone density ($p < 0.001$); this also applies to initially lower testosterone levels ($p = 0.03$). Putative influences on results by age, diagnosis, treatment modality, duration of treatment and gained testosterone levels were not significant ($p = 0.43$, $p = 0.88$, $p = 0.18$, $p = 0.15$, and $p = 0.65$, respectively), although initial bone density and testosterone levels showed a trend toward lower values in subjects with secondary hypogonadism in comparison to those with primary hypogonadism ($p = 0.07$) (Zitzmann *et al.* 2002).

These results suggest that bone density of androgen-deficient men is improved by testosterone therapy but may not reach the level of age-matched healthy eugonadal controls, especially in those patients with congenital disorders of hypogonadism, hence impaired pubertal skeletal maturation. This is confirmed by a smaller study in 33 secondary and 20 primary hypogonadal men using DXA (Schubert *et al.* 2003).

The potent synthetic androgen 7α-methyl-19-nortestosterone (MENT), which is resistant to 5α-reductase but is a substrate for aromatase and may therefore offer selective sparing of the prostate gland while supporting other androgen-dependent tissues, was tested in 16 hypogonadal men (Anderson *et al.* 2003). A prostate-sparing effect could, as intended, be demonstrated. Nevertheless, while androgen-dependent functions such as erythropoeisis (see Chapter 9) and psychosocial implications were adequately restored by MENT, a decrease in spinal bone mass was observed. Simultaneously, serum markers of bone formation decreased, while urinary excretion of bone resorption indicators increased. As it has been demonstrated that 5α-reduced androgens do not play a major role in bone metabolism, results point towards the importance of sufficient aromatization to maintain bone mass. It is suggested that aromatization-endproducts of MENT are less potent activators of estrogen receptors than estradiol itseslf (Anderson *et al.* 2003).

In conclusion, testosterone substitution in hypogonadal men restores bone mass. To this end, aromatization to estradiol is paramount in addition to intrinsic androgen activities.

7.3.3 Additional modalities for therapy of androgen-related bone loss

To date, testosterone substitution is the only form of therapy that has been systematically evaluated for the treatment of bone loss in male hypogonadism. However, some hypogonadal men may have contraindications to testosterone therapy, especially those with a prostate carcinoma. In addition, in some hypogonadal men, androgen therapy may not be fully sufficient to elevate bone mass into a safe range, which might be especially the case in persons receiving additional glucocorticoid therapy or being afflicted with chronic renal diseases. Thus, in addition to sex

steroids, other hormones or substances valuable for maintenance of bone mass can be considered.

For example, the osteoclast-inhibitor alendronate exerts beneficial effects on bone tissue in men with osteoporosis (Orwoll *et al.* 2000). Also injections of a fragment of PTH, teriparatide (rhPTH1-34), has stimulatory effects on bone formation in men, resulting in significant increase of bone mass (Orwoll *et al.* 2003). Men suffering from growth hormone deficiency may profit from respective supplementation in regard to maintenance of bone tissue (Baum *et al.* 1996; Grinspoon *et al.* 1995; Monson 2003; Ahmad *et al.* 2003).

It has to be stressed that sufficient supplementation of vitamin D and calcium to all these bone-protective agents has a measurable effect on their efficacy (Orwoll *et al.* 2000; 2003; Monson 2003).

7.4 Future research

As indicated by the trial involving MENT as a synthetic androgen, the future lies with substances which have a prostate-sparing effect but can exert the beneficial effects of androgens. This could be facilitated by selective androgen receptor modulators (SARMS), which are currently under development (see Chapter 20). It will be crucial for the efficacy of these substances that their aromatization-endproducts exert sufficient effects at the estrogen receptor. This is especially the case in regard to bone tissue. Future trials involving such SARMS will need to address bone metabolism and density as a pivotal endpoint.

7.5 Key messages

- Osteoporosis is common in men, especially with advancing age.
- Hypogonadism is a major risk factor for the development of osteoporosis in men.
- Androgens can stimulate osteoblasts and inhibit osteoclasts, thus preventing bone loss.
- The efficacy of testosterone substitution in hypogonadal men to prevent osteoporosis has been demonstrated.
- Aromatization of testosterone to estradiol is of pivotal importance for this effect, although testosterone itself exerts intrinsic activity on bone tissue.

7.6 REFERENCES

Ahmad AM, Thomas J, Clewes A, Hopkins MT, Guzder R, Ibrahim H, Durham BH, Vora JP, Fraser WD (2003) Effects of growth hormone replacement on parathyroid hormone sensitivity and bone mineral metabolism. J Clin Endocrinol Metab 88:2860–2868

Albagha OM, McGuigan FE, Reid DM, Ralston SH (2001) Estrogen receptor alpha gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. J Bone Miner Res 16:128–134

Anderson RA, Wallace AM, Sattar N, Kumar N, Sundaram K (2003) Evidence for tissue selectivity of the synthetic androgen 7 alpha-methyl-19-nortestosterone in hypogonadal men. J Clin Endocrinol Metab 88:2784–2793.

Andriole GL, Kirby R (2003) Safety and tolerability of the dual 5alpha-reductase inhibitor dutasteride in the treatment of benign prostatic hyperplasia. Eur Urol 44:82–88

Baum HB, Biller BM, Finkelstein JS, Cannistraro KB, Oppenhein DS, Schoenfeld DA, Michel TH, Wittink H, Klibanski A (1996) Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. Ann Intern Med 125:883–890

Becherini L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, Falchetti A, Gonnelli S, Fiorelli G, Tanini A & Brandi ML (2000) Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Gen 9: 2043–2050

Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E (1997) Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. J Clin Endocrinol Metab 82:2386–2390

Behre HM, Yeung CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E (2000) Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, eds. Andrology – Male reproductive health and dysfunction. Springer, Heidelberg, 2nd ed. pp 90–124

Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, Murray R, Manolagas SC (1995) Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J Clin Invest 95:2886–2895

Bertelloni S, Baroncelli GI, Federico G, Cappa M, Lala R, Saggese G (1998) Altered bone mineral density in patients with complete androgen insensitivity syndrome. Horm Res 50:309–314.

Bilezikian JP, Morishima A, Bell J, Grumbach MM (1998) Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599–603

Bondanelli M, Ambrosio MR, Margutti A, Franceschetti P, Zatelli MC, degli Uberti EC (2003) Activation of the somatotropic axis by testosterone in adult men: evidence for a role of hypothalamic growth hormone-releasing hormone. Neuroendocrinology 77:380–387

Braidman I, Baris C, Wood L, Selby P, Adams J, Freemont A, Hoyland J (2000) Preliminary evidence for impaired estrogen receptor-protein expression in osteoblasts and osteocytes from men with idiopathic osteoporosis. Bone 26:423–427

Campion JM, Maricic MJ (2003) Osteoporosis in men. Am Fam Physician 67:1521–1526

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER (1997) Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95

Cetin A, Gokce-Kutsal Y, Celiker R (2001) Predictors of bone mineral density in healthy males. Rheumatol Int 21:85–88

Chen Q, Kaji H, Sugimoto T, Chihara K (2001) Testosterone inhibits osteoclast formation stimulated by parathyroid hormone through androgen receptor. FEBS Lett 491:91–93

Colvard DS, Eriksen EF, Keeting PE, Wilson EM, Lubahn DB, French FS, Riggs BL, Spelsberg TC (1989) Identification of androgen receptors in normal human osteoblast-like cells. Proc Natl Acad Sci USA 86:854–857

Couse JF, Korach KS (1999) Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417

Daniell HW (1997) Osteoporosis after orchiectomy for prostate cancer. J Urol 157:439–444

Daniell HW, Dunn SR, Ferguson DW, Lomas G, Niazi Z, Stratte PT (2000) Progressive osteoporosis during androgen deprivation therapy for prostate cancer. J Urol 163:181–186

Erben RG, Eberle J, Stahr K, Goldberg M (2000) Androgen deficiency induces high turnover osteopenia in aged male rats: a sequential histomorphometric study. J Bone Miner Res 15:1085–1098

Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC, Riggs BL (1988) Evidence of estrogen receptors in normal human osteoblast-like cells. Science 241:84–86

Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S (2000) Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 106:1553–1560

Finkelstein JS, Klibanski A, Neer RM, Doppelt SH, Rosenthal DI, Segre GV, Crowley WF Jr (1989) Increases in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 69:776–783

Foresta C, Ruzza G, Mioni R, Guarneri G, Gribaldo R, Meneghello A, Mastrogiacomo I (1984) Osteoporosis and decline of gonadal function in the elderly male. Horm Res 19:18–22

Foresta C, Zanatta GP, Busnardo B, Scanelli G, Scandellari C (1985) Testosterone and calcitonin plasma levels in hypogonadal osteoporotic young men. J Endocrinol Invest 8:377–379

Fryburg DA, Weltman A, Jahn LA, Weltman JY, Samojlik E, Hintz RL, Veldhuis JD (1997) Short-term modulation of the androgen milieu alters pulsatile, but not exercise- or growth hormone (GH)-releasing hormone-stimulated GH secretion in healthy men: impact of gonadal steroid and GH secretory changes on metabolic outcomes. J Clin Endocrinol Metab 82:3710–3719

Fukayama S, Tashjian AH Jr (1989) Direct modulation by androgens of the response of human bone cells (SaOS-2) to human parathyroid hormone (PTH) and PTH-related protein. Endocrinology 125:1789–1794

Fukuda S, Jida H (2000) Effects of orchidectomy on bone metabolism in beagle dogs. J Vet Med Sci 62:69–73

Goldray D, Weisman Y, Jaccard N, Merdler C, Chen J, Matzkin H (1993) Decreased bone density in elderly men treated with the gonadotropin-releasing hormone agonist decapeptyl (D-Trp6-GnRH). J Clin Endocrinol Metab 76:288–290

Greendale GA, Edelstein S, Barrett-Connor E (1997) Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. J Bone Miner Res 12:1833–1843

Grinspoon SK, Baum HB, Peterson S, Klibanski A (1995) Effects of rhIGF-I administration on bone turnover during short-term fasting. J Clin Invest 96:900–906

Grumbach MM (2000) Estrogen, bone, growth and sex: a sea change in conventional wisdom. J Pediatr Endocrinol Metab 13:1439–1455

Gunness M, Orwoll E (1995) Early induction of alterations in cancellous and cortical bone histology after orchiectomy in mature rats. J Bone Miner Res 10:1735–1744

Hagenfeldt Y, Linde K, Sjoberg HE, Zumkeller W, Arver S (1992) Testosterone increases serum 1,25-dihydroxyvitamin D and insulin-like growth factor-I in hypogonadal men. Int J Androl 15:93–102

Ho AY, Yeung SS & Kung AW (2000) PvuII polymorphisms of the estrogen receptor alpha and bone mineral density in healthy southern Chinese women. Calc Tis Int 66:405–408

Hofbauer LC, Ten RM, Khosla S (1999) The anti-androgen hydroxyflutamide and androgens inhibit interleukin-6 production by an androgen-responsive human osteoblastic cell line. J Bone Miner Res 14:1330–1337

Hofbauer LC, Hicok KC, Chen D, Khosla S (2002) Regulation of osteoprotegerin production by androgens and anti-androgens in human osteoblastic lineage cells. Eur J Endocrinol 147:269–273

Huber DM, Bendixen AC, Pathrose P, Srivastava S, Dienger KM, Shevde NK, Pike JW (2001) Androgens suppress osteoclast formation induced by RANKL and macrophage-colony stimulating factor. Endocrinology 142:3800–3808

Jackson JA, Kleerekoper M, Parfitt AM, Rao DS, Villanueva AR, Frame B (1987) Bone histomorphometry in hypogonadal and eugonadal men with spinal osteoporosis. J Clin Endocrinol Metab 65:53–58

Jackson JA, Riggs MW, Spiekerman AM (1992) Testosterone deficiency as a risk factor for hip fractures in men: a case-control study. Am J Med Sci 304:4–8

Kasperk CH, Wakley GK, Hierl T, Ziegler R (1997) Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. J Bone Miner Res 12:464–471

Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A (1996) Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 81:4358–4365

Kawano H, Sato T, Yamada T, Matsumoto T, Sekine K, Watanabe T, Nakamura T, Fukuda T, Yoshimura K, Yoshizawa T, Aihara K, Yamamoto Y, Nakamichi Y, Metzger D, Chambon P, Nakamura K, Kawaguchi H, Kato S (2003) Suppressive function of androgen receptor in bone resorption. Proc Natl Acad Sci USA 100:9416–9421

Kenny AM, Prestwood KM, Gruman CA, Marcello KM, Raisz LG (2001) Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci 56:M266–272

Khosla S (2001) Minireview: the OPG/RANKL/RANK system. Endocrinology 142:5050–5055

Khosla S, Riggs BL (2003) Androgens, estrogens, and bone turnover in men. J Clin Endocrinol Metab 88:2352; author reply 2352–2353

Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM (2001) Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocrinol Metab 86:3555–3561

Komm BS, Terpening CM, Benz DJ, Graeme KA, O'Malley BW, Haussler MR (1988) Estrogen binding receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. Science 241:81–84

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC (2001) Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104:719–730

Kousteni S, Chen JR, Bellido T, Han L, Ali AA, O'Brien CA, Plotkin L, Fu Q, Mancino AT, Wen Y, Vertino AM, Powers CC, Stewart SA, Ebert R, Parfitt AM, Weinstein RS, Jilka RL, Manolagas SC (2002) Reversal of bone loss in mice by nongenotropic signaling of sex steroids. Science 298:843–846

Kousteni S, Han L, Chen JR, Almeida M, Plotkin LI, Bellido T, Manolagas SC (2003) Kinase-mediated regulation of common transcription factors accounts for the bone-protective effects of sex steroids. J Clin Invest 111:1651–1664

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA (1996) Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930

Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF (2000) A TA repeat polymorphism in the estrogen receptor gene is associated with osteoporotic fractures but polymorphisms in the first exon and intron are not. J Bone Miner Res 15:2222–2230

Leder BZ, Smith MR, Fallon MA, Lee ML, Finkelstein JS (2001) Effects of gonadal steroid suppression on skeletal sensitivity to parathyroid hormone in men. J Clin Endocrinol Metab 86:511–516

Leder BZ, LeBlanc KM, Schoenfeld DA, Eastell R, Finkelstein JS (2003) Differential effects of androgens and estrogens on bone turnover in normal men. J Clin Endocrinol Metab 88:204–210

Leifke E, Körner HC, Link TM, Behre HM, Peters PE, Nieschlag E (1998) Effects of testosterone replacement therapy on cortical and trabecular bone mineral density, vertebral body area and paraspinal muscle area in hypogonadal men. Eur J Endocrinol 138:51–58

Liegibel UM, Sommer U, Tomakidi P, Hilscher U, Van Den Heuvel L, Pirzer R, Hillmeier J, Nawroth P, Kasperk C (2002) Concerted action of androgens and mechanical strain shifts bone metabolism from high turnover into an osteoanabolic mode. J Exp Med 196: 1387–1392.

Marcus R, Leary D, Schneider DL, Shane E, Favus M, Quigley CA (2000) The contribution of testosterone to skeletal development and maintenance: lessons from the androgen insensitivity syndrome. J Clin Endocrinol Metab 85:1032–1037

Melton LJ 3rd, Chrischilles EA, Cooper C, Lane AW, Riggs BL (1992) Perspective. How many women have osteoporosis? J Bone Miner Res 7:1005–1010

Melton LJ 3rd, Alothman KI, Khosla S, Achenbach SJ, Oberg AL, Zincke H (2003) Fracture risk following bilateral orchiectomy. J Urol 169:1747–1750

Monson JP (2003) Long-term experience with GH replacement therapy: efficacy and safety. Eur J Endocrinol 148:S9–14

Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689–3698

Nguyen TV, Eisman JA, Kelly PJ, Sambrook PN (1996) Risk factors for osteoporotic fractures in elderly men. Am J Epidemiol 144:255–263

Ogata E, Shimazawa E, Suzuki H, Yoshitoshi Y, Asano H, Ando H (1970) Androgens and enhancement of hypocalcemic response to thyrocalcitonin in rats. Endocrinology 87:421–426

Orwoll E, Ettinger M, Weiss S, Miller P, Kendler D, Graham J, Adami S, Weber K, Lorenc R, Pietschmann P, Vandormael K, Lombardi A (2000) Alendronate for the treatment of osteoporosis in men. N Engl J Med 343:604–610

Orwoll ES, Scheele WH, Paul S, Adami S, Syversen U, Diez-Perez A, Kaufman JM, Clancy AD, Gaich GA (2003) The effect of teriparatide [human parathyroid hormone (1–34)] therapy on bone density in men with osteoporosis. J Bone Miner Res 18:9–17

Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL, Spelsberg TC (1991) Avian osteoclasts as estrogen target cells. Proc Natl Acad Sci USA 88:6613–6617

Oursler MJ, Pederson L, Fitzpatrick L, Riggs BL (1994) Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. Proc Natl Acad Sci USA 91:5227–5231

Patel MS, Cole DE, Smith JD, Hawker GA, Wong B, Trang H, Vieth R, Meltzer P & Rubin LA (2000) Alleles of the estrogen receptor alpha-gene and an estrogen receptor cotranscriptional activator gene, amplified in breast cancer-1 (AIB1), are associated with quantitative calcaneal ultrasound. Journal of Bone and Mineral Research 15:2231–2239

Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL, Oursler MJ (1999) Androgens regulate bone resorption activity of isolated osteoclasts in vitro. Proc Natl Acad Sci USA 96:505–510

Pettersson K, Grandien K, Kuiper GGJM, Gustafsson JA (1997) Mouse estrogen receptor beta forms estrogen response element binding heterodimers with estrogen receptor-alpha. Mol Endocrinol 11:1486–1496

Pilbeam CC, Raisz LG (1990) Effects of androgens on parathyroid hormone and interleukin-1-stimulated prostaglandin production in cultured neonatal mouse calvariae. J Bone Miner Res 5:1183–1188

Poor G, Atkinson EJ, O'Fallon WM, Melton LJ 3rd (1995) Determinants of reduced survival following hip fractures in men. Clin Orthop 319:260–265

Riggs BL, Khosla S, Melton LJ 3rd (2002) Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302

Rochira V, Faustini-Fustini M, Balestrieri A, Carani C (2000) Estrogen replacement therapy in a man with congenital aromatase deficiency: effects of different doses of transdermal estradiol on bone mineral density and hormonal parameters. J Clin Endocrinol Metab 85:1841–1845

Rochira V, Balestrieri A, Madeo B, Spaggiari A, Carani C (2002) Congenital estrogen deficiency in men: a new syndrome with different phenotypes; clinical and therapeutic implications in men. Mol Cell Endocrinol 193:19–28

Rudman D, Drinka PJ, Wilson CR, Mattson DE, Scherman F, Cuisinier MC, Schultz S (1994) Relations of endogenous anabolic hormones and physical activity to bone mineral density and lean body mass in elderly men. Clin Endocrinol (Oxf) 40:653–661

Saville PD (1969) Changes in skeletal mass and fragility with castration in the rat; a model of osteoporosis. J Am Geriatr Soc 17:155–166

Schoutens A, Verhas M, L'Hermite-Baleriaux M, L'Hermite M, Verschaeren A, Dourov N, Mone M, Heilporn A, Tricot A (1984) Growth and bone haemodynamic responses to castration in male rats. Reversibility by testosterone. Acta Endocrinol (Copenh) 107:428–432

Schubert M, Bullmann C, Minnemann T, Reiners C, Krone W, Jockenhövel F (2003) Osteoporosis in male hypogonadism: responses to androgen substitution differ among men with primary and secondary hypogonadism. Horm Res 60:21–28

Scopacasa F, Wishart JM, Need AG, Horowitz M, Morris HA, Nordin BE (2002) Bone density and bone-related biochemical variables in normal men: a longitudinal study. J Gerontol A Biol Sci Med Sci 57: 385–391

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056–1061

Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH, Dlewati A, Staley J, Santanna J, Kapoor SC, Attie MF, Haddad JG Jr, Strom BL (1999) Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Somjen D, Kaye AM, Harell A, Weisman Y (1989) Modulation by vitamin D status of the responsiveness of rat bone to gonadal steroids. Endocrinology 125:1870–1876

Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M (1999) Genetic markers, bone mineral density, and serum osteocalcin levels. J Bone Miner Res 14: 1411–1419

Stepan JJ, Lachman M, Zverina J, Pacovsky V, Baylink DJ (1989) Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab 69:523–527

Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD (2001) Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. J Clin Endocrinol Metab 86:3162–3165

Tenover JS (1992) Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP (1992) Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 75:1060–1065.

Tollin SR, Rosen HN, Zurowski K, Saltzman B, Zeind AJ, Berg S, Greenspan SL (1996) Finasteride therapy does not alter bone turnover in men with benign prostatic hyperplasia – a clinical research center study. J Clin Endocrinol Metab 81:1031–1034

Tomkinson A, Gevers EF, Wit JM, Reeve J, Noble BS (1998) The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res 13:1243–1250

Turner RT, Hannon KS, Demers LM, Buchanan J, Bell NH (1989) Differential effects of gonadal function on bone histomorphometry in male and female rats. J Bone Miner Res 4:557–563

van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW (2000) Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 85:3276–3282

Van Pottelbergh I, Goemaere S, Kaufman JM (2003) Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J Clin Endocrinol Metab 88:3075–3081

Vandenput L, Ederveen AG, Erben RG, Stahr K, Swinnen JV, Van Herck E, Verstuyf A, Boonen S, Bouillon R, Vanderschueren D (2001) Testosterone prevents orchidectomy-induced bone loss in estrogen receptor-alpha knockout mice. Biochem Biophys Res Commun 285: 70–76

Vandenput L, Swinnen JV, Van Herck E, Verstuyf A, Boonen S, Bouillon R, Vanderschueren D (2002) The estrogen receptor ligand ICI 182,780 does not impair the bone-sparing effects of testosterone in the young orchidectomized rat model. Calcif Tissue Int 70:170–175

Vanderschueren D, Van Herck E, Suiker AM, Visser WJ, Schot LP, Bouillon R (1992) Bone and mineral metabolism in aged male rats: short and long term effects of androgen deficiency. Endocrinology 130:2906–2916

Verhas M, Schoutens A, L'hermite-Baleriaux M, Dourov N, Verschaeren A, Mone M, Heilporn A (1986) The effect of orchidectomy on bone metabolism in aging rats. Calcif Tissue Int 39:74–77

Vidal O, Lindberg MK, Hollberg K, Baylink DJ, Andersson G, Lubahn DB, Mohan S, Gustafsson J, Ohlsson C (2000) Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. Proc Natl Acad Sci USA 97:5474–5479

Wakley GK, Schutte HD Jr, Hannon KS, Turner RT (1991) Androgen treatment prevents loss of cancellous bone in the orchidectomized rat. J Bone Miner Res 6:325–330

Wang C, Eyre DR, Clark R, Kleinberg D, Newman C, Iranmanesh A, Veldhuis J, Dudley RE, Berman N, Davidson T, Barstow TJ, Sinow R, Alexander G, Swerdloff RS (1996) Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men – a clinical research center study. J Clin Endocrinol Metab 81:3654–3662

Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2001) Effects of transdermal testosterone gel on bone turnover markers and bone mineral density in hypogonadal men. Clin Endocrinol (Oxf) 54:739–750

Wang X, Schwartz Z, Yaffe P, Ornoy A. (1999) The expression of transforming growth factor-beta and interleukin-1beta mRNA and the response to 1,25(OH)2D3' 17 beta-estradiol, and testosterone is age dependent in primary cultures of mouse-derived osteoblasts in vitro. Endocrine 11:13–22

Wink CS, Felts WJ (1980) Effects of castration on the bone structure of male rats: a model of osteoporosis. Calcif Tissue Int 32:77–82

Wüster C, Albanese C, De Aloysio D, Duboeuf F, Gambacciani M, Gonnelli S, Gluer CC, Hans D, Joly J, Reginster JY, De Terlizzi F, Cadossi R (2000) Phalangeal osteosonogrammetry study: age-related changes, diagnostic sensitivity, and discrimination power. The Phalangeal Osteosonogrammetry Study Group. J Bone Miner Res 15:1603–1614

Zachman M, Prader A, Sobel EH, Crigler Jr JF, Ritzen EM, Atares M, Fernandez A (1986) Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of estrogen in pubertal growth in girls. J Pediatr 108:694–697

Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001) The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. Clin Endocrinol (Oxf) 55:649–657

Zitzmann M, Brune M, Vieth V, Nieschlag E (2002) Monitoring bone density in hypogonadal men by quantitative phalangeal ultrasound. Bone 31:422–429

8

Testosterone effects on the skeletal muscle

S. Bhasin, T.W. Storer, A.B. Singh, L. Woodhouse, R. Singh, J. Artaza, W.E. Taylor, I. Sinha-Hikim, R. Jasuja and N. Gonzalez-Cadavid

Contents

8.1 Historical aspects of the anabolic steroid controversy

Soon after the biochemical synthesis of testosterone, several groups investigated the anabolic effects of testosterone in animal models. During the 1930s, Kochakian

(1935) first described the nitrogen-retaining properties of urinary androgens in the castrate dog. He recorded similar effects of androgens in the castrated male rat and found that the androgen stimulation resulted in dose-related increases in nitrogen retention and body weight (Kochakian 1935; 1937; 1950).

Shortly after the initial animal studies, Kenyon *et al.* (1940) studied the effects of testosterone propionate in eunuchoidal men, and eugonadal men and women. During androgen treatment, urinary nitrogen excretion diminished, with the greatest magnitude of effects observed in eunuchoidal men. Kenyon concluded presciently that "The . . . protein estimated as retained by these subjects is not accounted for by increases in the bulk of genital tissues and represents deposit of new material elsewhere in the body" (Kenyon 1940). These observations, combined with the results of the animal studies, allowed the early recognition of the anabolic effects of androgens. It is notable that Kenyon and others were not able to demonstrate sustained increases in nitrogen retention with testosterone supplementation in eugonadal men, an observation that sparked considerable skepticism for the next fifty years about the anabolic effects of supraphysiological doses of androgens in eugonadal men.

Although the use of performance-enhancing products dates back to antiquity, anabolic steroids have emerged as the most prevalent drugs of abuse among athletes in recent decades (Dawson 2001). Although the Russian power lifters were perhaps the first to abuse anabolic steroids in the early 1950s, this practice spread quickly to athletes in other countries. The systematic use of anabolic steroids by athletes in the former German Democratic Republic was an extreme example of state-sponsored malpractice; however, the abuse of performance-enhancing drugs is not limited to any one nation. Athletes and recreational bodybuilders who abuse androgenic steroids believe that these compounds increase muscle mass and performance and that higher doses of androgens produce greater effects on the muscle than lower doses (Wilson 1988). Hence, they take large doses and abuse multiple steroids simultaneously in a practice called stacking (Dawson 2001; Wilson 1988). Until a few years ago, the academic community was skeptical of these claims, and interpreted the available data to imply that only replacement doses of androgens in castrated males increased nitrogen retention, and that supraphysiologic doses of androgens did not further increase muscle mass and strength when given to eugonadal men. Considerable debate raged in the academic community for five decades on whether androgenic steroids had anabolic effects on the muscle, due in part to the shortcomings of previous studies; several reviews (Bardin 1996; Wilson 1988) have discussed these study design issues. Many of the previous studies that examined the effects of androgenic steroids were neither blinded nor randomized. Some studies included competitive athletes, whose desire to win might preclude compliance with a standardized regimen of diet and exercise. Nutritional intake

was not controlled in many of the studies; changes in energy and protein intake might have had independent effects on nitrogen balance. Exercise stimulus was not standardized and, in some studies, the participants were allowed to exercise *ad libitum*. Therefore, the effects of androgen administration could not be separated from the effects of resistance exercise training (Bhasin *et al.* 2001a). Most of the studies used relatively small doses of androgenic steroids (Bhasin *et al.* 2003a), in contrast, athletes use much larger doses of androgenic steroids. Not surprisingly, the results of these studies were inconclusive. However, studies published in the last decade by a number of groups have established that testosterone supplementation increases muscle mass and maximal voluntary strength (Bhasin *et al.* 1996; 1997; Brodsky *et al.* 1996; Katznelson *et al.* 1996; Snyder and Laurence 1980; Snyder *et al.* 2000; Wang *et al.* 1996; 2000).

8.2 Evidence that testosterone has direct anabolic effects on the mammalian skeletal muscle

8.2.1 Correlational studies demonstrating the relationship of serum testosterone concentrations and muscle mass and function

Healthy, hypogonadal men have lower fat free mass (FFM) and higher fat mass when compared to age-matched eugonadal men (Katznelson *et al.* 1996; 1998). The age-associated decline in serum testosterone levels correlates with decreased appendicular muscle mass and reduced lower extremity strength in Caucasian as well as African-American men (Baumgartner *et al.* 1999; Melton *et al.* 2000; Morley *et al.* 1997; Roy *et al.* 2002). Similarly, epidemiological studies have demonstrated an inverse correlation between serum testosterone levels and waist-to-hip ratio and visceral fat mass assessed by CT scan. In a cohort of 511 men aged 30 to 79 years in 1972 to 1974, levels of androstenedione, testosterone, and sex hormone-binding globulin measured at baseline were inversely related to subsequent central adiposity, estimated 12 years later using the waist-hip circumference ratio (Khaw and Barrett-Connor 1992). In another study, total and free testosterone concentrations were negatively correlated with waist/hip circumference ratio and visceral fat area and negatively associated with increased glucose, insulin, and C-peptide concentrations (Seidell *et al.* 1990). The correlation co-efficients were not high, suggesting that there are other important determinants of body composition besides testosterone.

8.2.2 The effects of lowering endogenous testosterone concentrations on body composition

Experimental suppression of serum testosterone levels by administration of a GnRH agonist analog in healthy young men is associated with a significant reduction in fat-free mass and an increase in fat mass, and a decrease in fractional muscle protein

synthesis (Mauras *et al.* 1998). In this study, gonadal suppression was also associated with a decrease in whole body leucine oxidation as well as non-oxidative leucine disappearance rates. Rates of lipid oxidation decreased after treatment, with parallel changes in resting energy expenditure.

8.2.3 The effects of physiologic testosterone replacement in healthy, young hypogonadal men

Testosterone replacement increases nitrogen retention in castrated males of several animal species (Kochakian 1935; 1937; 1950), eunuchoidal men, boys before puberty, and in women (Kenyon 1940). Several recent studies (Brodsky *et al.* 1996; Katznelson *et al.* 1996; Wang *et al.* 2000) have re-examined the effects of testosterone on body composition and muscle mass in hypogonadal men in more detail (Bhasin *et al.* 1996; 1997; Brodsky *et al.* 1996; Katznelson *et al.* 1996; Snyder *et al.* 2000; Wang *et al.* 1996; 2000). These studies are in agreement that replacement doses of testosterone, when administered to healthy, androgen-deficient men, increase fat-free mass, muscle size, and maximal voluntary strength. The muscle accretion during testosterone treatment is associated with an increase in fractional muscle protein synthesis (Brodsky *et al.* 1996).

8.2.4 The effect of supraphysiologic doses of testosterone on body composition and muscle strength

Intense controversy persisted until recently with respect to the effects of supraphysiologic doses of androgenic steroids on body composition and muscle strength (Bardin 1996; Bross *et al.* 1998; Wilson 1988). We conducted a placebo-controlled, double-blind, randomized, clinical trial to separately assess the effects of supraphysiologic doses of testosterone and resistance exercise on fat-free mass, muscle size and strength (Bhasin *et al.* 1996). Healthy men, 19–40 years of age, who were within 15% of their ideal body weight, were randomly assigned to one of four groups: placebo but no exercise; testosterone but no exercise; placebo plus exercise; and testosterone plus exercise. The men received 600 mg testosterone enanthate or placebo weekly for ten weeks. Serum total and free testosterone levels, measured seven days after each injection, increased five-fold; these were nadir levels and serum testosterone levels at other times must have been higher. Serum LH levels were markedly suppressed in the testosterone-treated but not the placebo-treated men providing additional evidence of compliance. Men in the exercise groups underwent weight lifting exercises thrice weekly; the training stimulus was standardized based on the subjects' initial 1-repetition maximum (1RM) and the sessions were supervised. Fat free mass by underwater weighing, muscle size by magnetic resonance imaging (MRI), and muscle strength of the arms and legs in bench press and squat exercises were measured before and after ten weeks of treatment.

The men given testosterone alone had greater gains in muscle size in the arm (mean (±SEM) change in triceps area 13.2 ± 3.3 vs. −2.1 ± 2.9%, $p < 0.05$) and leg (change in quadriceps area 6.5 ± 1.3 vs. −1.0 ± 1.1%, $p < 0.05$), than those given placebo injections (Bhasin *et al.* 1996). Testosterone treatment was also associated with greater gains in strength in the bench press (increase 10 ± 4 vs. −1 ± 2%, $p < 0.05$) and squat exercise capacity (increase 19 ± 6 vs. 3 ± 1%, $p < 0.05$) than placebo injections. Testosterone and exercise, given together, produced greater increases in fat free mass (+9.5 ± 1.0%) and muscle size (+14.7 ± 3.1% in triceps area and +14.1 ± 1.3% in quadriceps area) than either placebo or exercise alone, and greater gains in muscle strength (+24 ± 3% in bench press strength, and +39 ± 4% in squat exercise capacity) than either non-exercising group. These results demonstrate that supraphysiologic doses of testosterone, especially when combined with strength training, increase fat free mass, muscle size and strength in healthy men (Bhasin *et al.* 1996).

Other studies that have utilized supraphysiologic doses of testosterone esters in healthy volunteers, men with muscle dystrophy (Griggs *et al.* 1986; 1989), or older men undergoing hip surgery (Amory *et al.* 2002) have also demonstrated consistent gains in lean body mass during testosterone administration (Griggs *et al.* 1989). Collectively, these data demonstrate that when dietary intake and exercise stimulus are controlled, supraphysiologic doses of testosterone produce further increases in fat free mass and strength in eugonadal men. Strength training may augment androgen effects on the muscle.

8.3 Testosterone dose-response relationships in men

Testosterone increases muscle mass and strength, and regulates other physiologic processes, but we do not know whether testosterone effects are dose-dependent, and whether dose requirements for maintaining various androgen-dependent processes are similar (Bhasin *et al.* 2001). Androgen receptors in most tissues are either saturated or downregulated at physiologic testosterone concentrations (Antonio *et al.* 1999; Dahlberg *et al.* 1981; Rance and Max 1984; Wilson 1988), leading to speculation that there might be two separate dose-response curves: one in the hypogonadal range with maximal response at low normal testosterone concentrations, and a second in the supraphysiologic range, representing a separate mechanism of action (Bhasin *et al.* 2001; Wilson 1988). However, testosterone dose-response relationships for a range of androgen-dependent functions in humans have not been studied.

To determine the effects of graded doses of testosterone on body composition, muscle size, strength, power, sexual and cognitive functions, PSA, plasma lipids, hemoglobin, and IGF-1 levels, 61 eugonadal men, 18–35 years, were randomized

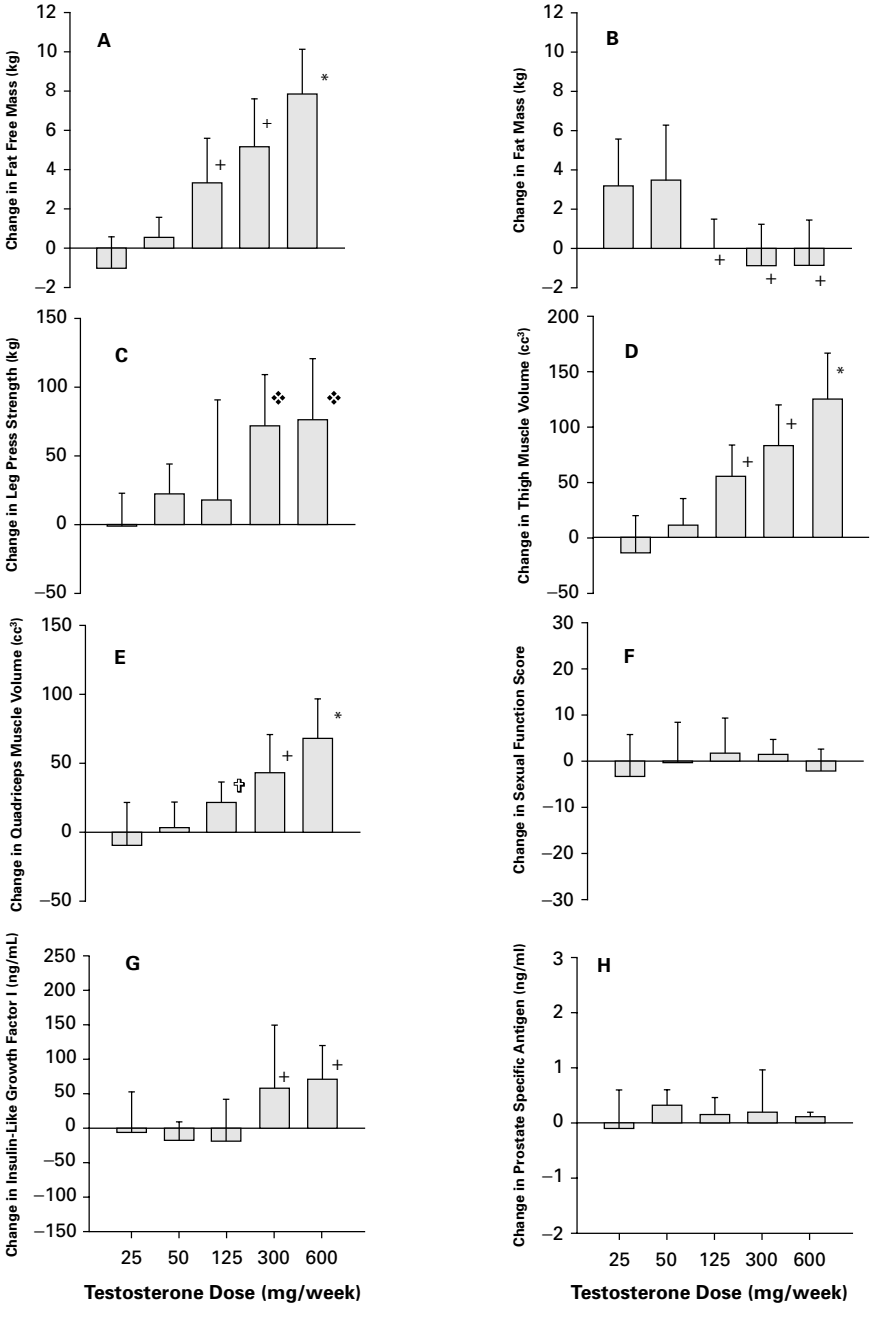


Fig. 8.1 Testosterone dose response relationships in young men

Change in fat-free mass (panel A), fat mass (panel B), leg press strength (panel C), thigh muscle volume (panel D), quadriceps muscle volume (panel E), sexual function (panel F), IGF-1 (panel G), and PSA (panel H). Data are mean ± SEM. * denotes significant differences from all other groups ($p < 0.05$); ❖ denotes significant difference from 25, 50 and 125 mg doses ($p < 0.05$); + denotes significant difference from 25 and 50 mg doses ($p < 0.05$); and ✞ denotes significant difference from the 25 mg dose ($p < 0.05$). (Reproduced with permission from Bhasin *et al*. 2001.)

to one of five groups to receive monthly injections of a long-acting GnRH agonist to suppress endogenous testosterone secretion, and weekly injections of 25, 50, 125, 300 or 600 mg testosterone enanthate for 20 weeks (Bhasin *et al.* 2001b; Singh *et al.* 2002; Woodhouse *et al.* 2003). Energy and protein intake were standardized. The administration of GnRH agonist plus graded doses of testosterone resulted in mean nadir testosterone concentrations of 253, 306, 542, 1345, and 2,370 ng/dL at the 25, 50, 125, 300, and 600 mg doses, respectively (Fig. 8.1). Fat free mass increased dose-dependently in men receiving 125, 300 or 600 mg of testosterone weekly (change +3.4, 5.2, and 7.9 kg, respectively). The changes in fat free mass were highly dependent on testosterone dose (Fig. 8.1, $P = 0.0001$) and correlated with log testosterone concentrations ($r = 0.73$, $P = 0.0001$). Changes in leg press strength, leg power, thigh and quadriceps muscle volumes, hemoglobin, and IGF-1 were positively correlated with testosterone concentrations, while changes in fat mass and plasma HDL cholesterol were negatively correlated. Sexual function, visual-spatial cognition and mood, and PSA levels did not change significantly at any dose. These data demonstrate that changes in circulating testosterone concentrations, induced by GnRH agonist and testosterone administration, are associated with testosterone dose- and concentration-dependent changes in fat free mass, muscle size, strength and power, fat mass, hemoglobin, HDL cholesterol, and IGF-1 levels, in conformity with a single linear dose-response relationship. However, different androgen-dependent processes have different testosterone dose-response relationships.

Despite strong correlation between testosterone dose and changes in fat free mass and muscle size, there was considerable heterogeneity in individual responses to a given testosterone dose. We investigated whether testosterone dose and/or any combination of baseline variables including concentrations of hormones, growth factors, age, measures of body composition, muscle function, muscle morphometry or polymorphisms in androgen receptor could explain the variability in anabolic response to testosterone (Woodhouse *et al.* 2003). Anabolic response was operationally defined as a change in whole body fat-free mass (FFM) (by DEXA), appendicular FFM (by DEXA) and thigh muscle volume (by MRI) during testosterone treatment. We used univariate and multivariate analysis to identify the subset of baseline measures that best explained the variability in anabolic response to testosterone supplementation. The three variable model of testosterone dose, age and baseline prostate specific antigen (PSA) level explained 67% of the variance in change in whole body FFM. Change in appendicular FFM was best explained (64% of the variance) by the linear combination of testosterone dose, baseline PSA and leg press strength, while testosterone dose, log of the ratio of luteinizing hormone (LH) to testosterone (T) concentration (LH/T) and age explained 66% of the variation in change in thigh muscle volume (MRI). The models were further validated

by using Ridge analysis and cross-validation in data subsets. Only the model using testosterone dose, age, and PSA was a consistent predictor of change in FFM in subset analyses. The length of CAG tract of the androgen receptor was only a weak predictor of change in thigh muscle volume and lean body mass (LBM) in this small sample. Thus, the anabolic response of healthy, young men to exogenous testosterone administration can be predicted largely by the testosterone dose (Woodhouse *et al.* 2003). Further studies are needed to elucidate the genetic basis of natural variation in androgen responsiveness and to test the generalizability of the proposed prediction models.

8.4 Testosterone effects on muscle performance

The data presented above have established that testosterone supplementation in men increases fat free mass, but it remains unclear whether measures of muscle performance such as maximal voluntary strength, power, fatigability, or specific tension are improved by androgen administration (Storer *et al.* 2003). Further, the extent to which these measures of muscle performance are related to testosterone dose or circulating concentration is unknown. To determine the dose-dependence of measures of muscle performance on testosterone dose and concentrations, we measured maximal voluntary strength, leg power, and muscle fatigability in our dose response study. Specific tension was estimated by the ratio of 1RM muscle strength to thigh muscle volume determined by MRI. Testosterone administration was associated with a dose-dependent increase in leg press strength and leg power, but muscle fatigability did not change significantly during treatment. Changes in leg press strength were significantly correlated with total ($r = 0.46$, $P = 0.005$) and free testosterone ($r = 0.38$, $P = 0.006$) as was leg power ($r = 0.38$, $P = 0.007$ for total and $r = 0.35$, $P = 0.015$ for free testosterone), but not muscle fatigability. Serum IGF-1 concentrations were not significantly correlated with leg strength, power, or fatigability. Specific tension did not change significantly at any dose.

These data led us to conclude that testosterone effects on muscle performance are domain-specific: it increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension. Failure to observe a significant testosterone-dose relationship with fatigability suggests that testosterone does not affect this domain of muscle performance and that different domains of muscle performance are regulated by different mechanisms. We also infer from these data that the gains in maximal voluntary strength during testosterone administration are proportional to the increase in muscle mass, and that testosterone does not improve the contractile properties of the skeletal muscle.

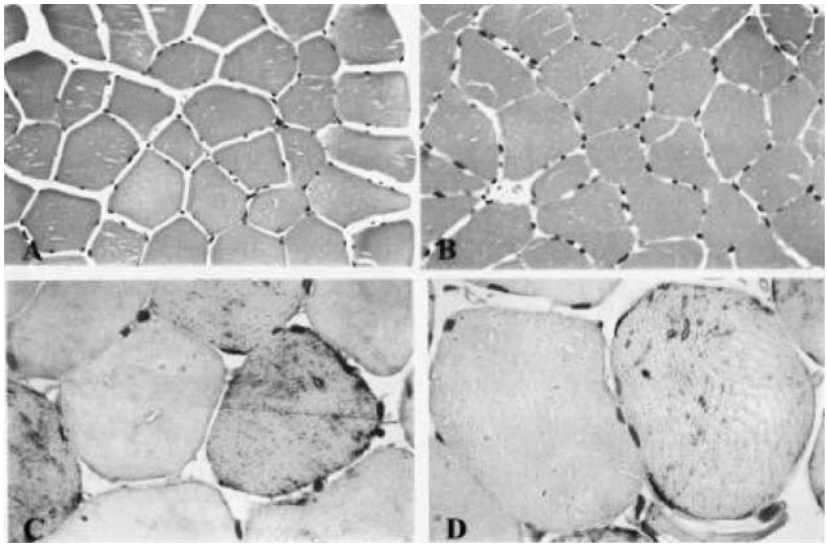


Fig. 8.2 Testosterone induces skeletal muscle fiber hypertrophy
The figure shows cross-sections of muscle biopsies obtained before and after 20 weeks of treatment in one man treated with GnRH agonist and 600 mg testosterone enanthate weekly. The left panels represent baseline sections, and the right panels sections obtained after 20 weeks of treatment. The magnification is 200-fold in panels A and B, and 1000-fold in panels C and D. (Reproduced with permission from Sinha-Hikim *et al*. 2002.)

8.5 Mechanisms of testosterone's anabolic effects on the muscle: pluripotent stem cell as the target of androgen action

8.5.1 Testosterone effects on muscle histomorphometry

In order to determine whether testosterone-induced increase in muscle size is due to muscle fiber hypertrophy or hyperplasia, muscle biopsies were obtained from *vastus lateralis* in 39 men before and after 20 weeks of combined treatment with GnRH agonist and weekly injections of 25, 50, 125, 300 or 600 mg testosterone enanthate (Sinha-Hikim *et al.* 2002). Changes in cross-sectional areas of both type I and II fibers were dependent on testosterone dose, and significantly correlated with total ($r = 0.35$, and 0.44, $P < 0.0001$ for type I and II fibers, respectively) and free ($r = 0.34$ and 0.35, $P < 0.005$) testosterone concentrations during treatment (Fig. 8.2). The men receiving 300 and 600 mg of testosterone enanthate weekly experienced significant increases from baseline in areas of type I (baseline vs. 20 wks, 3176 ± 163 vs. 4201 ± 163 μm^2, $P < 0.05$ at 300-mg dose, and 3347 ± 253 vs. 4984 ± 374 μm^2, $P = 0.006$ at 600-mg dose) muscle fibers; the men in the 600-mg group also had significant increments in cross-sectional areas of

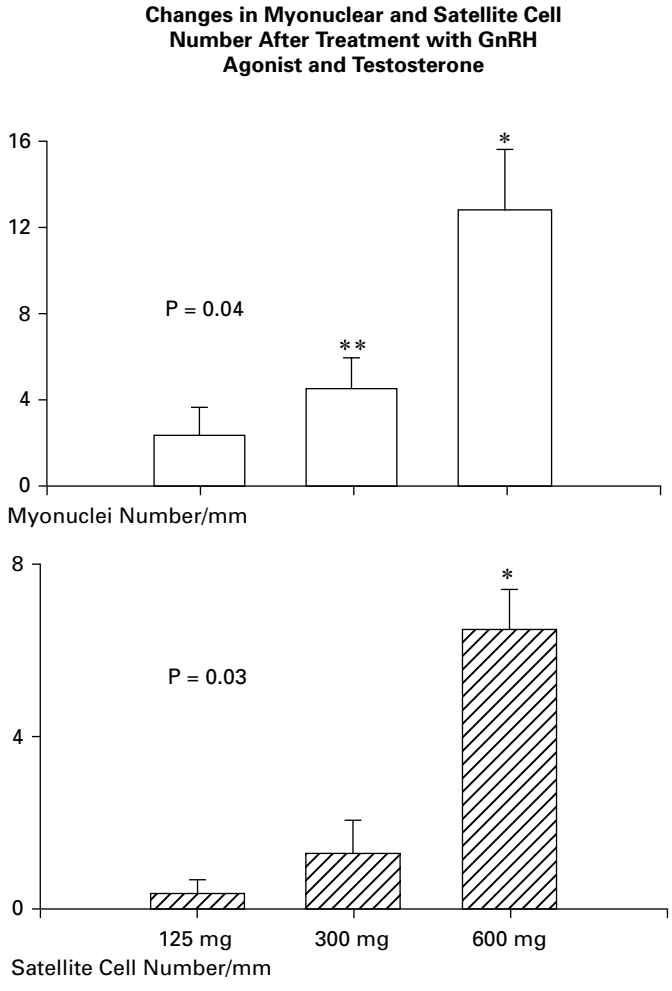


Fig. 8.3 The effect of testosterone administration on myonuclear number and absolute satellite cell number

The number of myonuclei (upper panel) and satellite cells per mm of muscle fiber length (middle panel) were computed by spatial distribution. Change was calculated as the difference between post-treatment and baseline values. Values significantly different from zero are marked by asterisks. The weekly dose of testosterone enanthate is shown at the bottom. *, P = 0.04 vs. zero change; **, P =0.03 vs. zero change. (Reproduced with permission from Sinha-Hikim *et al*. 2003.)

type II (4060 ± 401 vs. 5526 ± 544 μm^2, $P = 0.03$) fibers. The relative proportions of type I and type II fibers did not change significantly after treatment in any group. The myonuclear number per fiber increased significantly in men receiving the 300 and 600 mg doses of testosterone enanthate, and was significantly correlated with testosterone concentration, and muscle fiber cross-sectional area (Sinha-Hikim *et al.* 2002).

These data demonstrate that increases in muscle volume in healthy eugonadal men treated with graded doses of testosterone are associated with concentration-dependent increases in muscle fiber cross-sectional area and myonulcear number, but not muscle fiber number. We conclude that the testosterone-induced increase in muscle volume is due to muscle fiber hypertrophy. Testosterone-induced muscle fiber hypertrophy was also associated with an increase in satellite cell number and a proportionate increase in myonuclear number (Fig. 8.3) (Sinha-Hikim *et al.* 2002). The mechanisms by which testosterone might increase satellite cell number are not known. An increase in satellite cell number could occur by an increase in satellite cell replication, inhibition of satellite cell apoptosis, and/or increased differentiation of stem cells into the myogenic lineage. We do not know which of these processes is the site of regulation by testosterone.

8.5.2 Muscle protein synthesis as the target of androgen action

Induction of androgen deficiency by administration of a long acting GnRH agonist in healthy, young men is associated with decreased rates of ^{13}C-leucine appearance, a measure of proteolysis (Mauras *et al.* 1998). Lowering of testosterone concentrations in this study (Mauras *et al.* 1998) is also associated with a significant decrease in nonoxidative leucine disappearance, a marker for whole body protein synthesis. Conversely, testosterone supplementation stimulates the synthesis of mixed skeletal muscle proteins (Brodsky *et al.* 1996; Ferrando *et al.* 2002; Urban *et al.* 1995). All of these studies of protein turnover have been performed in the fasting state in which the net balance between protein synthesis and breakdown is negative. Testosterone administration improves the muscle protein balance and makes it less negative (Ferrando *et al.* 2002; Urban *et al.* 1995). However, none of the studies has demonstrated a clear improvement in muscle protein balance into the positive territory, which would indicate net protein accretion. It has been assumed, but never demonstrated, that during the fed state, testosterone administration leads to net protein accretion. Testosterone improves the efficiency of reutilization of amino acids in the muscle (Ferrando *et al.* 1998). The effects of testosterone administration on muscle protein breakdown have not been studied extensively. A recent study by Ferrando *et al.* (2002) reported a significant decrease in muscle protein breakdown following testosterone supplementation of older men. In this study, the proteasome peptidase activity was decreased by testosterone administration, a finding consistent with the

decrease in muscle protein degradation assessed by using labeled phenylalanine and measurements of arteriovenous differences.

Several observed effects of testosterone administration on body composition and muscle histomorphology are not easily explained by the muscle protein hypothesis. If muscle protein synthesis or degradation were the primary target of androgen action, then a separate mechanism would be required to explain the reduction in fat mass that occurs during androgen administration. Similarly, muscle protein hypothesis does not easily explain the observed increases in the number of myonuclei and satellite cells in the skeletal muscle during androgen treatment. Undoubtedly, muscle fiber hypertrophy could not occur without a net increase in protein accretion; however, it is likely that the increase in muscle protein synthesis is a secondary event in the cascade of molecular processes that culminate in muscle fiber hypertrophy.

8.5.3 Pluripotent stem cells as the target of androgen action

Because testosterone administration has reciprocal effects on muscle mass and fat mass, and increases satellite cell number, we considered the possibility that the target of androgen action might be a precursor cell from which muscle and fat cells are derived. We hypothesized that testosterone regulates body composition by promoting the commitment of mesenchymal pluripotent cells into myogenic lineage and inhibiting their differentiation into adipogenic lineage. To test this hypothesis, we treated pluripotent, mesenchymal C3H 10T1/2 cells with testosterone (0–300 nM) or dihydrotestosterone (0–30 nM) for 0–14 days (Singh *et al.* 2003). We evaluated myogenic conversion by immunochemical staining for early (MyoD) and late (myosin heavy chain II: MHC) myogenic markers, and measurements of MyoD and MHC mRNA and protein (Fig. 8.4). Adipogenic differentiation was assessed by adipocyte counting, and by measurements of PPARγ2 mRNA, and PPARγ2 and C/EBPα proteins. The number of MyoD+ myogenic cells and MHC+ myotubes, and MyoD and MHC mRNA and protein levels increased dose-dependently in response to testosterone and dihydrotestosterone treatment. Both testosterone and dihydrotestosterone decreased the number of adipocytes and downregulated the expression of PPARγ2 mRNA and PPARγ2 and C/EBPα proteins. Androgen receptor (AR) mRNA and protein levels were low at baseline, but increased after testosterone or dihydrotestosterone treatment. The effects of testosterone and dihydrotestosterone on myogenesis and adipogenesis were blocked by bicalutamide. Hence, testosterone and dihydrotestosterone regulate lineage determination in mesenchymal pluripotent cells by promoting their commitment to the myogenic lineage and inhibiting their differentiation into the adipogenic lineage through an AR-mediated pathway (Singh *et al.* 2003). The observation that differentiation of pluripotent cells is androgen-dependent provides a

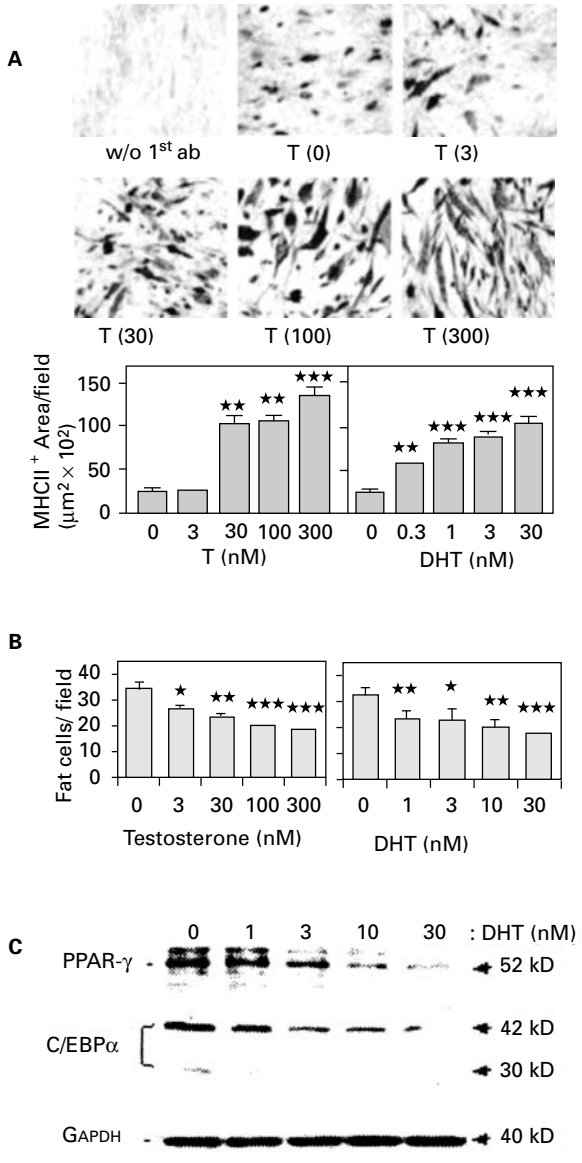


Fig. 8.4 Effects of testosterone supplementation on myogenic and adipogenic differentiation in C3H10T1/2 pluripotent cells. Panel A shows immunocytochemical staining of MHC+ myogenic cells. 10T1/2 cells, treated for 12 days with testosterone (0 to 300 nM) or DHT (0 to 30 nM, not shown), were analyzed by immunocytochemistry using an anti-MHC antibody. Negative control did not include the first antibody (w/o 1st ab). Total areas of MHC+ cells per field are plotted in the lower panel. P vs. control: **, <0.01; ***, <0.001. Magnification 100X. Panel B shows the effect of testosterone or DHT on adipocyte number. 10T1/2 cells were grown with increasing doses of testosterone or DHT for 12 days. The average number of adipocytes was calculated for each 100X field. P vs. control: *, $=0.02$; **, $P < 0.004$; ***, $P < 0.001$. Panel C shows the effect of DHT on PPARγ2 and C/EBPα protein expression. Cell extracts were analyzed by immunoblotting using anti-PPARγ, anti-C/EBPα or anti-GAPDH antibody. DHT concentrations (nM) and protein sizes (kD) are shown. (Adapted with permission from Singh *et al*. 2003.)

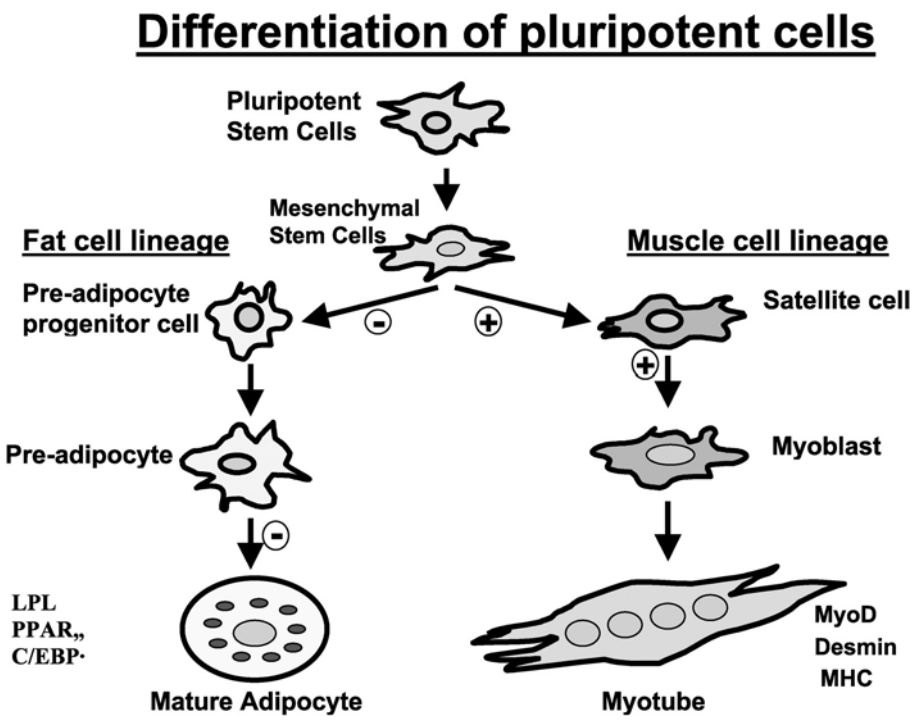


Fig. 8.5 A schematic representation of the hypothetical sites in pluripotent stem cell differentiation at which testosterone might act to affect body composition
Testosterone has been shown to stimulate mesenchymal pluripotent cell commitment into the myogenic lineage and inhibit the differentiation of these cells into the adipogenic lineage. In addition, testosterone has been reported to stimulate satellite cell replication and inhibit differentiation of preadipocytes into adipocytes. Thus, testosterone action at multiple sites in this cascade might serve to amplify androgen effects on myogenesis and adipogenesis. Testosterone supplementation also stimulates muscle protein synthesis and inhibits muscle protein degradation; these actions could also contribute to muscle fiber hypertrophy (Reproduced with permission from Bhasin *et al*. 2003).

unifying explanation for the reciprocal effects of androgens on muscle and fat mass in men. It is possible that androgens might also have effects on additional steps in the myogenic and adipogenic differentiation pathways (Fig. 8.5).

The molecular mechanisms which mediate androgen-induced muscle hypertrophy are not well understood. Urban *et al.* (1995) have proposed that testosterone stimulates the expression of insulin-like growth factor-I (IGF-I) and downregulates insulin-like growth factor binding protein-4 (IGFBP-4) in the muscle. Reciprocal changes in IGF-1 and its binding protein thus provide a potential mechanism for amplifying the anabolic signal.

It is not clear whether the anabolic effects of supraphysiologic doses of testosterone are mediated through an androgen receptor-mediated mechanism. in

vitro binding studies (Wilson 1988) suggest that the maximum effects of testosterone should be manifest at about 300 ng/dL, i.e., serum testosterone levels that are at the lower end of the normal male range. Therefore, it is possible that the supraphysiologic doses of androgen produce muscle hypertrophy through androgen-receptor independent mechanisms, such as through an anti-glucocorticoid effect (Konagaya and Max 1986). We cannot exclude the possibility that some androgen effects may be mediated through non-classical binding sites. Testosterone effects on the muscle are modulated by a number of other factors such as the genetic background, growth hormone secretory status (Fryburg *et al.* 1997), nutrition, exercise, cytokines, thyroid hormones, and glucocorticoids. Testosterone may also affect muscle function by its effects on neuromuscular transmission (Blanco *et al.* 1997; Leslie *et al.* 1991).

8.5.4 The role of 5α-reduction and aromatization of testosterone in the muscle

Although the enzyme 5α-reductase is expressed at low concentrations within the muscle (Bartsch *et al.* 1983), we do not know whether conversion of testosterone to dihydrotestosterone (DHT) is required for mediating androgen effects on the muscle. Men with benign prostatic hypertrophy who are treated with a 5α-reductase inhibitor do not experience muscle loss. Similarly, individuals with congenital 5α-reductase deficiency have normal muscle development at puberty. These data suggest that 5α-reduction of testosterone is not obligatory for mediating its effects on the muscle.

Sattler *et al.* (1998) have reported that serum dihydrotestosterone levels are lower and testosterone to dihydrotestosterone ratios higher in HIV-infected men than in healthy men. These investigators have proposed that a defect in testosterone to dihydrotestosterone conversion may contribute to wasting in a subset of HIV-infected men. If this hypothesis is true, then it would be rational to treat such patients with dihydrotestosterone rather than testosterone. A dihydrotestosterone gel is currently under clinical investigation. However, unlike testosterone, dihydrotestosterone is not aromatized to estradiol. Therefore, there is concern that suppression of endogenous testosterone and estradiol production by exogenous dihydrotestosterone may produce osteoporosis.

Studies of aromatase knock out mice have revealed higher fat mass and lower muscle mass in mice that are null for the P450-linked CYParomatse gene (Jones *et al.* 2000). Data from these gene-targeting experiments suggest that aromatization of testosterone might also be important in mediating androgen effects on the muscle.

After menopause, women tend to gain weight and experience an increase in body mass index (Gambacciani *et al.* 1997) mostly due to fat mass accumulation (Burger *et al.* 1995; Dallongeville *et al.* 1995); this weight gain is attenuated in women who receive estrogen replacement therapy. These data contradict the widely held

Table 8.1 Effects of testosterone supplementation in older men

Study	Subjects	Treatment Regimen	Changes in Body Composition	Changes in Muscle Function	Comments
Tenover (1992)	60–75 years, serum testosterone <400 ng/dL	Testosterone enanthate 100 mg weekly for 3 months	1.8 kg increase in fat-free mass; no change in fat mass	No change in grip strength	Mild increases in PSA and hematocrit
Morley *et al.* (1993)	69–89 years, bioavailable testosterone less than 75 ng/dL	Testosterone enanthate 200 mg every 2 weeks for 3 months	No change in fat mass or body weight	Increase in grip strength	
Sih *et al.* (1997)	Healthy men, 51–79 years, serum bioavailable testosterone <60 ng/dL	Testosterone cypionate 200 mg every 2 weeks for 12 months	0.9 cm (3%) increase in mid-arm circumference, no change in fat mass	4–5 kg increase in grip strength	No change in PSA, increase in hematocrit
Urban *et al.* (1995)	Healthy elderly, 67 + 2 years, testosterone <480 ng/dL	Testosterone enanthate weekly for 4 weeks to increase testosterone to 500–1000 ng/dL	Body composition not reported	Increase in hamstring and quadriceps work per repetition; no change in endurance	Approx. 2 fold increase in fractional muscle protein synthesis rate
Snyder *et al.* (1999)	Healthy older men, >65 years of age	Scrotal testosterone patch, 6 mg/day for 3 years	Lean body mass increased by 1.9 kg; fat mass decreased by 3 kg	No change in strength of knee extension and flexion	Improved perception of physical function
Tenover (2000)	Healthy older men	Testosterone enanthate, ~150 mg/2 weeks for 3 years	Fat-free mass increased and fat mass decreased	Improvements in some measures of muscle strength	
Kenny *et al.* (2001)	Healthy older men with bioavailable T<120 ng/dL	Testosterone patch 5 mg daily for one year	1 kg average gain in lean body mass and 1.7 kg decrease in fat mass	No significant change in muscle strength vs. placebo	Increased bone mineral density
Blackmann *et al.* (2002)	Healthy older men 65–88 years of age	Testosterone enanthate 100 mg every 2 weeks for 26 weeks, rhGH in a 2X2 factorial design	LBM increased 1.4 kg with testosterone, 3.1 kg with rhGH	No significant change in strength with testosterone or rhGH alone	
Steidle *et al.* (2003)	Healthy older men	Testosterone gel 5 or 10 g daily for 12 weeks	Fat free mass increased by 1.7 kg and fat mass decreased by 1.4%	Muscle strength not measured	
Ferrando *et al.* (2002)	Healthy older men >60 years	Varying doses of testosterone enanthate for 6 months	Increased in total and leg lean mass	Increased muscle strength	Increased muscle protein net balance

notion that hormone replacement therapy is associated with significant weight gain. Taken together, the collective body of experimental data suggests that aromatization of testosterone might also be important in mediating androgen effects on body composition. Further studies are needed to determine the important role of estrogens in regulation of body composition.

8.6 Potential clinical application of the anabolic effects of androgens

8.6.1 Effects of testosterone replacement in older men with low testosterone levels

Several studies (Blackman *et al.* 2002; Ferrando *et al.* 1998; Kenny *et al.* 2001; 2002; Morley *et al.* 1993; Sih *et al.* 1997; Snyder *et al.* 1999a; 1999b; Steidle *et al.* 2003; Tenover 1992; 2000; Urban *et al.* 1995) have established that increasing testosterone levels of older men with low testosterone levels to levels that are mid-normal for healthy, young men is associated with a significant increase in lean body mass and a reduction in fat mass (Table 8.1). Although testosterone supplementation is associated with greater gains in grip strength compared to placebo treatment, it remains unclear whether physiologic testosterone replacement can produce meaningful changes in muscle performance and physical function. In a study by Snyder *et al.* (1999) testosterone treatment of older men did not increase muscle strength or improve physical function, but these men were not uniformly hypogonadal and were unusually fit for their age. In addition, their muscle strength was measured by a method (Biodex dynamometer) which did not demonstrate a response even in frankly hypogonadal younger men treated with testosterone (Snyder *et al.* 2000). It is possible that testosterone might improve muscle strength and physical function in older men with clearly low testosterone levels. These studies also emphasize the need to use muscle function tests that are androgen-responsive, and to control for the confounding influence of the learning effect. A major source of debate has been the lack of data demonstrating improvements in measures of physical function in older men. Finally, most of the previous studies of testosterone supplementation in older men have been conducted in healthy, older men; we do not know whether similar beneficial effects can be achieved in older men with sarcopenia or frailty.

8.6.2 Why have previous studies of testosterone replacement in older men failed to demonstrate significant improvements in physical function?

Although testosterone replacement of androgen-deficient men increases fat-free mass and maximal voluntary strength, we do not know if testosterone improves physical function. Many previous studies of testosterone replacement in older men did not examine changes in physical function. The few studies that did examine this issue suffered from methodological problems in the measurements of physical function. We believe a major reason for the failure to demonstrate improvements in physical function is that the measures of physical function used in previous studies

were relatively insensitive and "threshold-dependent". The widely used measures such as 0.625 m stair climb, standing up from a chair, and 20-meter walk are tasks that require only a small fraction of an individual's maximal voluntary strength. In most healthy, older men, the baseline maximal voluntary strength is far higher than the threshold below which these measures would detect impairment. Given the low intensity of the tasks used, these relatively healthy older individuals show neither impairment in these threshold-dependent measures of physical function at baseline, nor an improvement in performance on these tasks during testosterone administration. Because testosterone improves maximal voluntary leg strength, we posit that it would improve measures of physical function that are threshold-independent, and require near-maximal strength of critical muscle groups such as the quadriceps. Another confounder of the effects of anabolic interventions on muscle function is the learning effect. For instance, subjects who are unfamiliar with weight lifting exercises often demonstrate improvements in measures of muscle performance simply because of increased familiarity with the exercise equipment and technique. Therefore, in efficacy trials of anabolic interventions, it is important to incorporate strategies to minimize the confounding influence of the learning effect. Because of the considerable test-to-test variability in tests of physical function, it is possible that previous studies (Blackman *et al.* 2002; Ferrando *et al.* 1998; Kenny *et al.* 2001; 2002; Morley *et al.* 1993; Sih *et al.* 1997; Snyder *et al.* 1999a; 1999b; Steidle *et al.* 2003; Tenover 1992; 2000; Urban *et al.* 1995) did not have adequate power to detect meaningful differences in measures of physical function between the placebo and testosterone-treated groups.

8.6.3 Effects of androgen replacement on body composition and muscle function in sarcopenia associated with chronic illnesses

Several studies on the effects of androgen supplementation in HIV-infected men have been reported (Bhasin *et al.* 1998; 2000; Coodley *et al.* 1994; Coodley and Coodley 1997; Dobs *et al.* 1999; Grinspoon *et al.* 1998; 2000; Sattler *et al.* 1999; 2002; Strawford *et al.* 1999a; 1999b; Van Loan *et al.* 1999). However, many of these studies were not controlled clinical trials. Most of the studies were of short duration ranging from 12–24 weeks. Several androgenic steroids have been studied in a limited fashion, including nandrolone decanoate, oxandrolone, oxymetholone, stanozolol, testosterone cypionate, and testosterone enanthate.

Of the five placebo-controlled studies of testosterone replacement in HIV-infected men with weight loss, three (Bhasin *et al.* 1998; 2000; Grinspoon *et al.* 1998) demonstrated an increase in fat-free mass and two (Coodley and Coodley 1997; Dobs *et al.* 1999) did not. The three studies (Bhasin *et al.* 1998; 2000; Grinspoon *et al.* 1998) that showed gains in fat-free mass selected patients with low testosterone levels. Coodley and Coodley (1997) examined the effects of

200 mg testosterone cypionate given every two weeks for three months to 40 HIV seropositive patients with weight loss of greater than 5% of usual body weight and CD4 cell counts of $<2 \times 10^5$/l in a double-blind, placebo controlled study. Among the 35 patients who completed the first three months of the study, there was no significant difference between the effects of testosterone and placebo treatment on weight gain. However, testosterone supplementation improved overall sense of well-being ($p = 0.03$). Body composition was not assessed.

In two placebo-controlled, double-blind, clinical trials, we have demonstrated that testosterone replacement in HIV-infected men with low testosterone levels is associated with significant gains in fat-free mass (Bhasin *et al.* 1998; 2000). There were no significant changes in liver enzymes, plasma HIV-RNA copy number, and CD4 and CD8+ T cell counts with testosterone administration in either of the two trials. In one study (Bhasin *et al.* 2000), we determined the effects of testosterone replacement, with or without a program of resistance exercise, on muscle strength and body composition in androgen-deficient, HIV-infected men with weight loss and low testosterone levels. This was a placebo-controlled, double-blind, randomized, clinical trial in HIV-infected men with serum testosterone less than 350 ng/dl, and weight loss of 5% or more in the previous six months. Participants were randomly assigned to one of four groups: placebo, no exercise; testosterone, no exercise; placebo plus exercise; or testosterone plus exercise (Bhasin *et al.* 2000). Placebo or 100 mg testosterone enanthate were given intramuscularly weekly for 16 weeks. The exercise program was a thrice-weekly, progressive, supervised strength-training program. Effort-dependent muscle strength in five different exercises was measured using the 1RM method. In the placebo-only group, muscle strength did not change in any of the five exercises (-0.3 to -4.0%). This indicates that this strategy was effective in minimizing the influence of the learning effect. Men treated with testosterone alone, exercise alone, or combined testosterone and exercise, experienced significant increases in maximum voluntary muscle strength in the leg press ($+22$ to 30%), leg curls ($+18$ to 36%), bench press ($+19$ to 33%), and latissimus dorsi pulldowns ($+17$ to 33%) exercises. The gains in strength in all the exercises were greater in men receiving testosterone, or exercise alone compared to those receiving placebo alone. The change in leg press strength was correlated with change in muscle volume ($R = 0.44$, $P = 0.003$) and change in fat free mass ($R = 0.55$, $P < 0.001$). We conclude that when the confounding influence of the learning effect is minimized and appropriate androgen-responsive measures of muscle strength are selected, testosterone replacement is associated with demonstrable increase in maximal voluntary strength in HIV-infected men with low testosterone levels.

Strength training also promotes gains in lean body mass and muscle strength (Bhasin *et al.* 1996; 2000). Further, supraphysiologic doses of androgens augment

the anabolic effects of resistance exercise on lean body mass and maximal voluntary strength (Sattler *et al.* 2002; Strawford *et al.* 1999).

These data suggest that testosterone can promote weight gain and increase in lean body mass, as well as muscle strength in HIV-infected men with low testosterone levels. We do not know, however, whether physiological androgen replacement can produce meaningful improvement in quality of life, utilization of health care resources, or physical function in HIV-infected men. Some studies have reported improvements in mood and depression indices in HIV-infected men after testosterone administration. Emerging data indicate that testosterone does not affect HIV replication, but its effects on virus shedding in the genital tract are not known.

There is a high frequency of low total and free testosterone levels, sexual dysfunction, infertility, delayed puberty, and growth failure in patients with end-stage renal disease (Handelsman *et al.* 1981; 1985; 1986). Fat free mass is decreased and physical function is markedly impaired in men with end-stage renal disease who are receiving maintenance hemodialysis (Johansen 1999; Painter and Johansen 1999). Androgen administration does not consistently improve sexual dysfunction in these patients (Handelsman 1985). Similarly, the effects of androgen treatment on growth and pubertal development in children with end-stage renal disease remain unclear (Jones *et al.* 1980; Kassmann *et al.* 1992). Controlled clinical trials of nandrolone decanoate have reported increased hemoglobin levels with androgen treatment in men with end-stage renal disease who are on hemodialysis (Buchwald *et al.* 1977; Berns *et al.* 1992; Johansen *et al.* 1999). Prior to the advent of erythropoietin, testosterone was commonly used to treat anemia associated with end-stage renal disease. Testosterone increases red cell production by stimulating erythropoietin, augmenting erythropoietin action, and by its direct action on stem cells. Further studies are needed to determine whether testosterone administration can reduce blood transfusion and erythropoietin requirements in patients with end-stage renal disease on hemodialysis.

Patients with autoimmune disorders, particularly those receiving glucocorticoids, often experience a reduction in circulating testosterone concentrations, muscle wasting and bone loss (MacAdams *et al.* 1986; Reid 1987; Reid *et al.* 1994; 1996). In a placebo-controlled study, Reid *et al.* (1996) administered a replacement dose of testosterone to men receiving glucocorticoids. Testosterone replacement was associated with a greater increase in fat free mass and bone density than placebo.

Chronic obstructive pulmonary disease (COPD) is a chronic debilitating disease for which there are few effective therapies. Muscle wasting and dysfunction are recognized as correctable causes of exercise intolerance in these patients. It has been speculated that low levels of anabolic hormones such as testosterone, growth hormone and insulin-like growth factor-1 may contribute to muscle atrophy and dysfunction (Casaburi *et al.* 1996). Human growth hormone increases nitrogen

retention and lean body muscle in patients with COPD; however, the effects of hrGH on respiratory muscle strength and exercise tolerance remain to be established (Burdet *et al.* 1997; Pape *et al.* 1991; Pichard *et al.* 1996). Schols *et al.* (1995) examined the effects of a low dose of nandrolone or placebo in 217 men and women with COPD; these authors reported modest increases in lean body mass and respiratory muscle strength. Casaburi *et al.* (2001) have recently demonstrated that physiologic testosterone replacement increases fat free mass, muscle size, and muscle strength in men with COPD who have low testosterone levels.

8.7 Testosterone effects on fat metabolism

Percent body fat is higher in hypogonadal men in comparison to eugonadal controls (Katznelson *et al.* 1998). Induction of androgen deficiency in healthy men by administration of a GnRH agonist leads to an increase in fat mass (Mauras *et al.* 1998). Some studies of young, hypogonadal men have reported a decrease in fat mass with testosterone replacement therapy (Brodsky *et al.* 1996; Katznelson *et al.* 1996; Snyder *et al.* 2000) while others (Bhasin *et al.* 1997; Wang *et al.* 1996) found no change. In contrast, long-term studies of testosterone supplementation of older men have consistently demonstrated a decrease in fat mass (Kenny *et al.* 2001; Snyder *et al.* 1999; Tenover 2000). Epidemiologic studies (Khaw and Barrett-Connor 1992; Seidell *et al.* 1990) have shown that serum testosterone levels are lower in middle-aged men with visceral obesity. Serum testosterone levels correlate inversely with visceral fat area and directly with plasma HDL levels. Testosterone replacement of middle-aged men with visceral obesity improves insulin sensitivity, decreases blood glucose and blood pressure (Marin *et al.* 1992; 1996). In our dose-response studies, administration of graded doses of testosterone to men was associated with a dose-dependent decrease in fat mass (Bhasin *et al.* 2001). Loss of fat mass at higher doses was evenly distributed in the trunk and appendices, and in the superficial and deep compartments. Thus, there was a decrease in intra-abdominal fat as well as intermuscular fat in association with high doses of testosterone. Testosterone is an important determinant of regional fat distribution and metabolism in men (Marin *et al.* 1996).

8.8 Key messages

- Androgens have direct anabolic effects on the muscle. Testosterone administration to healthy, young men is associated with dose-dependent increments in fat-free mass, muscle size, and maximal voluntary strength.
- In older men with low testosterone levels, testosterone replacement increases fat-free mass and decreases fat mass. We do not know whether testosterone replacement of older men improves

physical function or other health-related outcomes, and whether it reduces the risk of falls, fractures, and disability.
- Testosterone-induced increase in skeletal muscle mass is due to muscle fiber hypertrophy.
- Testosterone increases muscle mass and reduces fat mass by promoting the commitment and differentiation of mesenchymal, pluripotent stem cells into the myogenic lineage and inhibiting their differentiation into the adipogenic lineage.

8.9 REFERENCES

Amory JK, Chansky HA, Chansky KL, Camuso MR, Hoey CT, Anawalt BD, Matsumoto AM, Bremner WJ (2002) Preoperative supraphysiological testosterone in older men undergoing knee replacement surgery. J Am Geriatr Soc 50:1698–1701

Antonio J, Wilson JD, George FW (1999) Effects of castration and androgen treatment on androgen-receptor levels in rat skeletal muscles. J Appl Physiol 87:2016–2019

Bardin CW 1996 The anabolic action of testosterone. N Engl J Med 335:52–53

Bartsch W, Knabbe C, Voigt KD (1983) Regulation and compartmentalization of androgens in rat prostate and muscle. J Steroid Biochem 19:929–937

Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ (1999) Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev 107:23–136

Berns JS, Rudnick MR, Cohen RM (1992) A controlled trial of recombinant human erythropoietin and nandrolone decanoate in the treatment of anemia in patients on chronic hemodialysis. Clin Nephrol 37:264–267

Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R (1996) The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 335:1–7

Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ, Casaburi R (1997) Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 82:407–413

Bhasin S, Storer TW, Asbel-Sethi N, Kilbourne A, Hays R, Sinha-Hikim I, Shen R, Arver S, Beall G (1998) Effects of testosterone replacement with a nongenital, transdermal system, Androderm, in human immunodeficiency virus-infected men with low testosterone levels. J Clin Endocrinol Metab 83:3155–3162

Bhasin S, Storer TW, Javanbakht M, Berman N, Yarasheski KE, Phillips J, Dike M, Sinha-Hikim I, Shen R, Hays RD, Beall G (2000) Testosterone replacement and resistance exercise in HIV-infected men with weight loss and low testosterone levels. JAMA 283:763–770

Bhasin S, Woodhouse L, Storer TW (2001a) Proof of the effect of testosterone on skeletal muscle. J Endocrinol 170:27–38

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW (2001b) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:E1172–1181

Bhasin S, Woodhouse L, Storer TW (2003a) Androgen effects on body composition. Growth Horm IGF Res 13 Suppl: S63–71

Bhasin S, Taylor WE, Singh R, Artaza J, Sinha-Hikim I, Jasuja R, Choi H, Gonzalez-Cadavid NF (2003b). The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action. J Gerontol A Biol Sci Med Sci (in press)

Blackman MR, Sorkin JD, Munzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, Jayme J, O'Connor KG, Christmas C, Tobin JD, Stewart KJ, Cottrell E, St Clair C, Pabst KM, Harman SM (2002) Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA 288:2282–2292

Blanco CE, Popper P, Micevych P (1997) Anabolic-androgenic steroid induced alterations in choline acetyltransferase messenger RNA levels of spinal cord motoneurons in the male rat. Neuroscience 78:873–882

Brodsky IG, Balagopal P, Nair KS (1996) Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – a clinical research center study. J Clin Endocrinol Metab 81:3469–3475

Bross R, Casaburi R, Storer TW, Bhasin S (1998) Androgen effects on body composition and muscle function: implications for the use of androgens as anabolic agents in sarcopenic states. Baillieres Clin Endocrinol Metab 12:365–378

Buchwald D, Argyres S, Easterling RE, Oelshlegel FJ, Jr., Brewer GJ, Schoomaker EB, Abbrecht PH, Williams GW, Weller JM (1977) Effect of nandrolone decanoate on the anemia of chronic hemodialysis patients. Nephron 18:232–238

Burdet L, de Muralt B, Schutz Y, Pichard C, Fitting JW (1997) Administration of growth hormone to underweight patients with chronic obstructive pulmonary disease. A prospective, randomized, controlled study. Am J Respir Crit Care Med 156:1800–1806

Burger HG, Dudley EC, Hopper JL, Shelley JM, Green A, Smith A, Dennerstein L, Morse C (1995) The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. J Clin Endocrinol Metab 80:3537–3545

Casaburi R, Corsentino, G, Bhasin, S, Fournier, M, Lewis, M, Porszasz, J, Storer, T (2001) A randomized trial of strength training and testosterone supplementation in men with chronic obstructive pulmonary disease. Eur Respir J 18 (Suppl 33): 173S

Coodley GO, Coodley MK (1997) A trial of testosterone therapy for HIV-associated weight loss. Aids 11:1347–1352

Coodley GO, Loveless MO, Nelson HD, Coodley MK (1994) Endocrine function in the HIV wasting syndrome. J Acquir Immune Defic Syndr 7:46–51

Dahlberg E, Snochowski M, Gustafsson JA (1981) Regulation of the androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. Endocrinology 108:1431–1440

Dallongeville J, Marecaux N, Isorez D, Zylbergberg G, Fruchart JC, Amouyel P (1995) Multiple coronary heart disease risk factors are associated with menopause and influenced by substitutive hormonal therapy in a cohort of French women. Atherosclerosis 118:123–133

Dawson RT (2001) Drugs in sport – the role of the physician. J Endocrinol 170:55–61

Dobs AS, Cofrancesco J, Nolten WE, Danoff A, Anderson R, Hamilton CD, Feinberg J, Seekins D, Yangco B, Rhame F (1999) The use of a transscrotal testosterone delivery system in the

treatment of patients with weight loss related to human immunodeficiency virus infection. Am J Med 107:126–132

Ferrando AA, Tipton KD, Doyle D, Phillips SM, Cortiella J, Wolfe RR (1998) Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. Am J Physiol 275:E864–871

Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, LiebermanFerrando AA, Tipton K, Wolfe RR, Urban RJ (2002) Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. Am J Physiol Endocrinol Metab 282:E601–607

Fryburg DA, Weltman A, Jahn LA, Weltman JY, Samojlik E, Hintz RL, Veldhuis JD (1997) Short-term modulation of the androgen milieu alters pulsatile, but not exercise- or growth hormone (GH)-releasing hormone-stimulated GH secretion in healthy men: impact of gonadal steroid and GH secretory changes on metabolic outcomes. J Clin Endocrinol Metab 82:3710–3719

Gambacciani M, Ciaponi M, Cappagli B, Piaggesi L, De Simone L, Orlandi R, Genazzani AR (1997) Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. J Clin Endocrinol Metab 82:414–417

Griggs RC, Halliday D, Kingston W, Moxley RT, 3rd (1986) Effect of testosterone on muscle protein synthesis in myotonic dystrophy. Ann Neurol 20:590–596

Griggs RC, Pandya S, Florence JM, Brooke MH, Kingston W, Miller JP, Chutkow J, Herr BE, Moxley RT, 3rd (1989) Randomized controlled trial of testosterone in myotonic dystrophy. Neurology 39:219–222

Grinspoon S, Corcoran C, Askari H, Schoenfeld D, Wolf L, Burrows B, Walsh M, Hayden D, Parlman K, Anderson E, Basgoz N, Klibanski A (1998) Effects of androgen administration in men with the AIDS wasting syndrome. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 129:18–26

Grinspoon S, Corcoran C, Parlman K, Costello M, Rosenthal D, Anderson E, Stanley T, Schoenfeld D, Burrows B, Hayden D, Basgoz N, Klibanski A (2000) Effects of testosterone and progressive resistance training in eugonadal men with AIDS wasting. A randomized, controlled trial. Ann Intern Med 133:348–355

Handelsman DJ (1985) Hypothalamic-pituitary gonadal dysfunction in renal failure, dialysis and renal transplantation. Endocr Rev 6:151–182

Handelsman DJ, Ralec VL, Tiller DJ, Horvath JS, Turtle JR (1981) Testicular function after renal transplantation. Clin Endocrinol (Oxf) 14:527–538

Handelsman DJ, Spaliviero JA, Turtle JR (1986) Bioactive luteinizing hormone in plasma of uraemic men and men with primary testicular damage. Clin Endocrinol (Oxf) 24:259–266

Johansen KL (1999) Physical functioning and exercise capacity in patients on dialysis. Adv Ren Replace Ther 6:141–148

Johansen KL, Mulligan K, Schambelan M (1999) Anabolic effects of nandrolone decanoate in patients receiving dialysis: a randomized controlled trial. JAMA 281:1275–1281

Jones RW, El Bishti MM, Bloom SR, Burke J, Carter JE, Counahan R, Dalton RN, Morris MC, Chantler C (1980) The effects of anabolic steroids on growth, body composition, and metabolism in boys with chronic renal failure on regular hemodialysis. J Pediatr 97: 559–566

Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER (2000) Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA 97:12735–12740

Kassmann K, Rappaport R, Broyer M (1992) The short-term effect of testosterone on growth in boys on hemodialysis. Clin Nephrol 37:148–154

Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A (1996) Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 81:4358–4365

Katznelson L, Rosenthal DI, Rosol MS, Anderson EJ, Hayden DL, Schoenfeld DA, Klibanski A (1998) Using quantitative CT to assess adipose distribution in adult men with acquired hypogonadism. AJR Am J Roentgenol 170:423–427

Kenny AM, Prestwood KM, Gruman CA, Marcello KM, Raisz LG (2001) Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci 56:M266–272

Kenny AM, Bellantonio S, Gruman CA, Acosta RD, Prestwood KM (2002) Effects of transdermal testosterone on cognitive function and health perception in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci 57:M321–325

Kenyon A, Knowlton, K, Sandiford, I, Koch, FC, Lotwin, G (1940) A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. Endocrinology 26:26–45

Khaw KT, Barrett-Connor E (1992) Lower endogenous androgens predict central adiposity in men. Ann Epidemiol 2:675–682

Kochakian C (1937) Testosterone and testosterone acetate and the protein and energy metabolism of castrate dogs. Endocrinology 21:750–755

Kochakian C (1950) Comparison of protein anabolic property of various androgens in the castrated rat. Am J Physiol 60:53–58

Kochakian C, Murlin, J (1935) The effect of male hormone on the protein and energy metabolism of castrate dogs. J Nutrition 10:437–459

Konagaya M, Max SR (1986) A possible role for endogenous glucocorticoids in orchiectomy-induced atrophy of the rat levator ani muscle: studies with RU 38486, a potent and selective antiglucocorticoid. J Steroid Biochem 25:305–308

Leslie M, Forger NG, Breedlove SM (1991) Sexual dimorphism and androgen effects on spinal motoneurons innervating the rat flexor digitorum brevis. Brain Res 561:269–273

MacAdams MR, White RH, Chipps BE (1986) Reduction of serum testosterone levels during chronic glucocorticoid therapy. Ann Intern Med 104:648–651

Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, Lindstedt G, Bjorntorp P (1992a) The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord 16:991–997

Marin P, Krotkiewski M, Bjorntorp P (1992b) Androgen treatment of middle-aged, obese men: effects on metabolism, muscle and adipose tissues. Eur J Med 1:329–336

Marin P, Lonn L, Andersson B, Oden B, Olbe L, Bengtsson BA, Bjorntorp P (1996) Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. J Clin Endocrinol Metab 81:1018–1022

Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, Veldhuis JD, Urban RJ (1998) Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. J Clin Endocrinol Metab 83:1886–1892

Melton LJ, 3rd, Khosla S, Riggs BL (2000) Epidemiology of sarcopenia. Mayo Clin Proc 75 Suppl: S10–12; discussion S12–13

Morley JE, Kaiser FE, Perry HM, 3rd, Patrick P, Morley PM, Stauber PM, Vellas B, Baumgartner RN, Garry PJ (1997) Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. Metabolism 46:410–413

Morley JE, Perry, HM, 3rd, Kaiser FE, Kraenzle D, Jensen J, Houston K, Mattammal M, Perry HM, Jr (1993) Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. J Am Geriatr Soc 41:149–152

Painter P, Johansen K (1999) Physical functioning in end-stage renal disease. Introduction: a call to activity. Adv Ren Replace Ther 6:107–109

Pape GS, Friedman M, Underwood LE, Clemmons DR 1991 The effect of growth hormone on weight gain and pulmonary function in patients with chronic obstructive lung disease. Chest 99:1495–1500

Pichard C, Kyle U, Chevrolet JC, Jolliet P, Slosman D, Mensi N, Temler E, Ricou B (1996) Lack of effects of recombinant growth hormone on muscle function in patients requiring prolonged mechanical ventilation: a prospective, randomized, controlled study. Crit Care Med 24:403–413

Rance NE, Max SR (1984) Modulation of the cytosolic androgen receptor in striated muscle by sex steroids. Endocrinology 115:862–866

Reid IR (1987) Serum testosterone levels during chronic glucocorticoid therapy. Ann Intern Med 106:639–640

Reid IR, Veale AG, France JT (1994) Glucocorticoid osteoporosis. J Asthma 31:7–18

Reid IR, Wattie DJ, Evans MC, Stapleton JP (1996) Testosterone therapy in glucocorticoid-treated men. Arch Intern Med 156:1173–1177

Roy TA, Blackman MR, Harman SM, Tobin JD, Schrager M, Metter EJ (2002) Interrelationships of serum testosterone and free testosterone index with FFM and strength in aging men. Am J Physiol Endocrinol Metab 283:E284–294

Sattler F, Briggs W, Antonipillai I, Allen J, Horton R (1998) Low dihydrotestosterone and weight loss in the AIDS wasting syndrome. J Acquir Immune Defic Syndr Hum Retrovirol 18:246–251

Sattler FR, Jaque SV, Schroeder ET, Olson C, Dube MP, Martinez C, Briggs W, Horton R, Azen S (1999) Effects of pharmacological doses of nandrolone decanoate and progressive resistance training in immunodeficient patients infected with human immunodeficiency virus. J Clin Endocrinol Metab 84:1268–1276

Sattler FR, Schroeder ET, Dube MP, Jaque SV, Martinez C, Blanche PJ, Azen S, Krauss RM (2002) Metabolic effects of nandrolone decanoate and resistance training in men with HIV. Am J Physiol Endocrinol Metab 283:E1214–1222

Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF (1995) Physiologic effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo-controlled randomized trial. Am J Respir Crit Care Med 152:1268–1274

Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R (1990) Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. Metabolism 39:897–901

Sih R, Morley JE, Kaiser FE, Perry HM, 3rd, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L, Buchanan TA, Shen R, Bross R, Berman N, Bhasin S (2002) The effects of varying doses of T on insulin sensitivity, plasma lipids, apolipoproteins, and C-reactive protein in healthy young men. J Clin Endocrinol Metab 87:136–143

Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S (2003) Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. Endocrinology (July Epub ahead of print)

Sinha-Hikim I, Artaza J, Woodhouse L, Gonzalez-Cadavid N, Singh AB, Lee MI, Storer TW, Casaburi R, Shen R, Bhasin S (2002) Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am J Physiol Endocrinol Metab 283:E154–164

Snyder PJ, Lawrence DA (1980) Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 51:1335–1339

Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH, Dlewati A, Staley J, Santanna J, Kapoor SC, Attie MF, Haddad JG, Jr., Strom BL (1999a) Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL (1999b) Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A, Santanna J, Loh L, Lenrow DA, Holmes JH, Kapoor SC, Atkinson LE, Strom BL (2000) Effects of testosterone replacement in hypogonadal men. J Clin Endocrinol Metab 85:2670–2677

Steidle C, Schwartz S, Jacoby K, Sebree T, Smith T, Bachand R (2003) AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. J Clin Endocrinol Metab 88:2673–2681

Storer TW, Magliano L, Woodhouse L, Lee ML, Dzekov C, Dzekov J, Casaburi R, Bhasin S (2003) Testosterone dose-dependently increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension. J Clin Endocrinol Metab 88:1478–1485

Strawford A, Barbieri T, Neese R, Van Loan M, Christiansen M, Hoh R, Sathyan G, Skowronski R, King J, Hellerstein M (1999a) Effects of nandrolone decanoate therapy in borderline hypogonadal men with HIV-associated weight loss. J Acquir Immune Defic Syndr Hum Retrovirol 20:137–146

Strawford A, Barbieri T, Van Loan M, Parks E, Catlin D, Barton N, Neese R, Christiansen M, King J, Hellerstein MK (1999b) Resistance exercise and supraphysiologic androgen therapy in eugonadal men with HIV-related weight loss: a randomized controlled trial. JAMA 281:1282–1290

Tenover JS (1992) Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Tenover JL (2000) Experience with testosterone replacement in the elderly. Mayo Clin Proc 75 Suppl: S77–81; discussion S82

Urban RJ, Bodenburg YH, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, Ferrando A (1995) Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am J Physiol 269:E820–826

Van Loan MD, Strawford A, Jacob M, Hellerstein M (1999) Monitoring changes in fat-free mass in HIV-positive men with hypotestosteronemia and AIDS wasting syndrome treated with gonadal hormone replacement therapy. Aids 13:241–248

Wang C, Eyre DR, Clark R, Kleinberg D, Newman C, Iranmanesh A, Veldhuis J, Dudley RE, Berman N, Davidson T, Barstow TJ, Sinow R, Alexander G, Swerdloff RS (1996) Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men–a clinical research center study. J Clin Endocrinol Metab 81:3654–3662

Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–2853

Wilson J (1988) Androgen abuse by athletes. Endocr Rev 9:181–191

Woodhouse LJ, Reisz-Porszasz S, Javanbakht M, Storer TW, Lee M, Zerounian H, Bhasin S (2003) Development of models to predict anabolic response to testosterone administration in healthy young men. Am J Physiol Endocrinol Metab 284:E1009–1017

9

Androgens and Erythropoiesis

M. Zitzmann and E. Nieschlag

Contents

9.1 Introduction

Men exhibit a higher mass of red blood cells than women, which had already been demonstrated by spectrophotometry in 900 subjects almost 100 years ago (Williamson 1916). This fact cannot totally be accounted for by menstrual blood loss occuring in women and has been shown to be caused by higher androgen levels present in men (Vahlquist 1950). The marked influence of androgens on erythropoiesis was a major endocrinological research topic during the 1970s (for review: Shahidi 1973). Although the prime time of androgen therapy for anemia passed with the introduction of recombinant erythropoietin (rhEPO) in 1987, androgens continue to be widely used for testosterone substitution therapy in hypogonadal men who often present with markedly lowered hemoglobin and erythrocyte concentrations. Nevertheless, the general issue of androgens in relation to erythropoiesis seems to be experiencing a current revival. The effects of androgens on erythropoiesis are exerted via several pathways which will be discussed. Safety aspects

concerning increased red blood cell mass in terms of a putatively affected risk for ischemic vascular disease will also be considered.

9.2 Mechanisms of androgen action within the erythropoietic system

First intervention trials concerning androgens and erythropoiesis were performed in intact rats which exhibited a marked increase of hemoglobin concentrations and bone marrow activity upon testosterone administration (Vollmer and Gordon 1941). These results were confirmed in orchiectomized or hypophysectomized animals in which the resulting anemia could be successfully treated by injections with testosterone propionate (Crafts 1946; Steinglass *et al.* 1941). These results led to clinical application in treatment of women with breast cancer-related anemia (Kennedy and Gilbertsen 1957). Recent reports about anemia caused by androgen deficiency in men such as observed in a large cohort of patients with secondary hypogonadism (Ellegala *et al.* 2003) and men receiving androgen-blockade therapy (Strum *et al.* 1997) are explained by these early studies. The mechanisms by which androgens facilitate these effects on erythropoiesis are discussed below.

9.2.1 Stimulation of erythropoietin (EPO)

Androgens have been demonstrated to cause hypertrophy of renal tissue (Nathan and Gardner 1965), to increase EPO production and increase general RNA polymerase activity (Paulo *et al.* 1974). In addition, a corresponding effect of synergistic action of testosterone and oxygen-deprivation as experienced in high altitude-related hypobaria has been demonstrated in men (Klausen 1998). Nephrectomy abolishes the androgen-induced and EPO-related increase in erythropoiesis (Fried and Kilbridge 1969) and androgen effects on red blood cell mass are markedly mitigated by administration of EPO-antibodies (Schooley 1966).

Accordingly, treatment of hypogonadal Klinefelter patients with various doses of intramuscular testosterone undecanoate resulted in a significant and dose-related increase of EPO concentrations and, as a consequence, elevation of hemoglobin levels (Cui *et al.* 2003). *Vice versa*, the antiandrogen cyproterone acetate can antagonize the effect of testosterone on EPO secretion in mice. The resulting anemia can consequently be treated by synthetic EPO (Medlinsky *et al.* 1969). Such effects were later also seen in men receiving a combined androgen-blockade by a GnRH agonist and flutamide for the treatment of advanced prostate cancer. The marked anemia which had developed after a few months was significantly improved by rhEPO (Bogdanos *et al.* 2003). Nevertheless, androgen effects on erythropoiesis are not restricted to enhancement of EPO secretion but are exerted via some direct pathways as well.

9.2.2 Action on bone marrow

Testosterone has been demonstrated to stimulate erythroid colony formation dose-dependently in vitro. Hence, androgens might have a promoting effect on erythroid colony forming units in bone marrow (Moriyama and Fisher 1975a). As trials in rabbits demonstrated, testosterone may directly act on these colony forming units to enhance their differentiation into EPO-responsive cells, causing an increase of nucleated erythroid cell numbers. Thus, EPO is required to further increase the maturation of these cells (Moriyama and Fisher 1975b). There is also some evidence that, determined by the direct action of androgens on the cellular cycle of bone marrow stem cells, a prevailing differentiation towards the erythroid series and a resulting decrease of differentiation into leucocytes is caused by testosterone (Kozlov *et al.* 1979). This seems to be a pivotal and initial step of androgen action in erythropoiesis as has been demonstrated by bone marrow biopsies in some patients with renal failure receiving testosterone (Kalmanti *et al.* 1982). Activation of androgen receptors in erythroid cells appears to be necessary for testosterone to develop erythropoietic effects, as androgen receptors have been demonstrated in bone marrow erythroid cells and, as a consequence, the effects of testosterone can be completely abolished in cultures from rats pretreated with the androgen receptor antagonists cyproterone or flutamide. Interestingly, the effects of erythropoietin on further differentiated erythroid colony proliferation were also completely blocked by pretreatment with androgen receptor antagonists, suggesting a permissive effect of androgens on EPO action (Malgor *et al.* 1998). Since androgens may increase the sensitivity of the erythroid progenitors to erythropoietin by an independent but synergistic mechanism, effects of treatment by EPO in patients with renal anemia can be improved by additional androgen administration (Ballal *et al.* 1991; Gaughan *et al.* 1997).

A central role in the processes activated by androgens can be attributed to the endogenous retroviral mink cell focus-forming (MCF) genes which are involved in the regulation of bone marrow hemopoietic progenitor cell proliferation. The p15E protein encoded by one of the MCF genes is produced by early hemopoietic progenitors and expression can be stimulated by testosterone. Testosterone effects are mitigated when the respective gene expression is blocked by antisense-oligonucleotides (Chernukhin *et al.* 2000).

9.2.3 Iron incorporation

Early results in patients with iron-deficient anemia demonstrated the beneficial and synergistic effects of additional androgen administration (Victor *et al.* 1967). In the following, testosterone has been demonstrated to enhance iron (Fe) incorporation in red blood cells (Naets and Wittek 1968) and administration of testosterone propionate can increase the incorporation of ^{59}Fe by erythrocytes in mice after a

delay of three to four days (Molinari 1970; 1982; Molinari and Rosenkrantz 1971). Especially under conditions of hypoxia, iron incorporation can be stimulated by androgens (Alippi *et al.* 1985). There are indications that this process induced by androgenic steroids primarily affects the more mature erythroid precursors and needs the presence of EPO (Udupa *et al.* 1986). Testosterone may also facilitate intestinal iron resorption as high ferritin levels indicating low storage of iron are associated with low testosterone levels (Büttner *et al.* 2002).

9.2.4 Hemoglobin synthesis

Iron incorporation by erythrocytes is closely related to hemoglobin synthesis. It has been demonstrated that administration of androgens to human bone marrow cells can inhance hemoglobin synthesis (Necheles and Rai 1969). Likewise, there was a significant 23% increase in incorporation of ^{59}Fe into adult hemoglobin after the addition of testosterone in a primary cell culture system of human fetal liver, but beta-globin chain synthesis, measured as ^{3}H- or ^{14}C-leucine incorporation into globin chains, was identical in control and testosterone-treated cells. Administration of estradiol, on the contrary, led to a decrease of hemoglobin synthesis (Congote *et al.* 1977). Corresponding results were also described in models involving various animal cell cultures (Irving *et al.* 1976).

9.2.5 Red cell glycolysis

Erythrocyte-uptake of glucose subsequently results in glycolysis providing energy via phosphorylation products to enhance the activity of the general transcription machinery. This process is a prerequisite for red blood cell proliferation and is effective after 8–12 hours of glucose utilization (Molinari *et al.* 1976). Androgens can enhance the uptake of glucose (Molinari *et al.* 1976) once they have permeated the cell membrane, a process which is independent of various types of hemoglobin (Molinari 1982). In humans, oral ingestion of oxymetholone, a synthetic androgen, resulted in an increased rate of erythrocyte glycolysis as measured by quantitative determination of: fructose-1,6-diphosphate, dehydroxyacetone phosphate, 2,3-diphosphoglycerate and adenosine triphosphate as glycolysation products (Molinari and Neri 1978). Correspondingly, specific changes occure in the erythroid tissue following depletion of androgens, as studied in rats. The reduction of testosterone levels in the blood of orchiectomized animals caused a decline of erythrocyte glucose-6-phosphate and lactate levels. Subcutaneous administration of testosterone propionate to these animals restored parameters within 12 hours (Molinari *et al.* 1976).

9.2.6 Red cell 2,3-diphosphoglycerate

Androgens can increase erythrocyte 2,3-diphosphoglycerate levels by enhancing glycolysation (see above). This compound shifts the oxygen saturation curve of

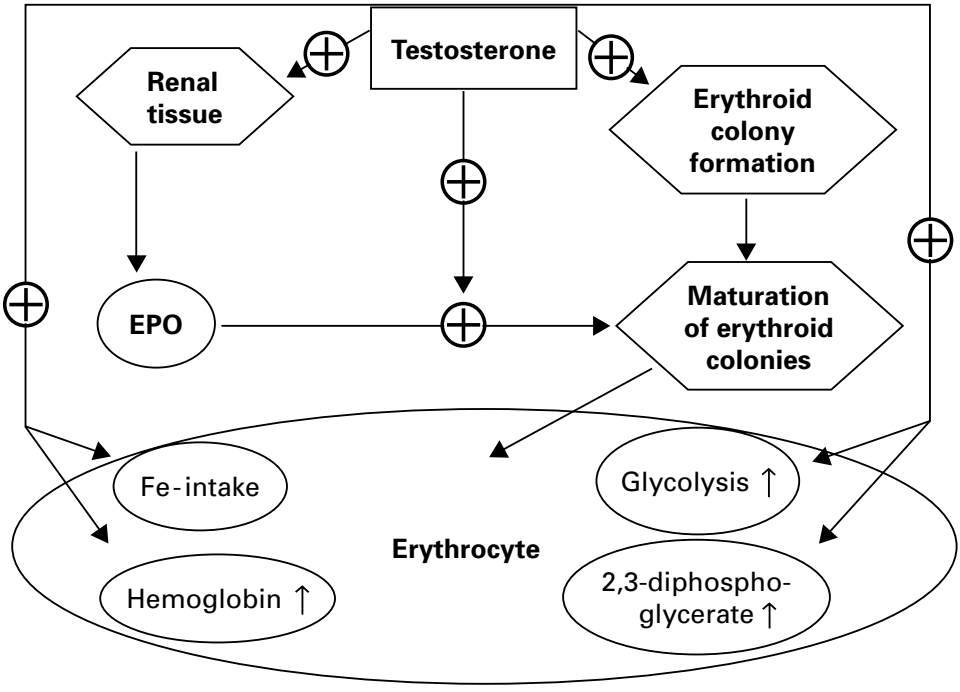


Fig. 9.1 Synopsis of testosterone-induced effects on erythropoiesis. Abbreviations: EPO: erythropoietin, Fe: iron.

hemoglobin to the right, which results in a greater unloading of oxygen in tissue. Placebo controlled trials in men have demonstrated that 2,3-diphosphoglycerate increases markedly and, as a consequence, an increase in oxygen P_{50} results, leading to enhanced oxygen delivery (Parker *et al.* 1972). In patients with renal disease being treated with androgens for anemia, a longer survival of erythrocytes, higher levels of 2,3-diphosphoglycerate and corresponding changes in oxygen affinity were observed (Solomon and Hendler 1987). However, in patients with atherosclerosis, no significant changes in oxygen affinity were detectable under testosterone treatment vs. placebo (Bille-Brahe *et al.* 1976). For a synopsis of androgen effects on erythropoiesis see Figure 9.1.

9.3 Androgen treatment in hypogonadism and effects on erythropoiesis

As mentioned above, early studies in artificially hypogonadal rats demonstrated the positive effects of testosterone on erythropoiesis by withdrawal and substitution trials; androgen administration to healthy men can cause a marked increment in erythropoiesis (Kamischke *et al.* 2002; Palacios *et al.* 1983; Wu *et al.* 1996). Correspondingly, hypogonadal men very often present with markedly lowered concentrations of erythrocytes and/or hemoglobin, hence anemia. Indeed,

anemia can be used as a diagnostic tool to evaluate whether a patient with borderline hypogonadism should receive androgen substitution therapy (e.g. Behre *et al.* 2000).

Various forms of androgen substitution can be used for treatment of male hypogonadism (see Chapter 10), ranging from oral testosterone undecanoate, to transdermal preparations, to long acting injected esters and testosterone implants. A parameter that assures the quality of androgen substitution is restoration of normal hemoglobin and erythrocyte concentrations. In addition, frequent assessment of red blood cell mass, hemoglobin content and also hematocrit is crucial in androgen therapy surveillance in order to detect overstimulation of the erythropoietic system resulting in polycythemia, which might cause adverse side effects (see below).

Therapy of hypogonadism with oral testosterone undecanoate (TU) (see Chapter 14) is effective in terms of restoring the red blood cell pool. This has been demonstrated in a mixed sample of men with primary or secondary hypogonadism receiving various treatment options (oral mesterolone, oral TU, injections with testosterone enanthate and implants with crystalline testosterone). Mesterolone, a non-aromatizable weak androgen did not have significant effects but such were demonstrated in those patients receiving oral TU: hemoglobin concentrations increased significantly (Jockenhövel *et al.* 1997). These effects were also seen for the other "full androgens" (see below). In agreement, the efficacy of oral TU to treat hypogonadism-related anemia was also demonstrated by a placebo-controlled trial in men with androgen deficiency and diabetes mellitus type 2 (Boyanov *et al.* 2003).

Concerning transdermal testosterone preparations and erythropoiesis, the effects of a non-scrotal transdermal patch system and intramuscular testosterone enanthate for the treatment of male hypogonadism were compared in a randomized study involving sixty-six adult hypogonadal men who were randomly assigned to receive either transdermal patches (two 2.5-mg systems applied nightly) or testosterone enanthate (200 mg injected every 2 weeks). Both treatment modalities stimulated erythropoiesis significantly. In patients receiving treatment with testosterone enanthate causing markedly higher serum concentrations of testosterone, abnormal hematocrit elevations (43.8% of patients) were seen more frequently compared with patch-treated men (15.4% of patients) (Dobs *et al.* 1999). Corresponding effects were also seen in 227 hypogonadal men receiving androgen substitution via the transdermal testosterone gel system in two different doses. Marked elevations in red blood cell mass were observed in a dose-dependent manner: those patients receiving the higher gel dose of 100 mg per day vs. those receiving 50 mg per day exhibited a significantly stronger increase in hemoglobin concentrations. Nevertheless, effects reached a plateau after several weeks of treatment (Wang *et al.* 2000). The significant positive effects of the intramuscularaly

injected testosterone enanthate on erythropoiesis in hypogonadal men, a substance used for 50 years, have been mentioned above (Dobs *et al.* 1999; Jockenhövel *et al.* 1997). A trial in 60 artificially hypogonadal men receiving androgen ablation by administration of a long-acting GnRH agonist and following treatment with various doses of intramuscularly injected testosterone enanthate demonstrated a non-linear dose-dependent effect on erythropoiesis (Bhasin *et al.* 2001). Nevertheless, a recent non-human primate study comparing the effects of various intramuscularly injected testosterone esters (testosterone undecanoate, enanthate or buciclate) demonstrated that pharmacokinetics of these different preparations also have a differential influence on androgen target tissues. Despite the higher total dose of testosterone enanthate, effects on erythropoiesis were not different from those observed in the long-acting esters which provided a much more stable environment of elevated androgen concentrations (Weinbauer *et al.* 2003).

As mentioned above, testosterone undecanoate is also available as an injectable ester with quite favorable kinetics allowing injection intervals of up to 12 weeks (also see Chapter 14). This long-acting depot preparation has been investigated in several trials for androgen replacement therapy in hypogonadal men. The effects on erythropoiesis were significant but exhibited a moderate pattern, thus avoiding polycythemia (Nieschlag *et al.* 1999; von Eckardstein and Nieschlag 2002). This is confirmed by Chinese investigations using the same substance in a different vehicle (see Chapter 14): a marked increment in hemoglobin levels was demonstrated and was accompanied by a corresponding elevation of EPO levels (Cui *et al.* 2003).

The modality with the most prolonged kinetics used for androgen substitution therapy is the subdermal implantation of crystalline testosterone pellets (Handelsman *et al.* 1997) (see chapter 14). Stimulation of erythropoiesis is effective and is, due to rather high androgen concentrations during the first months after implantation, comparable to effects achieved by intramuscular testosterone enanthate (Jockenhövel *et al.* 1997).

The synthetic androgen 7α-methyl-nortestosterone (MENT, see Chapters 13 and 14) has been recently tested for its efficacy in substitution therapy of hypogonadal men. The substance, which exhibits a markedly decreased 5α-reduction rate in comparison to testosterone, was able to maintain erythropoietic effects achieved by testosterone enanthate during a run-in phase, albeit in a dose-dependent manner (Anderson *et al.* 2003).

As demonstrated by all studies on androgen substitution and erythropoiesis, there is a certain amount of variation among patients despite similar regimens. This may be attributable to genetically determined modulations of androgen effects, facilitated by androgen receptor polymorphisms, such as the CAG repeat polymorphism

(see Chapter 3). Whether this polymorphism exerts a similar influence on the efficacy of testosterone therapy on erythropoiesis as has been demonstrated for prostate tissue (Zitzmann *et al.* 2003), remains speculative.

A mechanism by which androgen effects on erythropoiesis may be counter-regulated is the negative feedback by the red cell mass on EPO production, which is facilitated via the renal oxygen sensor (Lacombe *et al.* 1991). One could speculate that this oxygen sensor has different thresholds in various patients, which could explain the differences in dose-response-relationships of erythropoiesis to androgen exposure in men. In addition, due to cardiac insuffiency or chronic pulmonary diseases resulting in lower oxygen supply in target tissues, the development of polycythemia under androgen therapy might be promoted. Especially these patients would be put at risk due to increased blood viscosity (see next section). Finally, androgen therapy may also be useful in treatment of anemia of non-gonadal origin. See Section 15.3 for a review of publications.

9.4 Hematocrit and ischemic disease

Some men treated with testosterone respond with polycythemia, a phenomenon especially observed in older men (Drinka *et al.* 1995; Hajjar *et al.* 1997; Krauss *et al.* 1991; Sih *et al.* 1997). Especially the combination of increased red blood cell volume and decreased plasma volume results in a marked elevation of hematocrit, hence blood viscosity. In one study, three of nine patients with hematocrit >48% (53%, 58% and 64%) experienced central ischemic episodes such as basal ganglia stroke, brain stem stroke and transient ischemic attack (Krauss *et al.* 1991). Another small study reported polycythemia in two out of eight patients. These were characterized by the highest body mass index within the group and developed sleep apnea (Drinka *et al.* 1995).

While these reports of actual ischemic events under testosterone therapy are rare and were seen in small numbers of men, they have to be considered as important. Especially TE injected intramuscularly and causing supraphysiological peaks seems to cause high hematocrit (Dobs *et al.* 1999).

There is evidence from larger neurological studies unrelated to testosterone that elevated hematocrit can result in cerebral ischemia due to various mechanisms. Erythrocyte aggregation may lead to increased viscosity which in turn facilitates platelet activation and aggregation (Lowe 1999; Lowe and Forbes 1985; Wood *et al.* 1985). A retrospective analysis in 500 patients suggested a significant relation of hematocrit with the incidence of ischemic insults. A hemoglobin concentration >150 g/l or a hematocrit >44% was associated with a doubling of the incidence in cerebral infarctions (Niazi *et al.* 1994). An autopsy study in several hundred patients confirms these results and demonstrates that advanced age and atherosclerosis as

further adverse parameters can lower the critical threshold of hematocrit down to even 41%. Ischemic lesions were predominantly located in deep subcortical structures where artery calibers are small (Tohgi *et al.* 1978). In a later study, the critical threshold of hematocrit was seen to be higher (>50%) in an analysis of 320 patients suffering mortal cerebral ischemia (Lowe *et al.* 1983). A prospective study, which surveyed over 1000 residents during a 16-year period in regard to cerebral ischemic events, revealed increased hematocrit (>45%), also after correction for arterial hypertension and age, as independent risk factor (Kiyohara *et al.* 1986). Such results are confirmed by another prospective study involving 1000 patients: identifiable risk factors present in these stroke patients were arterial hypertension (64.3%), smoking (35.2%), diabetes mellitus (26.9%), hypercholesterolemia (24.1%), high hematocrit (> or = 50%) in 21.8% and a clinically identified potential cardiac sources of embolism in 18.3% (Lee *et al.* 2001). However, it remains unclear whether high hematocrit as caused by testosterone treatment in an otherwise healthy man has the same relevance.

Another important tissue involved in ischemic events is the heart. Coronary heart disease (CHD) is due to multifactorial events causing atherosclerosis. Involved are arterial hypertension, hyperlipoproteinemia, insulin resistance and inflammatory processes. All these factors are influenced by androgens but to date no conclusion can be drawn whether testosterone plays an adverse or beneficial role (see Chapter 10). It is most likely that androgens modulate these risk factors on an individual basis, a process possibly related to the CAG repeat androgen receptor polymorphism (see Chapter 3).

It can be assumed that high hematocrit needs the pathological basis of plaque rupture to become clinically significant in the form of increased coagulation. Elevated hematocrit (>49%) has been found in retrospective studies of patients with CHD (Burch and De Pasquale 1962; Sorlie *et al.* 1981). Such results are confirmed by a large prospective study in more than 8000 men followed over 10 years: those persons with hematocrit >45% had a significantly higher mortality rate due to CHD than to any other cause (Carter *et al.* 1983).

However, the relationship of hematocrit and CHD independent of other cardiovascular disease risk factors remained unclear until a large study involving data from 8896 adults aged 30–75 years showed that, although mortality rate per 10,000 population was 42.6, 31.9, and 46.3 among men with hematocrit in the lower, middle, and upper tertiles, respectively, this was not the case after adjustment for age, race, education, smoking status, hypertensive status, total serum cholesterol, body mass index, white blood cell count, previous history of CHD and diabetes mellitus (Brown *et al.* 2001). Hence, elevated hematocrit seems to present an epiphenomen in cardiac disease and the role of androgen-induced hematocrit elevation remains unresolved.

9.5 Key messages

- Androgens increase erythropoiesis.
- This is facilitated by several pathways involving enhancement of erythropoietin secretion and independently promoting differentiation of erythroid progenitor cells.
- Testosterone substitution therapy in hypogonadal men restores red blood cell mass and, hence, oxygen supply.
- Polycythemia can be induced during androgen substitution, especially when supraphysiological concentrations are reached or patients are older.
- Polycythemia or elevated hematocrit represent a risk factor for cerebral ischemia while its role in relation to cardiovascular disease remains unclear.

9.6 REFERENCES

Alippi RM, Barcelo AC, Bozzini CE (1985) Comparison of erythropoietic response to erythropoietin-secreting stimuli in mice following polycythemia induced by transfusion or hypoxia. Exp Hematol 13:159–162

Anderson RA, Wallace AM, Sattar N, Kumar N, Sundaram K (2003) Evidence for tissue selectivity of the synthetic androgen 7 alpha-methyl-19-nortestosterone in hypogonadal men. J Clin Endocrinol Metab 88:2784–2793

Ballal SH, Domoto DT, Polack DC, Marciulonis P, Martin KJ (1991) Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. Am J Kidney Dis 17:29–33

Behre HM, Yeung CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E (2000) Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM (eds.): Andrology: Male reproductive health and dysfunction, 2nd edition, Springer, Heidelberg, pp 89–124

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:E1172–1181

Bille-Brahe NE, Kehlet H, Madsbad S, Rorth M (1976) Effects of androgens on oxygen affinity in vivo and 2,3-diphosphoglycerate content of red cells in peripheral arterial insufficiency. Scand J Clin Lab Invest 36:801–804

Bogdanos J, Karamanolakis D, Milathianakis C, Repousis P, Tsintavis A, Koutsilieris M (2003) Combined androgen blockade-induced anemia in prostate cancer patients without bone involvement. Anticancer Res 23:1757–1762

Boyanov MA, Boneva Z, Christov VG (2003) Testosterone supplementation in men with type 2 diabetes, visceral obesity and partial androgen deficiency. Aging Male 6:1–7

Brown DW, Giles WH, Croft JB (2001) Hematocrit and the risk of coronary heart disease mortality. Am Heart J 142:657–663

Burch GE, De Pasquale NP (1962) The hematocrit in patients with myocardial infarction. JAMA 180: 63

Büttner R, Bollheimer LC, Zietz B, Drobnik W, Lackner K, Schmitz G, Schölmerich J, Palitzsch KD (2002) Definition and characterization of relative hypo- and hyperleptinemia in a large Caucasian population. J Endocrinol 175:745–756

Carter C, McGee D, Reed D, Yano K, Stemmermann G (1983) Hematocrit and the risk of coronary heart disease: the Honolulu Heart Program. Am Heart J 105:674–679

Chernukhin IV, Khaldoyanidi SK, Orlovskaya IA, Matrosova VY, Svinarchuk FP, Konevetz DA, Vlasov VV, Gaidul KV, Kozlov VA (2000) A role of endogenous retroviral MCF env gene in proliferation of pluripotent hemopoietic progenitors in mice. Russ J Immunol 5:19–26

Congote LF, Bruno F, Solomon S (1977) Effects of testosterone and estradiol on ratios of adult to fetal hemoglobin in cell cultures of human fetal liver. Biol Neonate 32:310–318

Crafts RC (1946) Effeccts of hypophysectomy, castration and testosterone propionate on hemopoeisis in the adult male rat. Endocrinology 39:401–413

Cui YG, Tong JS, Pan QQ, Di FS, Jia Y, Feng T, Liu Y, Wang XH, Zhang GY (2003) Effect of androgens on erythropoietin in patients with hypogonadism. Zhonghua Nan Ke Xue 9:248–251

Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA (1999) Pharmacokinetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in comparison with bi-weekly injections of testosterone enanthate for the treatment of hypogonadal men. J Clin Endocrinol Metab 84:3469–3478

Drinka PJ, Jochen AL, Cuisinier M, Bloom R, Rudman I, Rudman D (1995) Polycythemia as a complication of testosterone replacement therapy in nursing home men with low testosterone levels. J Am Geriatr Soc 43:899–901

Ellegala DB, Alden TD, Couture DE, Vance ML, Maartens NF, Laws ER Jr (2003) Anemia, testosterone, and pituitary adenoma in men. J Neurosurg 98:974–977

Fried W, Kilbridge T (1969) Effect of testosterone and of cobalt on erythropoietin production by anephric rats. J Lab Clin Med 74:623–629

Gaughan WJ, Liss KA, Dunn SR, Mangold AM, Buhsmer JP, Michael B, Burke JF (1997) A 6-month study of low-dose recombinant human erythropoietin alone and in combination with androgens for the treatment of anemia in chronic hemodialysis patients. Am J Kidney Dis 30:495–500

Hajjar RR, Kaiser FE, Morley JE (1997) Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. J Clin Endocrinol Metab 82:3793–3796

Handelsman DJ, Mackey MA, Howe C, Turner L, Conway AJ (1997) An analysis of testosterone implants for androgen replacement therapy. Clin Endocrinol (Oxf) 47:311–316

Irving RA, Mainwaring IP, Spooner PM (1976) The regulation of haemoglobin synthesis in cultured chick blastoderms by steroids related to 5beta-androstane. Biochem J 154:81–93

Jockenhövel F, Vogel E, Reinhardt W, Reinwein D (1997) Effects of various modes of androgen substitution therapy on erythropoiesis. Eur J Med Res 28:293–298

Kalmanti M, Dainiak N, Martino J, Dewey M, Kulkarni V, Howard D (1982) Correlation of clinical and in vitro erythropoietic responses to androgens in renal failure. Kidney Int 22:383–391.

Kamischke A, Heuermann T, Krüger K, von Eckardstein S, Schellschmidt I, Rübig A, Nieschlag E (2002) An effective hormonal male contraceptive using testosterone undecanoate with oral or injectable norethisterone preparations. J Clin Endocrinol Metab 87:530–539

Kennedy BJ, Gilbertsen AS (1957). Increased erythropoeisis induced by androgenic hormone therapy. N Engl J Med 86:308–314

Kiyohara Y, Ueda K, Hasuo Y, FujiiI, Yanai T, Wada J, Kawano H, Shikata T, Omae T, Fujishima M (1986) Hematocrit as a risk factor of cerebral infarction: long-term prospective population survey in a Japanese rural community. Stroke 17:687–692

Klausen T (1998) The feed-back regulation of erythropoietin production in healthy humans. Dan Med Bull 45:345–353

Kozlov VA, Tsyrlova IG, Zhuravkin IN (1979) Different effect of testosterone on polypotential stem hematopoietic stem cells and immunocompetent B-lymphocytes. Zh Mikrobiol Epidemiol Immunobiol 9:72–76

Krauss DJ, Taub HA, Lantinga LJ, Dunsky MH, Kelly CM (1991) Risks of blood volume changes in hypogonadal men treated with testosterone enanthate for erectile impotence. J Urol 146:1566–1570

Lacombe C, Da Silva JL, Bruneval P, Casadevall N, Camilleri JP, Bariety J, Tambourin P, Varet B (1991) Erythropoietin: sites of synthesis and regulation of secretion. Am J Kidney Dis 18:14–19

Lee BI, Nam HS, Heo JH, Kim DI; Yonsei Stroke Team (2001) Yonsei Stroke Registry. Analysis of 1,000 patients with acute cerebral infarctions. Cerebrovasc Dis 12:145–151

Lowe GD, Jaap AJ, Forbes CD (1983) Relation of atrial fibrillation and high haematocrit to mortality in acute stroke. Lancet 9:784–786

Lowe GD, Forbes CD (1985) Platelet aggregation, haematocrit, and fibrinogen. Lancet 16:395–396

Lowe GD (1999) Rheological influences on thrombosis. Baillieres Best Pract Res Clin Haematol 12:435–449

Malgor LA, Valsecia M, Verges E, De Markowsky EE (1998) Blockade of the in vitro effects of testosterone and erythropoietin on Cfu-E and Bfu-E proliferation by pretreatment of the donor rats with cyproterone and flutamide. Acta Physiol Pharmacol Ther Latinoam 48:99–105

Medlinsky JT, Napier CD, Gurney CW (1969) The use of an antiandrogen to further investigate the erythropoietic effects of androgens. J Lab Clin Med 74:85–92

Molinari PF (1970) Androgens and erythropoiesis in bone marrow. II. Effect of testosterone propionate on 59Fe concentration in erythrocytes and bone marrow. Experientia 26:531–532

Molinari PF (1982) Erythropoietic mechanism of androgens: a critical review and clinical implications. Haematologica 67:442–460

Molinari PF, Rosenkrantz H (1971) Erythropoietic activity and androgenic implications of 29 testosterone derivatives in orchiectomized rats. J Lab Clin Med 78:399–410

Molinari PF, Esber HJ, Snyder LM (1976) Effect of androgens on maturation and metabolism of erythroid tissue. Exp Hematol 4:301–309

Molinari PF, Neri LL (1978) Effect of a single oral dose of oxymetholone on the metabolism of human erythrocytes. Exp Hematol 6:648–654

Moriyama Y, Fisher JW (1975a). Effects of testosterone and erythropoietin on erythroid colony formation in human bone marrow cultures. Blood 45:665–670

Moriyama Y, Fisher JW (1975b) Increase in erythroid colony formation in rabbits following the administration of testosterone. Proc Soc Exp Biol Med 149:178–180

Naets JP, Wittek M (1968) The mechanism of action of androgens on erythropoiesis. Ann NY Acad Sci 149:366–376

Nathan DG, Gardner FH (1965) Effects of large doses of androgen on rodent erythropoiesis and body composition. Blood 26:411–420

Necheles TF, Rai US (1969) Studies on the control of hemoglobin synthesis: the in vitro stimulating effect of a 5-beta-H steroid metabolite on heme formation in human bone marrow cells. Blood 34:380–384

Niazi GA, Awada A, al Rajeh S, Larbi E (1994) Hematological values and their assessment as risk factor in Saudi patients with stroke. Acta Neurol Scand 89:439–445

Nieschlag E, Büchter D, von Eckardstein S, Abshagen K, Simoni M, Behre HM (1999) Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol (Oxf) 51:757–763

Palacios A, Campfield LA, McClure RD, Steiner B, Swerdloff RS (1983) Effect of testosterone enanthate on hematopoiesis in normal men. Fertil Steril 40:1000–104

Parker JP, Beirne GJ, Desai JN, Raich PC, Shahidi NT (1972) Androgen-induced increase in red-cell 2,3-diphosphoglycerate. N Engl J Med 287:381–383

Paulo LG, Fink GD, Roh BL, Fisher JW (1974) Effects of several androgens and steroid metabolites on erythropoietin production in the isolated perfused dog kidney. Blood 43:39–47

Schooley JC (1966) Inhibition of erythropoietic stimulation by testosterone in polycythemic mice receiving anti-erythropoietin. Proc Soc Exp Biol Med 122:402–403

Shahidi NT (1973) Androgens and erythropoiesis. N Engl J Med 289:72–80

Sih R, Morley JE, Kaiser FE, Perry HM 3rd, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667.

Solomon LR, Hendler ED (1987) Androgen therapy in haemodialysis patients. II. Effects on red cell metabolism. Br J Haematol 65:223–230

Sorlie PD, Garcia-Palmieri MR, Costas R Jr, Havlik RJ (1981) Hematocrit and risk of coronary heart disease: the Puerto Rico Health Program. Am Heart J 101:456–461

Steinglass P, Gordon AS, Charipper HA (1941) Effect of castration and sex hormones on blood of the rat. Proc Soc Exp Biol Med 48:169–177

Strum SB, McDermed JE, Scholz MC, Johnson H, Tisman G (1997) Anaemia associated with androgen deprivation in patients with prostate cancer receiving combined hormone blockade. Br J Urol 79:933–941

Tohgi H, Yamanouchi H, Murakami M, Kameyama M (1978) Importance of the hematocrit as a risk factor in cerebral infarction. Stroke 9:369–374

Udupa KB, Crabtree HM, Lipschitz DA (1986) In vitro culture of proerythroblasts: characterization of proliferative response to erythropoietin and steroids. Br J Haematol 62:705–714

Vahlquist B (1950) The cause of the sexual differences in erythrocyte, hemoglobin and serum iron levels in human adults. Blood 5:874–875

Victor G, Shanmugasundaram K, Krishnamurthi CA, Rex PM, Nagarajan D (1967) Haemoglobin response to anabolic steroid in iron-deficiency anaemia. J Assoc Physicians India 15:177–183

Vollmer EP, Gordon AS (1941) Effect os sex and gonadotropic hormones upon the blood picture of the rat. Endocrinology 29:828–837

von Eckardstein S, Nieschlag E (2002) Treatment of male hypogonadism with testosterone undecanoate injected at extended intervals of 12 weeks: a phase II study. J Androl 23:419–425

Wang C, Swedloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–2853

Weinbauer GF, Partsch CJ, Zitzmann M, Schlatt S, Nieschlag E (2003) Pharmacokinetics and degree of aromatization rather than total dose of different preparations determine the effects of testosterone: A nonhuman primate study in *macaca fascicularis*. J Androl 24:765–774

Williamson CS (1916) Influence of age and sex on hemoglobin: a spectrophotometric analysis of nine hundred and nineteen cases. Arch Intern Med 18:505–528

Wood JH, Kee DB Jr (1985) Hemorheology of the cerebral circulation in stroke. Stroke 16:765–767

Wu FC, Farley TM, Peregoudov A, Waites GM (1996) Effects of testosterone enanthate in normal men: experience from a multicenter contraceptive efficacy study. World Health Organization Task Force on Methods for the Regulation of Male Fertility. Fertil Steril 65:626–636

Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E (2003) Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: a longitudinal pharmacogenetic study. J Clin Endocrinol Metab 88:2049–2054.

10

Testosterone and cardiovascular diseases

A. von Eckardstein and F.C.W. Wu

Contents

10.1 Introduction

In industrialized countries, the average life expectancy is some eight years less in males than in females. Since cardiovascular disease is the most frequent cause of death and male gender is one of the classic risk factors for premature coronary artery disease (threefold excess in men before the age of 55 years), stroke (up to twofold excess in men), peripheral vascular disease (two to threefold excess in men) and heart failure, the lack of estrogens and the abundance of androgens have often been regarded as the proximate cause underlying this male disadvantage. Sex hormones may play a role in cardiovascular morbidity and mortality by modulating the risk factors of atherosclerosis and vascular function, by influencing the progression of subclincial coronary, cerebral and peripheral arterial vessel wall lesions to symptomatic cardiovascular disease including myocardial infarction, stroke, claudicatio intermittens and erectile dysfunction. Finally, sex hormones may influence the long-term clinical sequelae of coronary artery disease such as heart failure and arrhythmias.

The lack of an inflection point in the rate of increase in cardiovascular morbidity and mortality after menopause and the failure of controlled combined estrogen-progestin replacement intervention trials to show prevention of coronary events in postmenopausal women (Manson *et al.* 2003; Roussow *et al.* 2002) have shed doubts on the cardioprotective role of estrogens and increased the interest in testosterone. With the prospects of much wider therapeutic applications of testosterone for contraception, treatment of patients with aplastic anaemia, sarcopenic, osteopenic and dysphoric states, as well as physiological ageing, it has become increasingly important to address whether testosterone treatment might increase the risk or severity of cardiovascular diseases. This heightened interest is reflected by the recent publication of three reviews on this topic in international journals (Liu *et al.* 2003; Weidemann and Hanke 2002; Wu and von Eckardstein 2003).

10.2 Relationships between serum levels of testosterone and cardiovascular disease – observational studies

At the outset, it is important to emphasize the limitations of observational studies on associations between serum levels of endogenous androgens and cardiovascular

disease. The cardiovascular disease endpoints were extremely variable (mortality, morbidity such as myocardial infarction and angina, decompensated and compensated heart failure, completed stroke and transient ischemic attack, angiography, ultrasound, computer tomography, or post-mortem based diagnosis or unspecified events), study groups were heterogeneous and selection criteria diverse. Most cardiovascular disease patients will be on medications and have modified their lifestyle. In some studies, selection of poorly-matched controls may have introduced biases. The time interval from onset of disease to study varied from three months to several years and timing of blood sampling was not always standardised for diurnal variation of hormone levels. The majority of these studies did not adjust for confounding factors. For example, hypoandrogenemia in men and hyperandrogenemia in women are confounded with various metabolic disorders including obesity, insulin resistance, dyslipidemia and impaired fibrinolysis. Finally, chronic coronary artery disease and heart failure as well as acute myocardial infarction induce a fall in serum levels of testosterone (Kontoleon *et al.* 2003; Pugh *et al.* 2002; Wu and von Eckardstein 2003; Zitzmann and Nieschlag 2001).

10.2.1 Testosterone and cardiovascular disease in men

Sixteen of 32 cross-sectional studies found lower levels of testosterone in patients with coronary artery disease compared with healthy controls. Sixteen showed no difference in testosterone levels between cases and controls. In none were high levels of testosterone associated with coronary artery disease. All studies which measured levels of free or bioavailable testosterone found an inverse association with coronary artery disease (reviewed in Alexandersen *et al.* 1996; Wu and von Eckardstein 2003). None of six longitudinal studies in men showed any significant association between serum levels of testosterone and future coronary artery disease events (reviewed in Alexandersen *et al.* 1996; Wu and von Eckardstein 2003). With the limitations stated before, this suggests that testosterone plays a neutral or even beneficial role in the pathogenesis of coronary artery disease. This is also supported by the finding of a genetic case-control study, where we did not find any significant association of angiographically assessed coronary artery disease with the CAG repeat polymorphism in exon 1 of the androgen receptor gene (Hersberger and von Eckardstein, unpublished results), which determines testosterone sensitivity (for background information on this polymorphism, see Section 10.8 of this chapter as well as the Chapter 2 on the molecular biology of the androgen receptor).

Data of observational studies suggest that testosterone is a determinant of myocardial mass and may thereby influence the clinical course of heart failure. Also after adjustment for gender differences in body size and weight, men show higher left ventricular mass than women from puberty to the end of life (reviewed by Hayward *et al.* 2000; 2001; Liu *et al.* 2003). Moreover, serum levels of testosterone

were lower in men with cardiac failure (Kontoleon *et al.* 2003). The finding that chronic mechanical circulatory support leads to an increase in testosterone levels in men with end-stage cardiac failure (Kontoleon *et al.* 2003; Noirhomme *et al.* 1999) is compatible with the aforementioned importance of chronic disease as a cause of male hypogonadism.

In case-control studies, stroke survivors were found to have reduced concentrations of testosterone in both blood and cerebrospinal fluid (Elwan *et al.* 1990; Jeppesen *et al.* 1996). Upon ultrasound studies, increased carotid intima media thickness and the presence of carotid plaques (De Pergola 2003 *et al.*; Fukui 2003 *et al.*), which are strong risk factors of future stroke (and other cardiovascular events including myocardial infarction), showed an inverse association with serum levels of total or free testosterone.

Peripheral arterial disease is the consequence of atherosclerosis in the aorta and the arteries of the pelvis, the lower and upper extremities. A specific male variant of peripheral artery disease is vasculogenic erectile dysfunction, which is the most frequent cause of erectile dysfunction (see also Chapter 11). Observational studies on the association of testosterone with peripheral arterial disease are scarce. In the Rotterdam study, the presence of aortic calcifications were found to be associated with low total and free testosterone (Hak *et al.* 2002). With respect to erectile dysfunction, the role of hypotestosteronemia as a source of the vascular variant is difficult to assess since testosterone also affects neurovegetative and psychological aspects of sexual activity. At least, except the small subgroup of men with frank testosterone deficiency (<5%), men with erectile dysfunction were not found to have lower average testosterone levels than matched controls (Jannini *et al.* 1999; Liu *et al.* 2003; Lue 2000; Melman *et al.* 1999).

10.2.2 Testosterone and cardiovascular disease in women

By contrast to the neutral or even beneficial associations between endogenous testosterone levels and cardiovascular disease for men, the few retrospective or cross-sectional case-control studies in women revealed pro-atherogenic associations of androgens with CAD (Wu and von Eckardstein 2003). Only scanty prospective data is available on the importance of testosterone as a cardiovascular risk factor in women. Barrett-Connor and Goodman-Gruen (1995) reported a 19-year follow-up of 651 postmenopausal women. Serum levels of testosterone, bioavailable testosterone, and androstendione did not differ between those women with and those without a coronary artery disease history at baseline. Cardiovascular mortality during follow-up was not associated with any androgen serum level (Barrett-Connor and Goodman-Gruen 1995).

Indirect evidence for the atherogenicity of androgens in women was derived from the findings of clinical studies that women with coronary artery disease were affected

more frequently than control women by clinical symptoms of androgen excess such as hirsutism and polycystic ovaries (Amowitz and Sobel 1999; Dunaif 1997; Lobo and Carmina 2000; Rajkhova *et al.* 2000). Cross-sectional data consistently showed a strong obesity-independent association with a cluster of cardiovascular risk factors including insulin resistance, dyslipidaemia and impaired fibrinolysis. It was therefore suggested that the chronically abnormal hormonal and metabolic milieu in the polycystic ovary syndrome (PCOS), starting from adolescence, may predispose these women to premature atherosclerosis. Based on calculated risk profiles, women with polycystic ovary syndrome were predicted to have a 7-fold increased relative risk for myocardial infarction. In agreement with this concept, a combined angiography and pelvic ultrasound study of 143 women aged $\leq$60 years observed significant associations between the presence of polycystic ovaries with the presence and severity of coronary artery disease and a family history of myocardial infarction as well as with elevated levels of insulin and triglycerides and lower levels of HDL-C (Birdsall *et al.* 1997). In an electron beam computer tomography study, non-diabetic women with the polycystic ovary syndrome showed more coronary artery calcification, a marker of coronary atherosclerosis, than healthy controls matched by age and body mass index (Christian *et al.* 2003). Two case-control studies found significantly increased carotid artery intima-media thickness in women with PCOS compared to age-matched controls independently of BMI, fat distribution and other risk factors (Guzick *et al.* 1996; Talbott *et al.* 2000). By contrast to these data, the one and only long-term longitudinal study did not find any association between PCOS and coronary artery disease incidence. The authors compared the mortality and morbidity rates of 786 out of 1028 women diagnosed to have PCOS between 1930–1979 with 1060 age-matched control women over a mean period of 30 years. Despite significantly increased prevalences of diabetes, hypertension, and hypercholesterolemia among women with PCOS, the standardised odds ratios of coronary artery disease mortality and coronary artery disease morbidity were only little and insignificantly increased in women with PCOS (Pierpoint *et al.* 1998; Wild *et al.* 2000). Hence coronary artery disease risk in women with PCOS may have been overestimated previously. It may, however, also be that treatment of women with PCOS with estrogens have counteracted the effects of increased risk factors and pre-symptomatic disease.

10.3 Effects of testosterone on cardiovascular disease – interventional clinical studies

10.3.1 Endogenous androgen deprivation

Hamilton and Mestler (1969) investigated mentally handicapped castrated and non-castrated inmates of a psychiatric institution and found that castrated patients

lived longer. Nieschlag and colleagues compared the life span of castrated and intact singers and did not reveal any differences in total or cardiovascular mortality (Nieschlag *et al.* 1993). In agreement with a neutral or beneficial effect of endogenous androgens in men, bassos tended to live longer than tenors. By contrast and in agreement with an adverse effect of androgens in women altos had a reduced life expectancy as compared to sopranos (Nieschlag *et al.* 2003).

Cross-gender sex hormone treatment of 816 male-to-female transexuals aged 18–86 years (van Kestereren *et al.* 1997) with administration of ethinylestradiol 100 μg/day and cyproterone acetate 100 mg/day for 7734 patient-years was not associated with any significant difference in cardiovascular mortality or morbidity compared to the general male population despite a 20-fold increase in venous thromboembolic complications. These data were interpreted as an indication that the abolition of testicular androgens by pre- or postpubertal castration does not change cardiovascular mortality in men.

10.3.2 Androgen excess from anabolic steroid abuse

A formal case-control study of anabolic-androgenic steroid abuse in younger men presenting with acute myocardial infarction has not been performed. Nevertheless, a review of the literature covering a 12-year period from 1987 to 1998 (Sullivan *et al.* 1998) identified 17 case reports of cardiovascular events (11 acute myocardial infarction, 4 cardiomyopathy, 2 stroke) in young male bodybuilders using extremely high suprapharmacological doses of anabolic androgens. However, although the number of current and former anabolic androgen users has greatly increased since the 1960s to over 1 million in the USA alone, there does not seem to have been any increase in the frequency of reported vascular events amongst likely and ex-users of anabolic-androgenic steroids.

10.3.3 Exogenous testosterone treatment in men with cardiovascular disease

The longterm effects of exogenous testosterone on coronary event rates has not been investigated. However, in several small studies therapeutic doses of testosterone reduced the severity and frequency of angina pectoris events and improved electrocardiographic signs of myocardial ischemia. Webb and colleagues (Webb *et al.* 1999) showed that a single i.v. bolus of 2.3 mg of testosterone increased time to 1-mm ST segment depression by 66 sec in 14 men with coronary artery disease and low plasma testosterone. The plasma testosterone increased from 5.2 to 117 nmol/L. Infusion of testosterone over three minutes into the coronary arteries of 13 men with established coronary artery disease during coronary angiography at supraphyisological doses of 8 μmol/L but not the physiological dose of 8 nmol/L led to significant increases in coronary vessel diameter and blood flow at all four doses of testosterone. These results have been confirmed by a similar study

(Rosano *et al.* 1999) in 14 men with established coronary artery disease and have been interpreted as beneficial effects of testosterone on coronary artery vasoreactivity. However, it is important to note that these direct acute pharmacological effects of testosterone on the coronary vasculature have been only found upon acute injection of supraphysiological concentrations and may not be relevant to the physiological situation. Thus, in contrast to these acute experiments, flow-mediated and hence endothelium-dependent dilation of the brachial artery, which is strongly correlated with coronary endothelium-dependent vasoreactivity, was found worsened after three months testosterone substitution in hypogonadal men (Zitzmann *et al.* 2002).

Even less information is available on the effects of testosterone on other outcomes of cardiovascular disease. In a small placebo-controlled pilot study, treatment of 20 chronic heart failure patients with weekly injections of 100 mg testosterone enanthate led to improvements of left ventricular ejection fraction and exercise capacity (Unpublished, quoted after Liu *et al.* 2003). In agreement with this, treatment of 12 stable heart failure patients with 60 mg testosterone or placebo via the buccal route resulted in significant increases in serum levels of bioavailable testosterone and cardiac output and in a significant decrease of peripheral artery resistance with a maximal effect seen after three hours (Pugh *et al.* 2003). No study has been performed on the effects of testosterone on stroke and no study showed any positive effect of testosterone on subjective measures such as pain or walking distance or time and objective measures such as muscle blood flow, plethysmographic parameters or foot pulses (Liu *et al.* 2003). However, there is evidence that testosterone can induce nitric oxide production in penile vasculature in animals and humans and testosterone supplements can improve erectile response to sildenafil in men with low testosterone and erectile dysfunction (Aversa *et al.* 2000; 2003; Guay *et al.* 2001).

10.3.4 Exogenous androgen treatment in women

There is increasing interest in the use of testosterone as part of postmenopausal hormone replacement therapy, in particular to improve reportedly impaired sexual function (Davis and Tran 2001). Whether the concurrent use of testosterone will impact on the perceived benefits of estrogen hormone replacement therapy on the cardiovascular system is currently unknown. In a 20-year (1975–1994) retrospective survey of the Amsterdam Gender Dysphoria Clinic (van Kesteren 1997), 293 female-to-male transexuals aged 17–70 years (mean 34) were treated for two months to 41 years (total exposure of 2418 patient-years) with oral testosterone undecanoate 160 mg daily or testosterone (Sustanon) 250 mg i.m. every 2 weeks. There was no excess of cardiovascular (or all cause) mortality or morbidity compared with the general female Dutch population.

In an unmasked study, 40 postmenopausal women on conventional estorgen/progestin hormone replacement additionally received either placebo or 40 mg testosterone undecoanate per day for eight months. A small but significant increase in the pulsatility index of the middle cerebral artery but not in the internal carotid artery has been seen (Penotti *et al.* 2001).

10.4 Cardiovascular effects of testosterone in animal studies

10.4.1 Atherosclerosis

The influence of androgens on the development and progression of experimentally-induced atherosclerosis has been investigated in nine animal models. Four studies of castrated rabbits with diet-induced atherosclerosis showed either no or beneficial effects of testosterone on atherosclerotic lesion size in male animals and detrimental effects in female animals (Alexandersen *et al.* 1999; Bruck *et al.* 1997; Fogelberg *et al.* 1990; Larsen *et al.* 1993). Detrimental effects of testosterone were also seen in male chicks (Toda *et al.* 1984) and female ovariectomized cynomolgus monkeys (Adams *et al.* 1995). Interestingly, the sex-specific effects of testosterone in rabbits and monkeys occurred independently of changes in lipid levels (Alexandersen *et al.* 1999; Bruck *et al.* 1997) and, surprisingly, despite improved endothelial reactivity (Adams *et al.* 1995).

Three studies in apoE- or LDL-receptor deficient mice yielded discrepant results (Elhage *et al.* 1997; Nathan *et al.* 2001; von Dehn *et al.* 2001). In the study by Elhage and colleagues (1997), castration at the age of four weeks had no effect on atherosclerosis of either male or female mice. In both sexes, application of subcutaneous testosterone pellets for eight weeks significantly decreased serum levels of cholesterol and inhibited the development of fatty streak lesions in the sinus aortae by about 30%. In the study by von Dehn *et al.* (2001), suppression of testosterone by 100 μg of the GnRH antagonist Cetrorelix every 48 hours led to a decrease in atherosclerosis in both the sinus aortae and the ascending aorta despite increases of cholesterol in male and decreases of HDL-C in female mice. Implantation of a silastic implant with 35 mg testosterone led to increases of serum levels of testosterone and cholesterol and atherosclerotic lesion size in male mice. Despite an increase of testosterone levels to 10.1 ng/ml, female mice showed no change in lipids and fewer atherosclerotic lesions. The discrepancy between the two studies may have resulted from the higher dosages of testosterone in the second study. Another study performed in LDL-receptor knock-out mice also found an anti-atherogenic effect of testosterone which was blunted by the parallel use of an aromatase inhibitor. Therefore the anti-atherogenic effect was ascribed to estradiol rather than testosterone (Nathan *et al.* 2001).

Taken together the data of the animal experiments suggest the occurrence of sex-specific effects of exogenous testosterone on atherosclerosis which in male animals may be at least partially mediated by aromatization to estradiol.

10.4.2 Myocardial function and heart failure

In various rat models of cardiac hypertrophy or heart failure, female mice showed less or later cardiac dysfunction than male mice so that survival and cardiac adaptation were reduced in male animals compared to female animals. Also myocardial expression of the genes for β-myosin, sarcoplasmic reticulum Ca^{2+}-ATPase and acetylcholinesterase differed between pressure-overloaded male and female rats (Gardner *et al.* 2002; Hayward *et al.* 2001; 2002). Only a few animal studies have investigated the effects of testosterone on myocardial function giving contradictory results. Castration of either female or male rats resulted in decreases of heart weight and contractile function (Scheuer *et al.* 1988) which in both sexes were corrected by isosexual hormone replacement. Application of testosterone to rats also led to increased gene expression of the V1 myosin heavy chain isoenzyme. In rats with experimentally induced myocardial infarction, application of testosterone caused myocardial hypertrophy, improved or worsened ventricular function and increased the rate of acute cardiac rupture (Cavasin *et al.* 2003; Nahrendorf *et al.* 2003). After trauma and haemorrhage, male but not female mice were found to have depressed immune and cardiac function, the latter being improved by treatment with an anti-androgen (Remmers *et al.* 1997; 1998; Wichmann *et al.* 1997). Together the data indicate that testosterone contributes to gender differences in myocardial function but leave open the question of whether testosterone will exert beneficial or adverse effects in the treatment or prevention of heart failure.

10.5 Effects of testosterone on cardiovascular risk factors

The net effect of testosterone on cardiovascular risk is difficult to assess for at least six main reasons. First, the effects of testosterone on cardiovascular risk factors are contradictory depending on whether associations with endogenous testosterone or effects of exogenous testosterone have been investigated. Second, the associations between serum concentrations of endogenous testosterone and cardiovascular risk factors are confounded with mutual interactions between endogenous androgens, body fat distribution, and insulin sensitivity. Third, exogenous testosterone has profound effects on several risk factors, some of which at first sight appear beneficial, namely lipoprotein(a) (Lp(a)), insulin, fibrinogen, and plasminogen activator type 1 (PAI-1), while others are considered adverse, namely HDL-C. Fourth, the causal relationship between some of the aforementioned risk factors and atherosclerosis has not been proven. Of special importance are results of experimental and

clinical studies indicating that therapeutically-induced changes in HDL-C may not necessarily be accompanied by changes in cardiovascular risk (Hersberger and von Eckardstein 2003). Fifth, testosterone can exert its metabolic effects directly or by its metabolites estradiol and dihydrotestosterone. The effects of testosterone and estradiol, in particular, can be either additive (for example on Lp(a)) or counteractive (for example on HDL-C). Sixth, polymorphisms in the genes of the androgen and estrogen receptors, sex hormone binding globulin (SHBG), 5α-reductase and aromatase, regulate genomic effects and the bioavailability of testosterone, dihydrotestosterone and estradiol, respectively. Thus, at a given serum concentration the bioactivity and metabolic effects of testosterone can be diverse.

10.5.1 Associations of endogenous testosterone with cardiovascular risk factors

Several cross-sectional population studies found statistically significant correlations between plasma levels of testosterone and various risk factors, which, however, were opposite in men and women.

In men testosterone plasma levels were frequently found to have positive correlations with serum levels of HDL-C as well as inverse correlations with plasma levels of triglycerides, total cholesterol, LDL-C, fibrinogen and PAI-1. However, serum levels of testosterone have even stronger inverse correlations with BMI, waist circumference, waist-hip-ratio (WHR), amount of visceral fat and serum levels of leptin, insulin and free fatty acids. After adjustment for these measures of obesity and insulin resistance, the correlations between cardiovascular risk factors with testosterone but not with visceral fat or insulin lost their statistical significance (Hergenc *et al.* 1999; Tchernof *et al.* 1996; Tsai *et al.* 2000). These findings indicate that a low serum level of testosterone in eugonadal men is a component of the metabolic syndrome, which is characterized by the presence of obesity, glucose intolerance or overt type 2 diabetes mellitus, arterial hypertension, hypertriglyceridemia, low HDL-C, a pro-coagulatory and anti-fibrinolytic state and for which insulin resistance is thought to be an important etiological factor. Therefore, the frequently observed association of high testosterone levels with a more favourable cardiovascular risk factor profile in men probably does not reflect direct regulatory effects of testosterone on lipoprotein metabolism and the hemostatic system. Accordingly, in some populations these associations disappeared when serum levels of free testosterone (correcting for variations in SHBG) instead of total testosterone were correlated with lipids and other cardiovascular risk factors. Also in accordance with this, a low number of CAG repeats in the androgen receptor, which increases its sensitivity to testosterone, was associated with reduced levels of HDL-C and leptin as well as low body fat mass and body mass index (Zitzmann *et al.* 2001b; 2003). One reason for the discrepancy between the biological effects and the associations of endogenous testosterone with various cardiovascular risk factors is the negative

regulatory effect of insulin on the production of sex hormone binding globulin so that insulin resistance causes low levels of sex hormone binding globulin and thereby low levels of total testosterone (Hautanen 2000).

Women present the opposite relationships between endogenous androgens and obesity, insulin and cardiovascular risk factors. In cross-sectional studies, serum levels of testosterone were found to have significant positive correlations with BMI and leptin levels. Low serum levels of SHBG, which is an indirect measure of female hyperandrogenism, were associated with high BMI and WHR as well as with high serum levels of leptin and insulin and low serum levels of HDL-C (Hergenc *et al.* 1999). Moreover, in a prospective study, 20% of women with SHBG-levels below the 5th percentile developed diabetes mellitus type 2 within the 12-year follow-up period (Lindstedt *et al.* 1991). Thus, in women, hyperandrogenism, rather than hypoandrogenism as in men, is a component of the insulin resistance syndrome. In agreement with this, women with PCOS frequently present with hypercholesterolemia, low HDL-C, hypertriglyceridemia, elevated fibrinogen and PAI-1, and a family history of diabetes mellitus. Because many women with PCOS are overweight and most if not all are insulin resistant, it is a matter of debate whether these symptoms in women with PCOS are secondary to obesity and insulin resistance or whether hyperandrogenemia itself contributes to obesity, insulin resistance, and hyperinsulinemia (Amowitz and Sobel 1999; Dunaif 1997; Lobo and Carmina 2000; Rajkohowa *et al.* 2000).

10.5.2 Effects of puberty on cardiovascular risk factors

Longitudinal studies of puberty were informative on the effects of endogenous sex hormones on cardiovascular risk factors in children and adolescents. Prepubertal boys and girls do not differ significantly in their serum lipid and lipoprotein levels. In contrast to girls, in whom levels of HDL-C and LDL-C change little with puberty, sexually maturing boys experience a decrease in HDL-C and increases in LDL-C and triglycerides (Bagatell and Bremner 1995). However, these changes may not reflect effects of sex hormones only since they are confounded by other endocrine changes, for example in the growth hormone-IGF1 axis, which also regulates lipoprotein metabolism.

10.5.3 Effects of exogenous testosterone on cardiovascular risk factors

10.5.3.1 Lipoproteins

In the majority of studies, substitution of testosterone in hypogonadal men had no impact on total cholesterol, LDL cholesterol and triglycerides but decreased HDL-C and Lp(a) levels.

Treatment with supraphysiological doses of testosterone or androgen-like anabolic steroids in normal men decreased HDL-C by about 20% and more.

Conversely, castration as well as biochemical suppression of endogenous testosterone by GnRH antagonists increased HDL-C (reviewed in Wu and von Eckardstein 2003). In one study, exogenous testosterone only produced a fall in HDL-C in the presence of aromatase inhibitors. These observations and the finding of low HDL-C in men with aromatase deficiency or estrogen resistancy suggest that physiological tissue levels of estradiol play a role in maintaining physiological levels of HDL-C in men.

Since low HDL-C is an important coronary artery disease risk factor and since HDL exerts several potentially anti-atherogenic actions, lowering of HDL-C by testosterone is considered to increase cardiovascular risks (Hersberger and von Eckardstein 2003). However, the epidemiological association of low HDL-C with coronary artery disease has not been proven to be causal. Instead, low HDL-C frequently coincides with other components of the metabolic syndrome and markers of chronic inflammation, and may therefore merely be a surrogate marker for a separate but linked pro-atherogenic condition. Moreover, in transgenic animal models, only increases of HDL-C induced by apoA-I overproduction but not by inhibition of HDL catabolism were consistently found to prevent atherosclerosis (von Eckardstein *et al.* 2001, Hersberger and von Eckardstein 2003). Therefore, the mechanism of HDL modification rather than changes in levels of HDL-C per se appear to determine the (anti)-atherogenicity of HDL modification. Two genes involved in the catabolism of HDL are up-regulated by testosterone, namely scavenger receptor B1 and hepatic lipase. Scavenger receptor B1 mediates the selective uptake of HDL lipids into hepatocytes and steroidogenic cells including Sertoli and Leydig cells of the testes as well as cholesterol efflux from peripheral cells including macrophages. Testosterone up-regulates scavenger receptor B1 in the human hepatocyte cell line HepG2 and in macrophages thereby stimulating selective cholesterol uptake and cholesterol efflux, respectively (Langer *et al.* 2002). Hepatic lipase hydrolyses phospholipids on the surface of HDL thereby facilitating the selective uptake of HDL lipids by SR-B1. The activity of HL in postheparin plasma is increased after administration of exogenous testosterone (Tan *et al.* 1999) and slightly decreased by suppression of testosterone after GnRH antagonist treatment (Büchter *et al.* 1999). Increasing both scavenger receptor B1 and hepatic lipase activities are therefore consistent with the HDL lowering effect of testosterone. Interestingly, overexpression of SR-BI or HL in transgenic mice is associated with a dramatic fall in HDL-C which inhibited rather than enhanced atherosclerosis (von Eckardstein *et al.* 2001). This again demonstrates that the HDL lowering effect of testosterone may not increase and could even decrease cardiovascular risk.

Results of many case-control studies and most prospective population studies demonstrated that lipoprotein(a) (Lp(a)) levels higher than 30 mg/dl are an independent risk factor for coronary, cerebrovascular, and peripheral atherosclerotic

vessel diseases especially if they coexist with other cardiovascular risk factors (Danesh *et al.* 2000). Although Lp(a) levels are predominantly genetically determined, administration of testosterone to men decreased serum levels of Lp(a) significantly by 25% to 59%. Conversely Lp(a) levels were increased by 40% to 60% in controls and patients in whom endogenous testosterone was suppressed by treatment with the GnRH antagonist cetrorelix or the GnRH agonist buserelin (Angelin 1997; Wu and von Eckardstein 2003; von Eckardstein *et al.* 1997). The Lp(a) lowering effect of testosterone is independent of estradiol, which also reduces Lp(a) levels. It is not known how testosterone regulates Lp(a). It is also not known whether changes in Lp(a) induced by testosterone will affect cardiovascular risk.

10.5.3.2 The hemostatic system

In agreement with an important role of thrombus formation in the pathogenesis of acute coronary events and stroke, prospective studies have identified various hemostatic variables as cardiovascular risk factors, among them fibrinogen and the fibrinolysis inhibitor PAI-1 or tissue plasminogen activator antigen. Administration of supraphysiological dosages testosterone to 32 healthy men participating in a trial of male contraception, led to a sustained decrease of fibrinogen by 15 to 20% over 52 weeks of treatment (Anderson *et al.* 1995). In this study the doubling of testosterone levels initially also led to significant decreases of PAI-1, protein S, and protein C as well as to increases of antithrombin and β-thromboglobulin. Likewise PAI-1 was decreased in men who received the anabolic androgen stanozolol. Suppression of testosterone in patients with prostate cancer or benign prostate hypertrophy, however, by treatment with the nonsteroidal anti-androgen casodex or the GnRH agonist leuprolide exerted no significant effects on plasma fibrinogen levels (Eri *et al.* 1995). In agreement with the lowering effects of testosterone on PAI-1, testosterone inhibited the secretion of PAI-1 from bovine aortic endothelial cells in vitro. Taken together the current data indicate that testosterone lowers fibrinogen and PAI-1. However, these anti-coagulatory and pro-fibrinolytic effects may be opposed by pro-aggregatory effects on platelets since high dosages of androgens were found to decrease cyclooxygenase activity and thereby increase platelet aggregability.

10.5.3.3 Inflammation

Recent thinking on the pathogenesis of atherosclerosis has re-discovered the pathological observations from over 100 years ago that atherosclerosis is a chronic inflammatory disease (Libby 2002). This is supported by the epidemiological finding that serum levels of the acute phase reactant C-reactive protein (CRP) are positively associated with the risk of coronary events (Pepys and Hirschfield 2003). Of special importance is that postmenopausal hormone replacement with estrogens and progestins causes an increase in CRP levels (Pradhan *et al.* 2002). This effect has been

taken as one argument to explain the unexpected neutral or even adverse effect of postmenopausal hormone replacement on coronary artery disease. In two studies of healthy eugonadal men treatment with either increasing dosages of testosterone enanthate or dihydrotestosterone or recombinant chorionic gonadotropin as well as suppression of endogenous testosterone with a gonadotropin releasing hormone agonist had no effect on CRP levels. Neither had dihydrotestosterone any effect on serum levels of soluble adhesion molecules (Ng *et al.* 2002; Singh *et al.* 2002).

10.5.3.4 Obesity and insulin sensitivity

Numerous observations point to mutual relationships between androgens, body fat distribution, and insulin sensitivity, of which the latter two are also involved in the regulation of HDL and triglyceride metabolism (Björntorp 1996; Wu and von Eckardstein 2003). It is, however, not clear whether androgens regulate adipose tissue and insulin sensitivity or whether vice versa adipocytes and insulin regulate testosterone levels. Probably a bi-directional relationship exists.

Morbidly obese and insulin resistant *men* frequently have low serum levels of testosterone which increase upon weight loss (Leenen *et al.* 1994). Estradiol levels show the opposite changes to testosterone with obesity and weight loss. It has therefore been suggested that obesity causes hypotestosteronemia by increased aromatisation of testosterone to estradiol in the adipose tissue. Supporting a role of insulin in the determination of testosterone levels in men, infusion of insulin during euglycemic clamp increased testosterone levels in obese men but not in lean men (Pasquali *et al.* 1997). On the other hand, hypogonadal men are frequently obese with increased levels of leptin and insulin (Couillard *et al.* 2000). Body weight, leptin levels and insulin levels decrease upon substitution of testosterone in hypogonadal men (Behre *et al.* 1997). Even treatment of eugonadal obese men with testosterone led to a decrease of visceral fat mass and, in parallel, improved insulin sensitivity and corrected dyslipidemia (Wang *et al.* 2000). In the opposite experiment, suppression of testosterone by the GnRH-antagonist cetrorelix increased serum levels of leptin and insulin (Büchter *et al.* 1999). Moreover, male carriers of the testosterone-hypersensitive androgen receptor gene alleles with a low number of CAG repeats have less body fat than carriers with a high number of CAG repeats (Zitzmann *et al.* 2003). These data indicate that, *in men*, the dominant action in the bi-directional relationship is that testosterone reduces fat mass, especially in the abdomen, and improves insulin action. In agreement with this androgens activate the expression of β-adrenergic receptors, adenylate cyclase, protein kinase A and hormone sensitive lipase in adipocytes (Björntorp 1996). As a result, testosterone stimulates lipolysis and thereby reduces fat storage in adipocytes.

In *women*, mutual interrelationships have also been observed between testosterone, adipose tissue and insulin sensitivity, but in the opposite direction to

those seen in men. On the one hand, insulin sensitivity contributes to the pathogenesis of hyperandrogenemia in polycystic ovary syndrome. Insulin stimulates androgen synthesis in the ovaries via its cognate receptor and the inositolglycan pathway (Nestler *et al.* 1998). Since the ovaries remain sensitive to insulin when other tissues such as fat and muscle are resistant, hyperinsulinemia can augment the LH-dependent hyperandrogenism in insulin resistant women with polycystic ovary syndrome (Dunaif and Thomas 2001). In support of this, treatment of insulin resistance in women with polycystic ovary syndrome with metformin or the insulin sensitizer troglitazone significantly decreased serum levels of insulin as well as testosterone, independently of body mass index or gonadotropin levels (Kolodziejczyk *et al.* 2000; Pasquali and Filicori 1998). Concomittantly, plasma levels of HDL-cholesterol increased and plasma levels of PAI-1 decreased. These data imply that hyperinsulinemia contributes to the functional ovarian hyperandrogenism in polycystic ovary syndrome. Vice versa, lowering androgen levels with GnRH agonists and androgen receptor blockade in hyperandrogenic women were also found to improve insulin sensitivity and lipid profile (Dahlgren *et al.* 1998; Diamanti-Kandarakis *et al.* 1998). The magnitude of these changes however is less than that usually encountered in polycystic ovary syndrome. Since short-term lowering of ovarian androgens by laparoscopic ovarian cautery did not alter insulin or lipid levels (Lemieux *et al.* 1999), androgens probably only aggravate rather than account for the insulin resistance in women with polycystic ovary syndrome. This however, does not exclude the possibility that androgens have an etiological role in polycystic ovary syndrome. For example, experiments in rats and marmoset monkeys recently showed evidence for androgen imprinting. Transient intrauterine or perinatal exposure to testosterone predisposed female animals to central adiposity and insulin resistance in adult life (Eisner *et al.* 2000). Supraphysiological doses of exogenous testosterone or other androgens to women or female cynomolgus monkeys increased body mass index and the mass of both visceral fat and muscle and decreased insulin sensitivity (Adams *et al.* 1995). There appears to be a vicious circle where early androgen excess contributes to insulin resistance in adult women. The resulting hyperinsulinism contributes to the pathogenesis of polycystic ovary syndrome and aggravates the hyperandrogenism and the associated clinical phenotype.

10.6 Effects of testosterone on the function of vascular and cardiac cells

10.6.1 Vascular and cardiac expression of sex hormone receptors and testosterone converting enzymes: implications for genomic and non-genomic effects

In various cells of the vascular wall, testosterone can exert direct effects either by activation of the androgen receptor or by non-genomic effects on plasma

membrane receptors and channels (see Chapters 1 and 2). Thus testosterone can modulate calcium fluxes by mechanisms that are independent of androgen and estrogen receptors in macrophages and endothelial cells. (Benten *et al.* 1999; Guo *et al.* 2002; Lieberherr and Grosse 1994; Rubio-Gayosso *et al.* 2002). This could account for the effects of supraphysiological dosages of testosterone on vasoreactivity which are not inhibited by antagonists of the androgen and estrogen receptors in contrast to some effects seen with physiological dosages (reviewed in Liu *et al.* 2003; Wu and von Eckardstein 2003). There is also evidence that testosterone regulates macrophage function by non-genomic effects via a G-protein-coupled, agonist-sequestrable plasma membrane receptor which initiates calcium- and 1,4,5-trisphosphate-signaling pathways (Lieberherr and Grosse 1994).

The androgen receptor has been found to be expressed in endothelial cells, smooth muscle cells, macrophages, platelets and cardiomyocytes, all of which are relevant to atherosclerosis and heart failure. (reviewed in Hayward *et al.* 2000; Liu *et al.* 2003; Wu and von Eckardstein 2003). Expression of the androgen receptor in human arterial endothelial and smooth muscle cells has not been shown directly, although the association of endothelium-dependent and – independent vasoreactivity with the CAG repeat polymorphism in the androgen receptor provides some indirect evidence in support (Zitzmann *et al.* 2001b). It is also important to note that in several vascular cells, androgen receptor expression was higher if they were derived from male rather than female donors (Hanke *et al.* 2001; Higashiura *et al.* 1997; McCrohon *et al.* 2000). In addition, the expression of aromatase in smooth muscle cells, endothelial cells and macrophages opens the possibility of local conversion of testosterone into estradiol (Diano *et al.* 1999; Harada *et al.* 1999). Both the classic estrogen receptor ERα and the alternative estrogen receptor ERβ are expressed by various vascular cells, so that testosterone can also modulate vascular physiology indirectly via local estradiol production (Hayward *et al.* 2000; Ho and Liao 2002; Hodges *et al.* 2000; Rubanyi *et al.* 1997).

Cardiomyocytes express both androgen and estrogen receptors as well as aromatase and 5α-reductase. (Marsh *et al.* 1998; Thum and Borlak 2002; Weinberg *et al.* 1999) so that testosterone may regulate cardiomyocyte growth and function either directly through the androgen receptor or indirectly via the estrogen receptor.

10.6.2 Effects of testosterone on vascular reactivity

An early hallmark of atherosclerosis is decreased vascular responsiveness to various physiological stimuli due either to endothelial or to endothelium-independent disturbances in the vascular smooth muscle cell (Bonetti *et al.* 2003). As a result, decreased vasodilation and enhanced vasoconstriction can lead to vasospasm and angina pectoris. Moreover, endothelial dysfunction also contributes to coronary events by promoting plaque rupture and thrombosis (Libby 2002; Ross

1999). Testosterone can induce vasodilation or vasoconstriction via endothelium-dependent or endothelium-independent mechanisms and by genomic or non-genomic modes of action. The diversity of these findings appears to be due to differences in species, gender, concomitant disease and, most importantly, dosage of testosterone.

Suggestive of an adverse effect of testosterone, nitrate-induced and hence endothelium-independent dilation of the brachial arteries was significantly reduced in female-to-male transsexuals taking high-dose androgens (McCredie *et al.* 1998). In another case-control study, castrated patients with prostate cancer had a greater flow-induced (i.e. endothelium-dependent) dilation of brachial arteries than controls, whereas the endothelium-independent vasodilation by nitroglycerin did not differ between groups (Herman *et al.* 1997). In another study, untreated hypogonadal men were found to have increased flow-mediated vasodilatation of the brachial artery compared to matched eugonadal men. Upon three months of substitution treatment with 250 mg testosterone enanthate every two weeks, flow-mediated dilatation decreased significantly (Zitzmann *et al.* 2002). In a group of 110 healthy men, we observed a positive association between the number of CAG repeats in exon 1 of the androgen receptor gene and endothelium-dependent as well as endothelial–independent vasodilatation. Thus, the greater the sensitivity to testosterone, the less brachial arteries dilate in response to either flow or nitrate (Zitzmann *et al.* 2001b).

In contrast to these observational or long-term treatment studies, acute interventional studies with intravenous administration of testosterone to male patients with coronary artery disease revealed apparently beneficial vasodilatory effects of testosterone (see section 10.3.3). Likewise, in vivo studies in monkeys and dogs of both sexes as well as most in vitro studies with animal vessels suggest that testosterone exerts beneficial effects on vascular reactivity. After testosterone treatment for two years in ovariectomized female cynomolgus monkeys, intracoronary injections of acetylcholine caused significant endothelium-dependent vasodilation in treated but not in untreated animals. In contrast, endothelium-independent vasodilation occurred normally in both groups (Adams *et al.* 1995). In dogs, testosterone induced vasodilation of coronary arteries by endothelium-dependent and independent mechanisms (Chou *et al.* 1996; Costarella *et al.* 1996). In vitro studies with isolated rings of coronary arteries and/or aortas from rats, rabbits, and pigs also found that, in both sexes, testosterone improved both endothelium-dependent and/or endothelium-independent vascular responsiveness (Chou *et al.* 1996; Costarella *et al.* 1996; Yue *et al.* 1995). However, it must be emphasized that all these studies employed supraphysiological doses of testosterone in the micromolar range. Teoh *et al.* (2000) observed a direct vasodilatory effect of testosterone on porcine coronary artery rings at micromolar concentrations but no direct effect at nanomolar

dosages. In contrast, physiological doses of testosterone inhibited the vasodilatory effects of bradykinin and calcium ionophores. Similarly, testosterone inhibited the adenosine-mediated vasodilation of rat coronary arteries and impaired endothelium-dependent relaxation of aortic rings from rabbits which were either made hypercholesterolemic or exposed to tobacco smoke (Ceballos *et al.* 1999; Farhat *et al.* 1995; Hutchison *et al.* 1997).

The cellular and molecular mechanisms by which testosterone (and estradiol) regulate the vascular tone are little understood. Evidence for and against endothelium-dependent or endothelium-independent mechanisms have been found. Results of some studies suggest the involvement of endothelial nitric oxide (Chou *et al.* 1996; Costarella *et al.* 1996; Geary *et al.* 2000). In dog coronary arteries, rat aorta, and rat cerebral arteries the nitric oxide synthase inhibitor L-NMMA prevented testosterone-induced vasodilation. However, in another in vitro study L-NMMA had no effect on testosterone-induced vasodilation of rabbit aortas and coronary arteries (Yue *et al.* 1995). In agreement with the latter, in vitro expression of nitric oxide synthase in human aortic endothelial cells was stimulated by estradiol but not by testosterone (Hishikawa *et al.* 1995). The involvement of prostaglandins is suggested by the observation that testosterone increases the response of coronary arteries to prostaglandin F2α (Farhat *et al.* 1995) and by the finding that dihydrotestosterone increases the density of thromboxane receptors in rats and guinea pigs (Masuda *et al.* 1995). However, in some in vivo and in vitro animal studies, pretreatment with the prostaglandin synthesis inhibitor indomethacin had no effect on testosterone-induced vasodilation, so that the role of eicosanoids mediating the actions of testosterone on the arterial wall is still controversial.

Several observations also suggest that testosterone, modulates vascular tone via its secondary metabolites e.g. estradiol. Neither the aromatase inhibitor aminogluthemide nor the estrogen-receptor antagonist ICI 182,780 prevented the testosterone-induced vasodilation (Chou *et al.* 1996), so that the vasoactive mechanisms of testosterone do not appear to involve the estrogen receptors.

10.6.3 Effects of testosterone on endothelial cells

Endothelial cells play an important role in atherosclerosis not only by regulating vascular tone but also by forming a barrier which regulates the uptake of cells and macromolecules into the vessel wall and hemostasis (Libby 2002; Ross 1999). In the previous section, we discussed several arguments suggesting that testosterone affects endothelium-dependent vasodilatation via genomic effects mediated by the androgen receptor. Testosterone also suppresses the expression of the vascular cell adhesion molecule VCAM-1, which plays a pivotal role in the adhesion and hence immigration of leukocytes into the arterial wall. However, it is controversial whether this effect is mediated via testosterone and the androgen receptor (McCrohon *et al.*

1999; Zhang *et al.* 2002) or via the estrogen receptor after conversion of testosterone into estradiol (Hatakeyama *et al.* 2002; Mukherjee *et al.* 2002). Differences in the gender of cell donors or the stimulation procedures have been discussed as possible sources of the discrepant findings (Liu *et al.* 2003). Testosterone was also shown to enhance apoptosis of endothelial cells which were cultivated in the absence of serum, probably by a mechanism which involves the androgen receptor rather the estrogen receptor (Ling *et al.* 2002).

10.6.4 Effects of testosterone on arterial smooth muscle cell function

In addition to regulating vascular tone, arterial smooth muscle cells also play an important role in atherosclerosis by proliferation, migration and matrix production (Dzau *et al.* 2002; Libby 2002; Ross 1999). Whereas estradiol can inhibit proliferation and migration of smooth muscle cells, testosterone had no effect (Akishita *et al.* 1997; Kolodgie *et al.* 1996). Moreover, the protection of female rabbits by estradiol but not the protection of male rabbits by testosterone from atherosclerosis was associated with decreased incorporation of 5′-bromo-2′-deoxyuridine into DNA of neointimal cells, an in vivo marker of arterial smooth muscle cell proliferation (Bruck *et al.* 1997). The effect of testosterone on smooth muscle cell migration and matrix production by smooth muscle cells has not been investigated. Thus there is little indication of a role of testosterone in vascular smooth muscle cell function except the effects on vasomotor functions summarized above.

10.6.5 Effects of testosterone on macrophage functions

Monocytes which have immigrated into the vascular wall differentiate to macrophages and bind lipoproteins which have permeated the endothelium and become modified within the arterial wall, for example by oxidation. Unregulated uptake of oxidatively modified lipoproteins via type A scavenger receptors leads to the intracellular accumulation of cholesteryl esters in macrophages and thereby to foam cell formation. Foam cells together with T-lymphocytes, release inflammatory mediators which stimulate the proliferation and migration of smooth muscle cells (Glass and Witztum 2001; Li and Glass 2002; Ross 1999). Estradiol inhibits oxidation of LDL both in the presence and absence of cells including macrophages. By contrast, testosterone increases the oxidation of LDL by placental macrophages in vitro (Zhu *et al.* 1997). Moreover, dihydrotestosterone dose-dependently stimulates the uptake of acetylated LDL by scavenger receptor type A and, hence, the intracellular cholesteryl ester accumulation in macrophages. The stimulatory effect of dihydrotestosterone was only seen in macrophages of male but not female donors and was blocked by the androgen receptor antagonist hydroxyflutamide (McCrohon *et al.* 2000).

After internalization, oxidized LDL is transported via endosomes to lysosomes for degradation. Cholesteryl esters are hydrolysed by lysosomal acid lipase. The liberated cholesterol leaves the lysosome membrane to be re-esterified by acylCoA-cholesterol:acyltransferase. The formed cholesteryl esters can be stored in the cytosol giving the foamy appearance of lipid-laden macrophages. The transport of cholesterol from lysosomes to the site of re-esterification is inhibited in vitro by various steroids with an oxo-group at the C17 or C20 position such as progesterone, pregnenolone, and androstendione. 17-hydroxy-steroids including testosterone were less effective (Lange *et al.* 1996). Cytosolic cholesteryl esters can be hydrolysed by neutral cholesterol esterase which is activated by cylclic AMP. In adipose tissue of female rats, neutral cholesterol esterase is more active than in adipose tissue of male rats. Moreover, exogenous estradiol increases neutral cholesterol esterase activity in male rats and in female rats which have been ovariectomized. in vitro, estradiol but not testosterone increased the activity of neutral cholesterol esterase in the murine macrophage cell line J774, probably by increasing the activity of a cyclic AMP dependent protein kinase A (Tomita *et al.* 1996).

Non-hepatic and non-steroidogenic cells cannot metabolize cholesterol and, therefore, can only dispose off excess cholesterol by secretion. Cholesterol efflux from cells is hence central to the regulation of the cellular cholesterol homeostasis. Non-specific and passive (i.e. aqueous diffusion) as well as specific and active processes (i.e. receptor-mediated) are involved. To date, two plasma membrane proteins are known to facilitate cholesterol efflux. Interaction of the scavenger receptor B1 with mature lipid-containing HDL is thought to facilitate cholesterol efflux by re-organizing the distribution of cholesterol within bilayer plasma membrane. The ATP binding cassette transporter A1 mediates phospholipid and cholesterol efflux to extracellular lipid-free apolipoproteins by translocating these lipids from intracellular compartments to the plasma membrane and/or by forming a pore within the plasma membrane, through which the lipids are secreted (Oram 2002; von Eckardstein *et al.* 2001). Testosterone up-regulates the expression of the scavenger receptor B1 in human monocyte-derived macrophages thereby stimulating HDL-induced cholesterol efflux. No effect of testosterone was seen on the expression of the ATP binding cassette transporter A1 (Langer *et al.* 2002).

Activated macrophages produce various cytokines including chemotactic protein 1, interleukins (IL) 1 and 10, and tumour necrosis factor α (TNFα), as well as growth factors such as platelet-derived growth factor 1. These bioactive molecules induce or inhibit various processes which contribute to atherosclerosis, e.g. recruitment of macrophages into the vascular wall and smooth muscle cell proliferation and migration (Glass and Witztum 2001; Li and Glass 2002; Ross 1999). Effects of testosterone on the production of cytokines and growth factors have not been studied in macrophage foam cell models but only in unstimulated or

lipopolysacharide-stimulated macrophages. Whether these results are also valid for macrophages in the arterial wall is not known. For example, estradiol but not testosterone inhibited the migration of monocytes in response to chemotactic protein 1. In J774 macrophages, testosterone exerted potentially anti-inflammatory effects by stimulating IL-10 synthesis and inhibiting the production of TNFα and nitric oxide (D'Agostini 1999; Friedl *et al.* 2000).

10.6.6 Effects of testosterone on platelet function

Aggregation of platelets is a prerequisite for thrombus formation and hence a critical step in acute coronary events (Ruggeri 2002). Administration of testosterone cypionate to eugonadal men led to enhanced *ex vivo* platelet aggregation in response to the thromboxane analogue I-BOP but not in response to thrombin (Ajayi *et al.* 1995). Testosterone increases the expression of the androgen receptor in a megakaryocyte cell line, as well as in platelets (Matsuda *et al.* 1993; 1994). Flutamide inhibited the stimulatory effect of testosterone on thromboxane receptor expression (Matsuda *et al.* 1993; 1994) suggesting that the effect is mediated via the androgen receptor.

10.6.7 Effects of testosterone on cardiomyocytes

In agreement with the association of testosterone and male sex with higher left ventricular mass as well as with the stimulating effect of exogenous testosterone on heart weight and contractility, testosterone was found to induce proliferation of isolated cardiomyocytes. This is in contrast to the antiproliferative effect of estrogens, which therefore suggests a direct effect of testosterone. This was also shown directly (Marsh *et al.* 1998). By contrast, the similar effect of testosterone and estradiol on the expression of the V1 myosin heavy chain isoenzyme may point to the importance of estradiol and the estrogen receptor for mediating this effect, although this was not shown directly (Lengsfeld *et al.* 1988; Morano *et al.* 1990).

10.7 Lessons from genetic studies on the role of testosterone in atherosclerosis

In the absence of controlled intervention studies and in view of the conflicting data presented above, it is difficult to predict the net effects of testosterone on cardiovascular disease. Further difficulties arise from the fact that associations found in observational studies do not prove causal relationships and that in theory several effects of testosterone on intermediate phenotypes can be exerted via either non-genomic or genomic mechanisms, the latter being mediated either directly via testosterone and dihydrotestosterone or indirectly via estradiol. Genetic studies on the associations or effects of genetic variation in the androgen receptor and estrogen

receptors may answer these questions. In addition, variations in these genes as well in genes or enzymes (e.g. aromatase and 5α-reductase) and transport proteins (e.g. sex hormone binding globulin) regulating the bioavailability of these hormones may modulate the effects of testosterone.

10.7.1 Variation in the androgen receptor

A variable number of CAG repeats in exon 1 of the androgen receptor gene on the X-chromosome, which normally ranges between 9 and 35 encodes for a variable number of glutamine residues in the aminoterminal domain of the receptor and is inversely associated with the transcriptional activity of testosterone-responsive target genes. Abnormal expansion of the CAG repeats beyond the number of 36 leads to Kennedy disease, which is accompanied by signs of hypoandrogenism (see Chapter 2 for details). Within the physiological range of 9 to 35, the number of CAG repeats was shown to be inversely associated with the risk of prostate cancer, benign prostatic hyperplasia, sperm production, and bone density, and depression (Dowsing *et al.* 1999; Ferro *et al.* 2002; Seidmann *et al.* 2001; von Eckardstein *et al.* 2001; Zitzmann *et al.* 2001a). With respect to cardiovascular disease it is important to emphasize that the number of CAG repeats is positively correlated with flow-mediated vasoreactivity and HDL-cholesterol levels, body fat mass and serum levels of insulin and leptin (Zitzmann *et al.* 2001b; 2003). These findings underscore the fact that the physiological function of testosterone is to reduce endothelial-dependent vasodilatation, to lower HDL cholesterol and body fat mass and to increase the sensitivity towards insulin and leptin. Because of the multitude of these opposing pro- and anti-atherogenic actions, it is not surprising that we did not find any significant association of the CAG repeat polymorphism with coronary artery disease (Hersberger *et al.*, unpublished observation). Data of similar genetic association studies on possible associations of the CAG polymorphism with other cardiovascular phenotypes and events such as ventricular mass and ejection fraction, stroke and claudicatio will be useful to estimate the clinical importance of testosterone for these entities.

10.7.2 Variation in the estrogen receptor

The importance of locally produced estrogens from aromatisation of testosterone in males for cardiovascular health is highlighted by recent human and transgenic mouse models of aromatase deficiency and estrogen resistance. In two men with undetectable circulating estradiol and estrone and high testosterone due to P450 aromatase deficiency (Carani *et al.* 1997; Morishima *et al.* 1995), dyslipidaemia with elevated total and LDL-C and triglyceride and decreased HDL-C was associated with insulin resistance (in one patient). These metabolic abnormalities were correctable by low dose oral or transdermal estrogen replacement. Insulin resistance, impaired

glucose tolerance, and low HDL-C were also apparent in a 28-year old male with a null mutation in ERα gene causing estrogen resistance (Smith *et al.* 1994). Intact hepatic ERβ may have prevented full expression of dyslipdaemia. Ultrafast electron beam computer tomography imaging showed calcium deposition in the proximal left anterior descending coronary artery, indicating the presence of premature atherosclerosis. Flow-mediated brachial artery endothelial dependent was absent, showing marked endothelial dysfunction (Sudhir *et al.* 1997).

These rare experiments of nature suggest that estrogens are important in maintaining normal carbohydrate and lipid metabolism as well as normal endothelial-dependent nitric oxide mediated vasodilatation in men. They are compatible with data from transgenic knockout models confirming that estrogen receptor α is important in preventing adipocyte hypertrophy, obesity, insulin resistance and hypercholesterolaemia (Heine *et al.* 2000) and maintaining basal nitric oxide release from vascular endothelium in male animals. Estrogen receptor β in vascular smooth muscle may also regulate vascular sensitivity to estradiol (Hodges *et al.* 2000; Nilsson *et al.* 2000; Rubanyi *et al.* 1997). The favourable effects of estrogens on HDL-C demonstrated are also in accord with clinical studies using aromatase inhibitors in normal men.

Several polymorphisms of intronic sequences of the estrogen receptor α gene which are in linkage dysequilibrium with each other were previously found to modulate the response of HDL-cholesterol levels to estrogen replacement therapy in menopause (Herrington *et al.* 2002). We did not find any significant association of ER polymorphisms with cardiovascular risk factors or the presence of coronary artery disease in men (Hersberger and von Eckardstein, unpublished data). In an autopsy study, a *pvuI* polymorphism in the estrogen receptor α was found associated with the extent of complicated coronary artery atherosclerotic lesions in men older than 53 years (Lehtimaki *et al.* 2002).

10.7.3 Variation in other genes

Polymorphisms in the genes for 5α-reductase, aromatase and sex hormone binding globulin were previously associated with various phenotypes including risk of prostate cancer or osteoporosis (Forsti *et al.* 2002; Hogeveen *et al.* 2002; Igaz *et al.* 2002; Novelli *et al.* 2001; van Pottelbergh *et al.* 2003). To our knowledge the importance of these polymorphisms for cardiovascular diseases and risk factors has not yet been investigated.

10.8 Clinical implications

Current evidence indicates that the gender difference in the incidence of cardiovascular diseases cannot be explained on the basis of ambient testosterone exposure. It

has therefore been speculated that exposure to testosterone in pre- or perinatal life is responsible or contributes to the male gender disadvantage in cardiovascular disease (Liu *et al.* 2003). In adults, androgens can exert both beneficial and deleterious actions on a multitude of factors implicated in the pathogenesis of atherosclerosis and heart failure so that, at present, it is not possible to determine the net effect of testosterone on coronary artery disease, stroke, peripheral artery disease and heart failure.

What are the clinical implications of this ongoing uncertainty? In our view the answer to this question must differentiate between the concern for the possibility of cardiovascular side effects in androgen treatment of endocrine and non-endocrine conditions on the one hand, and whether testosterone may be used for the prevention or even treatment of coronary artery disease on the other.

Efforts to exploit the therapeutic benefits of testosterone in the treatment of hypogonadism, osteoporosis, wasting, and chronic consumptive disease or for contraception in a wider male population should not be deterred or hampered by concerns regarding increased cardiovascular risks. However, the possibility that spontaneous or induced hyperandrogenaemia may increase the risks for coronary artery disease in women needs to be seriously considered.

Some clinicians argue that androgen replacement in the elderly male, in addition to possible benefits on muscle, bone, sexual and mental functions, has the potential to prevent atherosclerotic vessel diseases. However, androgens have such an extraordinary array of effects in vivo that it is hazardous to extrapolate isolated experimental findings to the wider clinical setting. It is premature to assume clinical benefits from manipulation of the sex steroid milieu based on biologically plausible mechanisms, or indeed on cross-sectional risk factor observational data in a complex multifactorial condition such as coronary artery disease. Interpretations of effects of pharmacological doses of androgens on arterial compliance and flow-mediated dilatation in particular must also be treated with circumspection. The lessons from estrogen hormone replacement in postmenopausal women are especially salutary. Despite the overwhelmingly positive but indirect evidence on risk factors and disease incidence, controlled interventional studies recently have not confirmed estrogens to be an effective secondary preventative treatment for established coronary artery disease in women (Manson *et al.* 2003; Roussow *et al.* 2002). There is an analogous need for randomised controlled trials which assess clinical endpoints for male hormone replacement therapy. In the absence of such evidence on testosterone, priority must be given to treatment modalities of proven efficacy in the prevention or treatment of coronary artery disease (e.g. weight reduction, smoking cessation, exercise, aspirin, statins, anti-hypertensives, and vasodilators).

10.9 Key messages

Significant and independent associations between *endogenous* testosterone levels and cardiovascular events in men and women have *not* been confirmed in large prospective studies, even though cross-sectional data suggested cardiovascular diseases can be associated with low testosterone in men. However, hypoandrogenemia in men and hyperandrogenemia in women are associated with visceral obesity, insulin resistance, low HDL cholesterol, elevated triglycerides, LDL cholesterol and PAI-1. These gender differences and confounders render the precise role of *endogenous* testosterone in atherosclerosis unclear.

The effects of *exogenous* testosterone on cardiovascular mortality or morbidity have not been extensively investigated in prospective controlled studies; preliminary data suggest that supraphysiological dosages of testosterone result in short-term improvements in electrocardiographic changes in men with coronary artery disease.

In the majority of animal experiments, *exogenous* testosterone exerts either neutral or beneficial effects on the development of atherosclerosis in male animals, possibly by conversion into estradiol, but adverse effects in females.

Exogenous androgens induce both apparently beneficial and deleterious effects on cardiovascular risk factors by decreasing serum levels of HDL-C, PAI-1 (apparently deleterious) Lp(a), fibrinogen, insulin, leptin and visceral fat mass (apparently beneficial) in men as well as women. However, androgen-induced declines in circulating HDL-C should not automatically be assumed to be pro-atherogenic, since they may reflect accelerated reverse cholesterol transport instead.

Supraphysiological concentrations of testosterone stimulate vasorelaxation; but at physiological concentrations, beneficial, neutral, and detrimental effects on vascular reactivity have been observed. Testosterone exerts 'pro-atherogenic' effects on macrophage function by facilitating the uptake of modified lipoproteins and an 'anti-atherogenic' effect by stimulating efflux of cellular cholesterol to HDL.

In conclusion, the inconsistent data, which can only be partly explained by differences in dose and source of androgens, do not allow any meaningful assessment of the net effect of testosterone on atherosclerosis. Based on current evidence, the therapeutic use of testosterone in men need not be restricted by concerns regarding cardiovascular side effects.

10.10 REFERENCES

Adams, MR, Williams JK, Kaplan JR (1995) Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. Arterioscler Thromb Vasc Biol 15:562–570

Ajayi AA, Mathur R, Halushka PV (1995) Testosterone increases human platelet thromboxane A2 receptor density and aggregation responses. Circulation 91:2742–2747

Akishita M, Ouchi Y, Miyoshi H, Kozaki K, Inoue S, Ishikawa M, Eto M, Toba K, Orimo H (1997) Estrogen inhibits cuff-induced intimal thickening of rat femoral artery:

effects on migration and proliferation of vascular smooth muscle cells. Atherosclerosis 130:1–10

Alexandersen P, Haarbo J, Christiansen C (1996) The relationship of natural androgens to coronary heart disease in males: a review. Atherosclerosis 125:1–13

Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C (1999) Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. Circ Res 84: 813–819

Amowitz LL, Sobel BE (1999) Cardiovascular consequences of polycystic ovary syndrome. Endocrinol Metab Clin North Am 28:439–458

Anderson RA, Ludlam CA, Wu FCW (1995) Haemostatic effects of supraphysiological levels of testosterone in normal men. Thromb Haemost 74:693–697

Angelin B (1997) Therapy for lowering lipoprotein(a) levels. Curr Opin Lipidol 8:337–341

Aversa A, Isidori AM, Spera G, Lenzi A, Fabbri A (2003) Androgens improve cavernous vasodilation and response to sildenafil in patients with erectile dysfunction. Clin Endocrinol (Oxf) 58:632–638

Aversa A, Isidori AM, De Martino MU, Caprio M, Fabbrini E, Rocchietti-March M, Frajese G, Fabbri A (2000) Androgens and penile erection: evidence for a direct relationship between free testosterone and cavernous vasodilation in men with erectile dysfunction. Clin Endocrinol (Oxf) 53:517–522

Bagatell CJ, Bremner WJ (1995) Androgen and progestagen effects on plasma lipids. Prog Cardiovasc Dis 38:255–271

Barrett-Connor E, Goodman-Gruen D (1995) Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. BMJ 311:1193–1196

Behre HM, Simoni M, Nieschlag E (1997) Strong association between serum levels of leptin and testosterone in men. Clin Endocrinol 47:237–240

Benten WP, Lieberherr M, Stamm O, Wrehlke C, Guo Z, Wunderlich F (1999) Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Mol Biol Cell 10:3113–3123

Birdsall M, Farquhar C, White H (1997) Association between polycystic ovaries and extent of coronary artery disease in women having cardiac catheterization. Ann Intern Med 126: 32–35

Björntorp P (1996) The regulation of adipose tissue distribution in human. Int J Obesity 20:291–302

Bonetti PO, Lerman LO, Lerman A (2003) Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 23:168–175

Bruck B, Brehme U, Gugel N, Hanke S, Finking G, Lutz C, Benda N, Schmahl FW, Haasis R, Hanke H (1997) Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 17: 2192–2199

Büchter D, Behre HM, Kliesch S, Chirazi A, Nieschlag E, Assmann G, von Eckardstein A (1999) Effects of testosterone suppression in young men by the gonadotropin releasing hormone antagonist cetrorelix on plasma lipids, lipolytic enzymes, lipid transfer proteins, insulin, and leptin. Exp Clin Endocrinol Diabet 107:522–529

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER (1997) Effect of testosterone and oestradiol in a man with aromatase deficiency. New Eng J Med 337:91–95

Cavasin MA, Sankey SS, Yu AL, Menon S, Yang XP (2003) Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction. Am J Physiol Heart Circ Physiol 284:H1560–569

Ceballos G, Figueroa L, Rubio I, Gallo G, Garcia A, Martinez A, Yanez R, Perez J, Morato T, Chamorro G (1999) Acute and nongenomic effects of testosterone on isolated and perfused rat heart. J Cardiovasc Pharmacol 33:691–697

Chou TM, Sudhir K, Hutchison SJ, Ko E, Amidon TM, Collins P, Chatterjee K (1996) Testosterone induces dilatation of canine coronary conductance and resistance arteries in vivo. Circulation 94:2614–2619

Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF 2nd, Fitzpatrick LA (2003) Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:2562–2568

Costarella CE, Stallone JN, Rutecki GW, Whittier FC (1996) Testosterone causes direct relaxation of rat thoracic aorta. J Pharmacol Experiment Therap 27:34–39

Couillard C, Gagnon J, Bergnon J *et al.* (2000) Contribution of body fatness and adipose tissue distribution to the age variation in plasma steroids hormone concentration in men: the HERITAGE family study. J Clin Endocrinol Metab 85:1026–1031

De Pergola G, Pannacciulli N, Ciccone M, Tartagni M, Rizzon P, Giorgino R (2003) Free testosterone plasma levels are negatively associated with the intima-media thickness of the common carotid artery in overweight and obese glucose-tolerant young adult men. Int J Obes Relat Metab Disord 7:803–807

D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, Farruggio R, Miceli DM, Miele M, Castagnetta L, Cillari E (1999) Sex hormones modulate inflammatory mediators produced by macrophages. Ann N Y Acad Sci 876:426–429

Dahlgren E, Landin K, Krotkiewski M, Holm G, Janson PO (1998) Effects of two antiandrogen treatments on hirsutism and insulin sensitivity in women with polycystic ovary syndrome. Hum Reprod 13:2706–2711

Danesh J, Collins R, Peto R (2000) Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 102:1082–1085

Davis SR, Tran J (2001) Testosterone influences libido and well being in women. Trends in Endocrinology & Metabolism 12:33–37

Diamanti-Kandarakis E, Mitrakou A, Raptis S, Tolis G, Duleba AJ (1998) The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome. J Clin Endocrinol Metab 83:2699–2705

Diano S, Horvath TL, Mor G, Register T, Adams M, Harada N, Naftolin F (1999) Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects. Menopause Spring 6:21–28

Dowsing AT, Yong EL, Clark M, McLachlan RI, de Kretser DM, Trounson AO (1999) Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet 354:640–643

Dunaif A (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 18:774–800

Dunaif A, Thomas A (2001) Current concepts in the polycystic ovarian syndrome. Ann Rev Med 52:401–419

Dzau VJ, Braun-Dullaeus RC, Sedding DG (2002) Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. Nat Med 11:249–256

Eisner JR, Dumesic DA, Kemnitz JW, Abbott DH (2000) Timing of prenatal androgen excess determines differential impairment in insulin secretion and action in adult female rhesus monkeys. J Clin Endocrinol Metab 85:1206–1210

Elhage R, Arnal JF, Pieraggi M-T, Duverger N, Fiévet C, Faye JC, Bayard, F (1997) 17β-estradiol prevents fatty streak formation in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 17:2679–2684

Elwan O, Abdallah M, Issa I, Taher Y, el-Tamawy M (1990) Hormonal changes in cerebral infarction in the young and elderly. J Neurol Sci 98:235–243

Eri LM, Urdal P, Bechensteen AG (1995) Effects of the luteinizing hormone-releasing hormone agonist leuprolide on lipoproteins, fibrinogen and plasminogen activator inhibitor in patients with benign prostatic hyperplasia. J Urol 154:100–104

Farhat MY, Wolfe R, Vargas R, Foegh ML, Ramwell (1995) Effect of testosterone treatment on vasoconstrictor response of left anterior descend coronary artery in male and female pigs. J Cardiovasc Pharmacol 25:495–500

Ferro P, Catalano MG, Dell'Eva R, Fortunati N, Pfeffer U (2002) The androgen receptor CAG repeat: a modifier of carcinogenesis? Mol Cell Endocrinol 193:109–120

Forsti A, Jin Q, Grzybowska E, Soderberg M, Zientek H, Sieminska M, Rogozinska-Szczepka J, Chmielik E, Utracka-Hutka B, Hemminki K (2002) Sex hormone-binding globulin polymorphisms in familial and sporadic breast cancer. Carcinogenesis 8:1315–1320

Fogelberg M, Björkhem I, Diczfalusy U, Henriksson P (1990) Stanozolol and experimental atherosclerosis: atherosclerosis development and blood lipids during anabolic steroid therapy of New Zealand white rabbits. Scand J Clin Lab Invest 50:693–700

Friedl R, Brunner M, Moeslinger T, Spieckermann PG (2002) Testosterone inhibits expression of inducible nitric oxide synthase in murine macrophages. Life Sci 68:417–429

Fukui M, Kitagawa Y, Nakamura N, Kadono M, Mogami S, Hirata C, Ichio N, Wada K, Hasegawa G, Yoshikawa T (2003) Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. Diabetes Care 6:1869–1873

Gardner JD, Brower GL, Janicki JS (2002) Gender differences in cardiac remodeling secondary to chronic volume overload. J Card Fail 8:101–107

Geary GG, Krause DN, Duckles SP (2000) Gonadal hormones affect diameter of male rat cerebral arteries through endothelium-dependent mechanisms. Am J Physiol Heart Circ Physiol 279:H610–618

Glass CK, Witztum JL (2001) Atherosclerosis. The road ahead. Cell 104:503–16.

Guay AT, Perez JB, Jacobson J, Newton RA (2001) Efficacy and safety of sildenafil citrate for treatment of erectile dysfunction in a population with associated organic risk factors. J Androl 22:793–797

Guo Z, Benten WP, Krucken J, Wunderlich F (2002) Nongenomic testosterone calcium signaling. Genotropic actions in androgen receptor-free macrophages. J Biol Chem 277:29600–29607

Guzick DS, Talbott EO, Sutton-Tyrrell K, Herzog HC, Kuller LH, Wolfson SK Jr (1996) Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. Am J Obstet Gynecol 174:1224–1229

Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA (2002) Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. J Clin Endocrinol Metab 287:3632–3639

Hamilton JB, Mestler GE (1969) Mortality and survival: comparison of eunuchs with intact men and women in a mentally retarded population. J Gerontol 24:395–411

Hanke H, Lenz C, Hess B, Spindler KD, Weidemann W (2001) Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. Circulation 103:1382–1385

Harada S, Murakami H, Ohkuma T, Nagura H, Takagi Y (1999) Localised expression of aromatse in human vascular tissue. Circ Res 84:1285–1291

Hatakeyama H, Nishizawa M, Nakagawa A, Nakano S, Kigoshi T, Uchida K (2002) Testosterone inhibits tumor necrosis factor-alpha-induced vascular cell adhesion molecule-1 expression in human aortic endothelial cells. FEBS Lett 530:129–132

Hautanen A (2000) Synthesis and regulation of sex hormone-binding globulin in obesity. Int J Obes Relat Metab Disord 24 (Suppl 2):S64–70

Hayward CS, Webb CM, Collins P(2001) Effect of sex hormones on cardiac mass. Lancet 357:1354–1356

Hayward CS, Kelly RP, Collins P (2000) The roles of gender, the menopause and hormone replacement on cardiovascular function. Cardiovasc Res 46:28–49

Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS (2000) Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci USA 97:12729–12734

Hergenc G, Schulte H, Assmann G, von Eckardstein A (1999) Associations of obesity markers, insulin, and sex hormones with HDL-cholesterol levels in Turkish and German individuals. Atherosclerosis 145:147–156

Herman SM, Robinson JTC, McCredie RJ, Adams MR, Boyer MJ, Celermajer DS (1997) Androgen deprivation is associated with enhanced endothelium-dependent dilatation in adult men. Arterioscler Thromb Vasc Biol 17:2004–2009

Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Brosnihan KB, Meyers DA, Bleecker ER (2002) Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. N Engl J Med 346:967–974

Hersberger M and von Eckardstein A (2003) Low High Density Lipoprotein Cholesterol: Physiological Background, Clinical Importance and Drug Treatment. Drugs 63:1907–1945

Higashiura K, Mathur RS, Halushka PV (1997) Gender-related differences in androgen regulation of thromboxane A2 receptors in rat aortic smooth-muscle cells. J Cardiovasc Pharmacol 29:311–315

Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Saruta T (1995) Up regulation of nitric oxide synthase by estradiol in human aortic endothelial cells. FEBS Lett 360:291–295

Ho KJ, Liao JK (2003) Nonnuclear actions of estrogen. Arterioscler Thromb Vasc Biol 22:1952–1961

Hodges YK, Tung L, Yan XD, Graham JD, Horwitz KB, Horwitz LD (2000) Estrogen receptors alpha and beta: prevalence of estrogen receptor beta mRNA in human vascular smooth muscle and transcriptional effects. Circulation 101:1792–1798

Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL (2002) Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. J Clin Invest 109:973–981

Hutchison SJ, Sudhir K, Chou TM, Sievers RE, Zu BQ, Sun YP, Deedwania PC, Glntz SA, Parmely WW, Chatterjee K (1997) Testosterone worsens endothelial dysfunction associated with hypercholesterolemia and environmental tobacco smoke exposure in male rabbit aorta. J Am Coll Cardiol 29:800–807

Igaz P, Pap E, Patocs A, Falus A, Tulassay Z, Racz K (2002) Genomics of steroid hormones: in silico analysis of nucleotide sequence variants (polymorphisms) of the enzymes involved in the biosynthesis and metabolism of steroid hormones. J Steroid Biochem Mol Biol 82:359–367

Jannini EA, Screponi E, Carosa E, Pepe M, Lo Giudice F, Trimarchi F, Benvenga S (1999) Lack of sexual activity from erectile dysfunction is associated with a reversible reduction in serum testosterone. Int J Androl 22:385–392

Jeppesen LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K (1996) Decreased serum testosterone in men with acute ischemic stroke. Arterioscler Thromb Vasc Biol 16:749–754

Kolodgie FD, Jacob A, Wilson PS, Carlson GC, Farb A, Verma A, Virmani R (1996) Estradiol attenuates directed migration of vascular smooth muscle cells in vitro. Am J Pathol 148:969–976

Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L (2000) Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril 73:1149–1154

Kontoleon PE, Anastasiou-Nana MI, Papapetrou PD, Alexopoulos G, Ktenas V, Rapti AC, Tsagalou EP, Nanas JN (2003) Hormonal profile in patients with congestive heart failure. Int J Cardiol 87:179–183

Lange Y, Duan H, Mazzone T (1996) Cholesterol homeostasis is modulated by amphiphiles at transcriptional and post-transcriptional loci. J Lipid Res 37:534–539

Langer C, Gansz B, Goepfert C, Engel T, Uehara Y, von Dehn G, Jansen H, Assmann G, von Eckardstein A (2002) Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages. Biochem Biophys Res Commun 296:1051–1057

Larsen BA, Nordestgaard BG, Stender S, Kjeldsen K (1993) Effect of testosterone on atherogenesis in cholesterol-fed rabbits with similar plasma cholesterol levels. Atherosclerosis 99:79–86

Leenen R, van der Kooy K, Seidell JC, Deurenberg P, Koppeschaar HP (1994) Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy J Clin Endocrinol Metab 78:1515–1520

Lehtimaki T, Kunnas TA, Mattila KM, Perola M, Penttila A, Koivula T, Karhunen PJ (2002) Coronary artery wall atherosclerosis in relation to the estrogen receptor 1 gene polymorphism: an autopsy study. J Mol Med 80:176–180

Lemieux S, Lewis GF, Ben-Chetrit A, Steiner G, Greenblatt EM (1999) Correction of hyperandrogenemia by laparoscopic ovarian cautery in women with polycystic ovarian syndrome is

not accompanied by improved insulin sensitivity or lipid-lipoprotein levels. J Clin Endocrinol Metab 84:4278–4282

Lengsfeld M, Morano I, Ganten U, Ganten D, Ruegg JC (1988) Gonadectomy and hormonal replacement changes systolic blood pressure and ventricular myosin isoenzyme pattern of spontaneously hypertensive rats. Circ Res 63:1090–1094

Li AC, Glass CK (2002) The macrophage foam cell as a target for therapeutic intervention. Nat Med 8:1235–1242

Libby P (2002) Inflammation in atherosclerosis. Nature 420:868–874

Lieberherr M, Grosse B (1994) Androgens increase intracellular calcium concentration and inositol 1,4,5-trisphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. J Biol Chem 269:7217–7223

Lindstedt G, Lundberg P, Lapidus L, Lundgren H, Bengtsson C, Björntorp P (1991) Low sex hormone binding globulin concentration as an independent risk factor for development of NIDDM:12 yr follow up of population study of women in Gothenburg. Diabetes 40:123–128

Ling S, Dai A, Williams MR, Myles K, Dilley RJ, Komesaroff PA, Sudhir K (2002) Testosterone enhances apoptosis-related damage in human vascular endothelial cells. Endocrinology 143:1119–1125

Liu PY, Death AK, Handelsman DJ (2003) Androgens and cardiovascular disease. Endocr Rev 24:313–340

Lobo RA, Carmina E (2000) The importance of diagnosing the polycystic ovary syndrome. Ann Intern Med 132:989–993

Lue TF (2000) Erectile dysfunction. N Engl J Med 342:1802–1813

Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Wong ND, Crouse JR, Stein E, Cushman M (2003) Women's Health Initiative Investigators. Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med 349:523–534

Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ (1998) Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 98:256–261

Masuda A, Mathur A, Halushka PV (1995) Testosterone increases thromboxane A2 receptors in cultured rat smooth muscle cells. Circ Res 69:638–643

Matsuda K, Mathur RS, Duzic E, Halushka PV (1993) Androgen regulation of thromboxane A2/prostaglandin H2 receptor expression in human erythroleukemia cells. Am J Physiol 265:E928–934

Matsuda K, Ruff A, Morinelli TA, Mathur RS, Halushka PV (1994) Testosterone increases thromboxane A2 receptor density and responsiveness in rat aortas and platelets. Am J Physiol 267:H887–893

McCredie RJ, McCrohon JA, Turner L, Griffiths KA, Handelsman DJ, Celermajer DS (1998) Vascular reactivity is impaired in genetic females taking high-dose androgens. J Am Coll Cardiol 32:1331–1335

McCrohon JA, Death AK, Nakhla S, Jessup W, Handelsman DJ, Stanley KK, Celermajer DS (2000) Androgen receptor expression is greater in macrophages from male than from female donors. A sex difference with implications for atherogenesis. Circulation 101:224–226

Melman A, Gingell JC (1999) The epidemiology and pathophysiology of erectile dysfunction. J Urol 161:5–11

Morano I, Gerstner J, Ruegg JC, Ganten U, Ganten D, Vosberg HP (1990) Regulation of myosin heavy chain expression in the hearts of hypertensive rats by testosterone. Circ Res 66:1585–1590

Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689–3698

Mukherjee TK, Dinh H, Chaudhuri G, Nathan L (2002) Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. Proc Natl Acad Sci U S A 99:4055–4060

Nahrendorf M, Frantz S, Hu K, von zur Muhlen C, Tomaszewski M, Scheuermann H, Kaiser R, Jazbutyte V, Beer S, Bauer W, Neubauer S, Ertl G, Allolio B, Callies F (2003) Effect of testosterone on post-myocardial infarction remodeling and function. Cardiovasc Res 57:370–378

Nathan L, Shi W, Dinh H, Mukherjee TK, Wang X, Lusis AJ, Chaudhuri G (2001) Testosterone inhibits early atherogenesis by conversion to estradiol: Critical role of aromatase. Proc Natl Acad Sci U S A 98:3589–3593

Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F (1998) Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 83:2001–2005

Ng MK, Liu PY, Williams AJ, Nakhla S, Ly LP, Handelsman DJ, Celermajer DS (2002) Prospective study of effect of androgens on serum inflammatory markers in men. Arterioscler Thromb Vasc Biol 22:1136–1141

Nieschlag E, Nieschlag S, Behre HM (1993) Lifespan and testosterone. Nature 366:215

Nieschlag E, Kramer U, Nieschlag S (2003) Androgens shorten the longevity of women: sopranos last longer. Exp Clin Endocrinol Diabetes 111:230–231

Nilsson BO, Ekblad E, Heine T, Gustafsson J (2000) Increased magnitude of relaxation to oestrogen in aorta from oestrogen receptor beta knock-out mice. J Endocrinol 166: R5–9

Noirhomme P, Jacquet L, Underwood M, El Khoury G, Goenen M, Dion R (1999) The effect of chronic mechanical circulatory support on neuroendocrine activation in patients with end-stage heart failure. Eur J Cardiothorac Surg 16:63–67

Novelli G, Margiotti K, Sangiuolo F, Reichardt JK (2001) Pharmacogenetics of human androgens and prostatic diseases. Pharmacogenomics 2:65–72

Oram JF (2002) Molecular basis of cholesterol homeostasis: lessons from Tangier disease and ABCA1. Trends Mol Med 8:168–173

Pasquali R, Macor C, Vicennati V, Novo F, De lasio R, Mesini P, Boschi S, Casimirri F, Vettor R (1997) Effects of acute hyperinsulinemia on testosterone serum concentrations in adult obese and normal-weight men. Metabolism 46:526–529

Pasquali R, Filicori M (1998) Insulin sensitizing agents and polycystic ovary syndrome. Eur J Endocrinol 138:253–254

Penotti M, Sironi L, Cannata L, Vigano P, Casini A, Gabrielli L, Vignali M (2001) Effects of androgen supplementation of hormone replacement therapy on the vascular reactivity of cerebral arteries. Fertil Steril 76:235–240

Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. J Clin Invest 111:1805–1812

Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS (1998) Mortality of women with polycystic ovary syndrome at long-term follow-up. J Clin Epidemiol 51:581–586

Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Ridker PM (2002) Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA 288:980–987

Pugh PJ, Channer KS, Parry H, Downes T, Jone TH (2002) Bio-available testosterone levels fall acutely following myocardial infarction in men: association with fibrinolytic factors. Endocr Res 28:161–173

Pugh PJ, Jones TH, Channer KS (2003) Acute haemodynamic effects of testosterone in men with chronic heart failure. Eur Heart J 24:909–915

Rajkhowa M, Glass MR, Rutherford AJ, Michelmore K, Balen AH (2000) Polycystic ovary syndrome: a risk factor for cardiovascular disease? B J Obs Gynecol 107:11–8

Remmers DE, Cioffi WG, Bland KI, Wang P, Angele MK, Chaudry IH (1998) Testosterone: the crucial hormone responsible for depressing myocardial function in males after trauma-hemorrhage. Ann Surg 227:790–799

Remmers DE, Wang P, Cioffi WG, Bland KI, Chaudry IH (1997) Testosterone receptor blockade after trauma-hemorrhage improves cardiac and hepatic functions in males. Am J Physiol 273:H2919–2925

Rosano GM, Leonardo F, Pagnotta P, Pellicia F, Panina G, Cerquetani E, della Monica PL, Bonfigli B, Volpe M, Chierchia SL (1999) Acute anti-ischemic effect of testosterone in men with coronary artery disease. Circulation 99:1666–1670

Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340:115–126

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288:321–333

Rubanyi GM, Frey AD, Kauser K, Sukovich D, Burton G, Lubahn DB, Couse JF, Curtis SW, Korach KS (1997) Vascular estrogen receptors and endothelium-derived nitric oxide production in the mouse aorta. J Clin Invest 99:2429–2437

Rubio-Gayosso I, Garcia-Ramirez O, Gutierrez-Serdan R, Guevara-Balcazar G, Munoz-Garcia O, Morato-Cartajena T, Zamora-Garza M, Ceballos-Reyes G (2002) Testosterone inhibits bradykinin-induced intracellular calcium kinetics in rat aortic endothelial cells in culture. Steroids 67:393–397

Ruggeri ZM (2002) Platelets in atherothrombosis. Nat Med 8:1227–1234

Scheuer J, Malhotra A, Schaible TF, Capasso (1988) Effects of gonadectomy and hormonal replacement on rat hearts. Circ Res 61:12–9

Seidma SN, Araujo AB, Roose SP, McKinlay JB (2001) Testosterone level, androgen receptor polymorphism, and depressive symptoms in middle-aged men. Biol Psychiatry 50:371–376

Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L, Buchanan TA, Shen R, Bross R, Berman N, Bhasin S (2002) The effects of varying doses of T on insulin sensitivity plasma

lipids, apolipoproteins, and C-reactive protein in healthy young men. J Clin Endocrinol Metab 87:136–143

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056–1061

Sudhir K, Chou TM, Messina LM, Hutchison SJ, Korach KS, Chatterjee K, Rubanyi GM (1997) Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. Lancet 349:1146–1147

Sullivan ML, Martinez CM, Gennis P, Gallagher EJ (1998) The cardiac toxicity of anabolic steroids. Prog Cardiovas Dis 41:1–15

Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH (2000) Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thromb Vasc Biol 20:2414–2421

Tan KC, Shiu SW, Kung AW (1999) Alterations in hepatic lipase and lipoprotein subfractions with transdermal testosterone replacement therapy. Clin Endocrinol (Oxf) 51:765–769.

Teoh H, Quan A, Leung SW, Man RY (2000) Differential effects of 17beta-estradiol and testosterone on the contractile responses of porcine coronary arteries. Br J Pharmacol 129:1301–1308

Tchernof A, Labrie F, Belanger A, Despres JP (1996) Obesity and metabolic complications: contribution of dehydroepiandrosterone and other steroid hormones. J Endocrinol 150:S155–164

Thum T, Borlak J (2002) Testosterone, cytochrome P450, and cardiac hypertrophy. FASEB J 16:1537–1549

Toda T, Toda Y, Cho BH, Kummerow FA (1984) Ultrastructural changes in the comb and aorta of chicks fed excess testosterone. Atherosclerosis 51:47–53

Tomita T, Sawamura F, Uetsuka R, Chiba T, Miura S, Ikeda M, Tomita I (1996) Inhibiion of cholesteryl ester accumulation by 17β-estradiol in macrophges through activation of neutral cholesterol esterase. Biochim Biophys Acta 1300:210–218

Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY (2000) Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obes Relat Metab Disord 24:485–491

van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ (1997) Mortality and morbidity in transsexual subjects treated with cross-sex hormones. Clin Endocrinol 47:337–342

Van Pottelbergh I, Goemaere S, Kaufman JM (2003) Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J Clin Endocrinol Metab. 88:3075–3081

von Dehn G, von Dehn O, Völker W, Langer C, Weinbauer GF, Behre HM, Nieschlag E, Assmann G, von Eckardstein A (2001) Atherosclerosis in apolipoprotein E-deficient mice is increased by exogenous androgens and decreased by suppression of endogenous testosterone. Horm Metab Res 33:110–114

von Eckardstein A, Kliesch S, Nieschlag E, Chirazi A, Assmann G, Behre HM (1997) Suppression of endogenous testosterone in young men increases serum levels of HDL-subclass LpA-I and lipoprotein(a) Clin Endocrinol Metab 82:3367–3372

von Eckardstein A, Nofer JR, Assmann G (2001) HDL and coronary heart disease: Role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 20:13–27

von Eckardstein S, Syska A, Gromoll J, Kamischke A, Simoni M, Nieschlag E (2001) Inverse correlation between sperm concentration and number of androgen receptor CAG repeats in normal men. J Clin Endocrinol Metab 86:2585–2590

Wang C, Swedloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–2853

Webb CM, Adamson DL, de Zeigler D, Collins P (1999) Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. Am J Cardiol 83:437–9

Weidemann W, Hanke H (2002) Cardiovascular effects of androgens. Cardiovasc Drug Rev 20:175–198

Weinberg EO, Thienelt CD, Katz SE, Bartunek J, Tajima M, Rohrbach S, Douglas PS, Lorell BH (1999) Gender differences in molecular remodeling in pressure overload hypertrophy. J Am Coll Cardiol 34:264–273

Wichmann MW, Ayala A, Chaudry IH (1996) Male sex steroids are responsible for depressing macrophage immune function after trauma-hemorrhage. Am J Physiol 273:C1335–1340

Wild S, Pierpoint T, McKeigue P, Jacobs HS (2000) Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf) 52:595–600

Wu FC, von Eckardstein A Androgens and coronary artery disease. Endocr Rev (2003) 24:183–217

Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P (1995) Testosterone relaxes rabbit coronary arteries and aorta. Circulation 91:1154–1160

Zhang X, Wang LY, Jiang TY, Zhang HP, Dou Y, Zhao JH, Zhao H, Qiao ZD, Qiao JT (2002) Effects of testosterone and 17-beta-estradiol on TNF-alpha-induced E-selectin and VCAM-1 expression in endothelial cells. Analysis of the underlying receptor pathways. Life Sci 71:15–29

Zhu XD, Bonet B, Knopp RH (1997) 17ß-estradiol, progesterone, and testosterone inversely modulate low density lipoprotein oxidation and cytotoxicity in cultured placental trophoblast and macrophages. Am J Obstet Gynecol 177:196–209

Zitzmann M, Nieschlag E (2001) Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 144:183–197

Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001a) The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. Clin Endocrinol (Oxf) 55:649–657

Zitzmann M, Brune M, Kornmann B, Gromoll J, von Eckardstein S, von Eckardstein A, Nieschlag E (2001b) The CAG repeat polymorphism in the AR gene affects high density lipoprotein cholesterol and arterial vasoreactivity. J Clin Endocrinol Metab 86:4867–4873

Zitzmann M, Brune M, Nieschlag E (2002) Vascular reactivity in hypogonadal men is reduced by androgen substitution. J Clin Endocrinol Metab 87:5030–5037

Zitzmann M, Gromoll J, Von Eckardstein A, Nieschlag E (2003) The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. Diabetologia 46:31–39

11

Testosterone and erection

H.M. Behre

Contents

11.1 Introduction

Erectile dysfunction has been defined by the NIH Consensus Development Panel on Impotence as "the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance" (NIH Consensus Development Panel on Impotence 1993). Today, it is generally accepted that the pathogenesis of erectile dysfunction is multifactorial, and several emotional, physical and medical factors contribute to the degree of the dysfunction. The prevalence of erectile dysfunction increases with age, and results of various surveys indicate an overall prevalence in males aged between 30 and 80 years of approximately 20% (Shabsigh and Anastasiadis 2003; Braun *et al.* 2003).

11.2 Physiology of erection

Erection can be regarded as a complex neurovascular process that can be initiated by recruitment of penile afferent signals (*reflexogenic erection*) and by visual, acoustic, tactile, olfactory and imaginary stimuli (*psychogenic erection*). Several brain regions have been identified that are involved in the initiation of penile erection. The effect of testosterone on these central mechanisms is described in depth in Chapter 4.

At the penile level, the erection is determined by the contractile state of the smooth muscles. Contracted smooth muscle cells in the flaccid penis minimize the blood flow into the sinuses of the corpora cavernosa. With sexual stimulation, three hemodynamic factors are essential for achievement of erection with full tumescence and rigidity:

(1) Relaxation of cavernosal smooth muscle cells which leads to intracavernosal reduction of resistance,
(2) increased arterial inflow into the sinuses of the corpora cavernosa by relaxation of smooth muscles of the arterial vessels, and
(3) restriction of venous outflow by compression of intracavernosal and subtunical venous plexus (for review van Ahlen and Hertle 2000; Shabsigh and Anastasiadis 2003).

In normal physiology, nitric oxide seems to be the key factor for smooth muscle cell relaxation in the penis. *Neurogenic* nitric oxide is considered as the most important factor for immediate relaxation of penile vessels. *Endothelial* nitric oxide seems to be responsible for maintaining erection (Andersson 2003). Within the muscle cell, nitric oxide activates a soluble guanylyl cyclase, which raises the intracellular level of cyclic GMP (Shabsigh and Anastasiadis 2003). cGMP activates a specific protein kinase which finally leads to a decrease of intracellular cytosolic calcium concentration and relaxation of the smooth muscle cells. Various other signal transduction pathways, such as the Rho-kinase pathway, are involved in erectile function which have been reviewed elsewhere (e.g., Andersson 2003; Kandeel *et al.* 2001; Wingard *et al.* 2003). However, sufficient levels of nitric oxide as well as the integrity of the smooth muscles of the penile arteries and the corpora cavernosa seem to be the predominant physiological factors for erection.

11.3 Direct effects of testosterone on erection

Androgens and a functioning androgen receptor are necessary for normal development of the human penis. In humans, the penis grows in phases, initially during early gestation and then continuing until approximately the age of five. A latency period follows until puberty, when penile size responds to the increase of testosterone

levels. Growth ceases at the completion of pubertal growth despite continued high levels of circulating testosterone. The exact mechanism of penile growth cessation remains unresolved, but is probably not only due to down regulation of androgen receptors (Baskin *et al.* 1997; Levy *et al.* 1996).

Numerous studies in animal models have demonstrated a direct testosterone-dependency of erection. In castrated rats, the intracavernosal blood pressure of the penis was insufficient in response to induced erection by electrical field stimulation of the cavernosal nerve. Testosterone replacement restored the normal erectile response (Mills *et al.* 1994). In the rat, the primary mode of action of testosterone for erectile function seems to be the stimulation of *neurogenic* and *endothelial* nitric oxide synthesis (e.g. Baba *et al.* 2000a; 2000b; Chamness *et al.* 1995; Garban *et al.* 1995; Marin *et al.* 1999; Park *et al.* 1999; Penson *et al.* 1996; Reilly *et al.* 1997; Schirar *et al.* 1997; Seftel 1997; Zvara *et al.* 1995). This effect is mediated by testosterone or dihydrotestosterone, but not by estradiol (Lugg *et al.* 1995). In addition, castration induces programmed smooth muscle cell death in the rat penis, indicating that androgens may have an important role in maintaining smooth muscle growth and functional integrity (Shabsigh 1997).

In a different species, the New Zealand white rabbit, castration similarly reduces intracavernosal blood pressure during stimulation of the cavernosal nerve for induced erection (Traish *et al.* 1999). Testosterone, but not estradiol treatment prevented the effects of castration and restored intracavernosal pressure to values comparable to those obtained in intact animals. Interestingly, no change of the neuronal nitric oxide synthase protein expression and total activity were observed after castration or testosterone replacement (Traish *et al.* 1999; 2003). However, testosterone deficiency induced by castration or administration of GnRH agonists reduced trabecular smooth muscle content, and this reduction was restored by testosterone, and not by estradiol (Traish *et al.* 2003). The imbalance between smooth muscle and extracellular matrix in testosterone deficiency can lead to veno-occlusive dysfunction of the penis, and thereby cause erectile dysfunction (Mills *et al.* 1998). Comparable results indicating an androgen-dependent mechanism of veno-occlusive erectile dysfunction have recently been described in a castrated mouse model (Palese *et al.* 2003).

In humans, dynamic colour duplex ultrasound after pharmaco-stimulation of erection indicates that in men with erectile dysfunction low free testosterone levels correlate independently of age with impaired relaxation of cavernous smooth muscle cells (Aversa *et al.* 2000). Other studies demonstrated a significant increase of testosterone concentration in blood samples taken from the corpus cavernosum in healthy males during the tumescence and rigidity phase of erection, whereas no significant change was detected in patients with organogenic erectile dysfunction (Becker *et al.* 2000; 2001).

11.4 Effects of testosterone therapy on erection in hypogonadal men

Since the early beginning of testosterone therapy of hypogonadal patients it has been known that testosterone restores normal male sexual behaviour and erectile function (see Chapter 4). A meta-analysis on testosterone therapy for erectile dysfunction in hypogonadal patients confirmed the significant improvement of erections after initiation of testosterone therapy (Jain *et al.* 2000). It should be noted that most of the clinical trials included only small numbers of patients, in most cases fewer than 20. Pooled data on placebo-controlled studies showed an improvement of erectile function in 36 of 55 men treated with testosterone, whereas significantly fewer men responded to placebo treatment (9 out of 45) (Jain *et al.* 2000).

A recent large study involving 227 hypogonadal men randomly assigned to therapy with non-scrotal testosterone patches (two testosterone patches per day) or testosterone gel (5–10 g testosterone gel per day) demonstrated significant improvement of erectile function during a treatment period of up to 42 months (Wang *et al.* 2000; 2002). Sexual performance, "per-cent full erection" and satisfaction with erection were assessed by a simple self-report diary (Lee *et al.* 2003). Overall, testosterone replacement improved sexual performance, "per-cent full erection" and erection satisfaction significantly. Maximal increases were observed at the first assessment 30 days after initiation of therapy, and erectile function remained constantly improved thereafter. No significant differences were detected between treatment groups (Fig. 11.1).

Early studies on the relationship between androgens and erectile response in men have postulated a difference between spontaneous, sleep-related erections (nocturnal penile tumescence, NPT), which are impaired in terms of duration and degree in hypogonadism and enhanced by testosterone replacement therapy, and erections in response to visual erotic stimuli (VES), which have not been influenced by testosterone withdrawal or replacement (Bancroft and Wu 1983; Kwan *et al.* 1983). In a later study, nine hypogonadal men showed not only significant increases of penile circumference and rigidity of sleep-related erections after three months of androgen replacement, but also a minor, but significant improvement of both duration of erection and maximum level of rigidity following visual erotic stimuli (Carani *et al.* 1995).

11.5 Testosterone and erection in normal men

So far, only small-scale studies have been performed testing the effects of testosterone on erection in normal men. In addition, these studies do not allow exact differentiation between effects on sexual behaviour and direct effects on the penis.

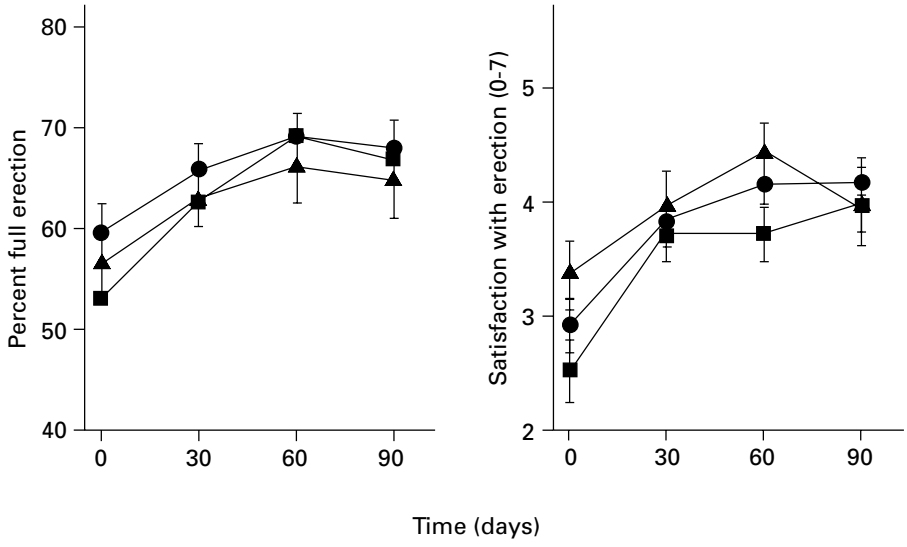


Fig. 11.1 Effects of treatment of hypogonadal men (n = 227) with testosterone gel (*squares*, 50 mg/d; *circles*, 100 mg/d) and non-scrotal testosterone patches (*triangles*, 5 mg/d) on erection as assessed by a questionnaire on percentage of full erection (left panel) and satisfaction with erection (right panel) (modified with permission from Wang *et al*. 2000, copyright 2000, The Endocrine Society).

One controlled study involving 11 normal men tested the effects of varying serum testosterone concentrations within the normal range (Buena *et al.* 1993). All men received the depot GnRH agonist leuprolide acetate for suppression of endogenous testosterone to the hypogonadal range. Six volunteers received 4 mg/d of a testosterone microcapsule formulation to restore testosterone levels to the low normal range (mean values 10.5 ± 1.7 nmol/l), whereas five volunteers received a dose of 8 mg/d resulting in testosterone levels in the middle to high normal range (mean values 26.5 ± 3.4 nmol/l). Despite significantly different testosterone levels, albeit in the normal range, there was no difference in the number of spontaneous nocturnal erections during rapid eye movement (REM) periods as well as no difference in the magnitude and duration of tumescence as measured by NPT recordings at the base and the tip of the penis.

When ten healthy, young adult males received the depot GnRH agonist leuprolide acetate or placebo for 12 weeks, but remained without androgen substitution, the suppression of endogenous testosterone to the low hypogonadal range resulted in a significant decrease of sleep-related erections (Hirshkowitz *et al.* 1997). Whereas sleep efficiency and REM sleep measures did not differ between groups, the total tumescence time of sleep-related erections at the penile base decreased significantly compared to placebo. The observed reduction of maximal circumference increase and frequency of erections did not reach statistical significance.

Comparable results were seen in studies concentrating on sexual behaviour (see Chapter 4). In short, suppression of endogenous testosterone by the GnRH antagonist Nal-Glu in normal men and substitution of exogenous testosterone with low (50 mg/week) and high doses (100 mg/week) of testosterone enanthate did not change the frequency of sexual desire, sexual fantasies, intercourse, or spontaneous erections. Addition of the aromatase inhibitor testolactone had no effect on sexual behaviour, whereas significant effects on sexual behaviour were noted in those men receiving the GnRH antagonist and placebo (Bagatell *et al.* 1994).

Weekly administration of 25, 50, 125, 300 and 600 mg of testosterone enanthate for 20 weeks to eugonadal men who had received a GnRH agonist for suppression of endogenous testosterone did not change scores for sexual activity and sexual desire (Bhasin *et al.* 2001), whereas other androgen-dependent parameters, such as fat-free mass, muscle size, strength and power, haemoglobin, HDL cholesterol and IGF 1, showed significant dose-response relationships. Similarly, administration of high doses of weekly 200 mg testosterone enanthate for eight weeks in a single-blind, placebo-controlled study in normal men did not induce changes in parameters of sexual activity (Anderson *et al.* 1992). One early placebo-controlled study in eight normal eugonadal men found enhanced rigidity of nocturnal penile tumescence after administration of 150 mg testosterone enanthate, but no effect on frequency of erections or circumference increase of the penis (Carani *et al.* 1990).

These experimental studies in normal men indicate that variations of testosterone levels within the normal range or serum levels exceeding the upper limit of normal have no or very limited influence on erectile function. This conclusion is in agreement with the results of two larger studies correlating serum levels of testosterone with erectile function in normal men. In one study involving 201 men, serum levels of testosterone in the normal range did not show any significant association with parameters of nocturnal penile tumescence and rigidity monitoring, whereas men with serum levels lower than 200 ng/dl had significantly lower values of the respective erectile parameters (Granata *et al.* 1997). In a recent large study involving 1071 mainly eugonadal men aged from 40 to 90 years, no significant association was detected between serum testosterone levels and the prevalence or severity of erectile dysfunction as assessed by the questionnaire of the simplified International Index of Erectile Function (IIEF-5) (Rhoden *et al.* 2002).

11.6 Prevalence of testosterone deficiency in patients with erectile dysfunction

Various studies have estimated the prevalence of testosterone deficiency in patients with erectile dysfunction. A systematic multidisciplinary assessment of 256 men

with erectile dysfunction showed a prevalence of hypothalamic-pituitary-gonadal axis abnormalities of 17.5%. In only 12.1% did the testosterone deficiency clearly contribute to erectile dysfunction (Nickel *et al.* 1984). Another routine hormonal screening in 300 men presenting with a primary complaint of erectile dysfunction showed a prevalence of only 1.7% (Maatman and Montague 1986). A similar low prevalence of 2.1% was detected in 330 consecutive patients with erectile dysfunction screened for testosterone deficiency (Johnson and Jarow 1992).

More recently, endocrine screening of 1022 men with erectile dysfunction detected serum concentrations of testosterone < 3 ng/ml in 8.0% of men. However, 40% of these patients had normal serum levels at repeated determination (Buvat and Lemaire 1997). Pituitary tumors were discovered in two men with low testosterone. Determination of testosterone only in cases of low sexual desire or abnormal physical examination would have overlooked 40% of men with low testosterone, and 37% of men subsequently improving during testosterone substitution therapy (Buvat and Lemaire 1997). The largest study involving 3547 men with erectile dysfunction revealed a prevalence of testosterone deficiency (serum concentration less than 2.8 ng/ml) of 18.7% (Bodie *et al.* 2003).

The marked difference in the prevalence of testosterone deficiency in patients with erectile dysfunction could be explained by different patient populations, different age of men with erectile dysfunction, differences in primary, secondary or tertiary care centres, different definitions of low testosterone levels, or single versus repeated testosterone determinations. It should be noted that none of these studies really fulfils the principles of evidence-based medicine, as no study included a control group of age-matched men without erectile dysfunction. Nevertheless, recent consensus conferences on erectile dysfunction, such as the "Third International Conference on the Management of Erectile Dysfunction" (Nehra *et al.* 2003), or the "2nd International Consultation on Erectile and Sexual Dysfunction" held in Paris in 2003 recommend screening for testosterone deficiency in all patients with erectile dysfunction.

11.7 Combined therapy with testosterone and phosphodiesterase type 5 inhibitors in patients with erectile dysfunction

Oral therapy with inhibitors of the phosphodiesterase type 5, e.g. sildenafil, vardenafil, and tadalafil, is highly effective for therapy of erectile dysfunction (Shabsigh and Anastasiadis 2003). However, in placebo-controlled phase III clinical trials and post-marketing evaluation approximately 15 to 40% of patients do not respond to this medication. There is some evidence that patients with erectile dysfunction and testosterone deficiency respond poorly to therapy with phosphodiesterase type 5 inhibitors (Guay *et al.* 2001; Shabsigh 2003).

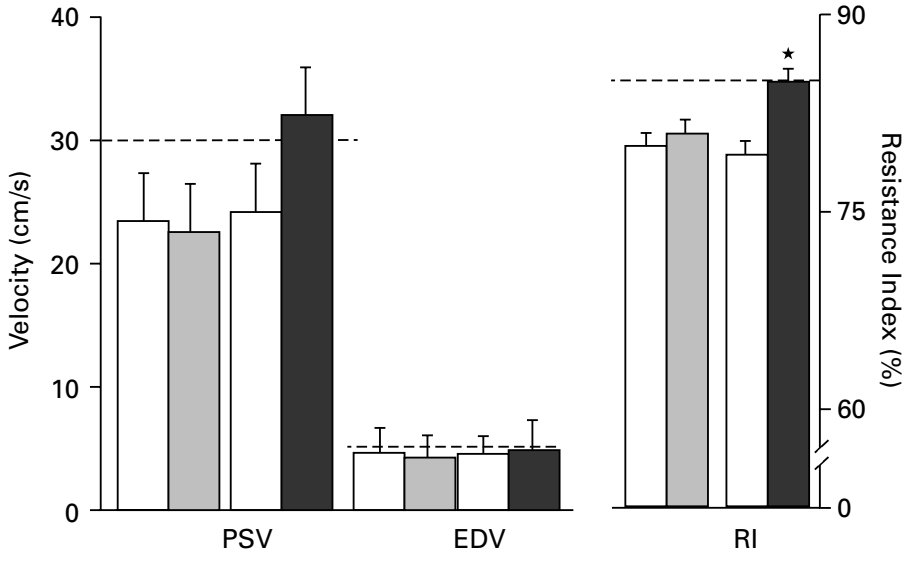


Fig. 11.2 Blood flow parameters of the cavernous arteries assessed by duplex sonography after a standardized pharmacostimulation of erection with 10 μg prostaglandin E1. *PSV*, peak systolic velocity; *EDV*, end diastolic velocity; *RI*, resistance index; *open bars*, baseline values; *grey bars*, placebo plus sildenafil group; *filled bars*, testosterone plus sildenafil group (modified with permission from Aversa *et al*. 2003, copyright 2003, Blackwell Publishing).

Two prospective, randomized placebo-controlled studies have been performed recently to test whether testosterone substitution can improve the response to sildenafil in patients with erectile dysfunction and testosterone serum levels in the low-normal or hypogonadal range. The first study included 20 patients with arteriogenic erectile dysfunction as diagnosed by dynamic colour duplex ultrasound. These men had normal sexual desire, serum levels of total and free testosterone in the lower quartile of the normal range and had not responded to the highest dose of sildenafil (100 mg) on six consecutive attempts (Aversa *et al.* 2003). Patients were randomized to transdermal non-scrotal testosterone patches (5 mg/d; n = 10) or placebo patches (n = 10), and received 100 mg sildenafil tablets on demand. Dynamic colour duplex ultrasound revealed a significant increase of arterial inflow to cavernous arteries of the penis in the testosterone-treated men, whereas this parameter remained unchanged in the placebo-group (Fig. 11.2). Effects on erectile function were assessed by the International Index of Erectile Function (IIEF) questionnaire (Rosen *et al.* 1997). Compared to placebo plus sildenafil, treatment with testosterone and sildenafil resulted in a significantly increased score of the erectile function domain, the intercourse satisfaction domain, and the overall satisfaction domain of the IIEF. The scores of the sexual desire domain and orgasmic function

domain remained unchanged, indicating that the treatment effect of testosterone was not only due to central effects on sexual desire. The Global Assessment Question (GAQ) "Has the treatment you received . . . improved your erections" was positively affirmed by 80% of men in the testosterone/sildenafil group compared to 10% in the placebo/sildenafil group at the end of treatment (Aversa *et al.* 2003).

This pilot study was followed by a randomized, double-blind, placebo-controlled 12-week multicentre study in 70 men with low or low-normal serum testosterone (morning levels before 10 a.m.: < 400 ng/dl) and non-responders to 100 mg of sildenafil during a four-week run-in period (Shabsigh *et al.* 2003). Patients were randomly assigned to therapy with placebo gel and sildenafil (group I, n = 33) or 5 g/d of testosterone gel and sildenafil (group II, n = 37). While the severity of erectile dysfunction was similar in both groups at baseline, the erectile function domain as well as the orgasmic function domain of the IIEF improved significantly in group II at study week 4, and scores remained at this level up to the end of the study. However, it should be noted that the difference between both groups lost significance following week 4 because of improvement of the total IIEF score and score of the erectile function domain in group I. In addition, no control group treated only with testosterone and oral placebo was included in this study.

One further, however not properly controlled study in patients with diabetes mellitus and erectile dysfunction not responding to sildenafil therapy showed similar results (Kalinchenko *et al.* 2003). 120 diabetic patients, aged 43 to 73 years, with low testosterone levels and erectile dysfunction who had failed to respond to 100 mg sildenafil at least three times were given 80–120 mg/d of oral testosterone undecanoate and sildenafil for four to six weeks. Androgen replacement in combination with sildenafil medication significantly improved erectile function and libido as assessed by the IIEF. After cessation of testosterone therapy, scores of the IIEF decreased to baseline within two weeks.

The two randomized, placebo-controlled studies provide preliminary evidence that patients with erectile dysfunction and low-normal or subnormal testosterone levels who do not respond to high-dose sildenafil therapy might benefit from a combined therapy with testosterone and phosphodiesterase type 5 inhibitors. However, further large-scale studies are needed to test the long-term benefit of this interesting combination therapy for erectile dysfunction.

11.8 Effects of treatment of erectile dysfunction on testosterone

There is some evidence that not only testosterone is relevant for effective therapy of erectile dysfunction, but conversely that effective therapy of erectile dysfunction can also increase serum concentrations of testosterone. In normal healthy men and

patients with erectile dysfunction, serum levels of testosterone increase significantly during the tumescence as well as the rigidity phase of penile erection, and return to baseline in the detumescence phase (Becker *et al.* 2000; 2001).

A controlled, non-randomized study demonstrated that effective psychological, medical (prostaglandin E1, yohimbine) or mechanical (vascular surgery, penile prostheses, vacuum devices) therapy of erectile dysfunction leads to a sustained increase of serum testosterone levels (Jannini *et al.* 1999). This increase could be caused by increased LH bioavailability (Carosa *et al.* 2002). However, randomized controlled studies are awaited to prove this interesting hypothesis.

11.9 Conclusion

There is clear evidence from experimental studies that testosterone influences erectile function not only indirectly by increased libido, but has direct effects on the penis. Whereas testosterone substitution therapy is effective for treatment of erectile dysfunction in hypogonadal patients, the effects of testosterone on erectile function in normal men seem to be marginal. Recent studies suggest that therapy combining testosterone and phosphodiesterase type 5 inhibitors could be useful in so-called "sildenafil non-responders" with low-normal or subnormal testosterone levels.

11.10 Key messages

- Positive effects of testosterone on erection are mediated by central stimulation of libido and sexual activity, but also by direct effects on the penis.
- Experimental studies suggest that the integrity of the smooth muscles of the penile arteries and the corpora cavernosa as well as the biological activity of nitric oxide, the predominant cellular transmitter for normal erection, are androgen-dependent.
- Impaired erectile function is a classical symptom of hypogonadism. Testosterone therapy of hypogonadal patients significantly improves erectile function. Testosterone not only enhances spontaneous sleep-related erections, but – to a lesser degree – also erectile response to visual erotic stimuli.
- In eugonadal men, variations of testosterone levels within the normal range or levels exceeding the upper limit of normal have no or very limited influence on erectile function.
- The true prevalence of testosterone deficiency as a cause for erectile dysfunction is not known, but seems to be less than 20%. Recent consensus statements recommend screening for testosterone deficiency in patients with erectile dysfunction.
- Randomized, placebo-controlled studies indicate that patients with erectile dysfunction and low-normal or subnormal testosterone levels might benefit from therapy combining testosterone and phosphodiesterase type 5 inhibitors.
- Preliminary, non-randomized studies suggest that effective therapy of erectile function might increase serum testosterone levels.

11.11 REFERENCES

Anderson RA, Bancroft J, Wu FC (1992) The effects of exogenous testosterone on sexuality and mood of normal men. J Clin Endocrinol Metab 75:1503–1507

Andersson KE (2003) Erectile physiological and pathophysiological pathways involved in erectile dysfunction. J Urol 170(2 Pt 2):S6–13

Aversa A, Isidori AM, De Martino MU, Caprio M, Fabbrini E, Rocchietti-March M, Frajese G, Fabbri A (2000) Androgens and penile erection: evidence for a direct relationship between free testosterone and cavernous vasodilation in men with erectile dysfunction. Clin Endocrinol (Oxf) 53:517–522

Aversa A, Isidori AM, Spera G, Lenzi A, Fabbri A (2003) Androgens improve cavernous vasodilation and response to sildenafil in patients with erectile dysfunction. Clin Endocrinol (Oxf) 58:632–638

Baba K, Yajima M, Carrier S, Akkus E, Reman J, Nunes L, Lue TF, Iwamoto T (2000a) Effect of testosterone on the number of NADPH diaphorase-stained nerve fibers in the rat corpus cavernosum and dorsal nerve. Urology 56:533–538

Baba K, Yajima M, Carrier S, Morgan DM, Nunes L, Lue TF, Iwamoto T (2000b) Delayed testosterone replacement restores nitric oxide synthase-containing nerve fibres and the erectile response in rat penis. BJU Int 85:953–958

Bagatell CJ, Heiman JR, Rivier JE, Bremner WJ (1994) Effects of endogenous testosterone and estradiol on sexual behavior in normal young men. J Clin Endocrinol Metab 78:711–716

Bancroft J, Wu FC (1983) Changes in erectile responsiveness during androgen replacement therapy. Arch Sex Behav 12:59–66

Baskin LS, Sutherland RS, DiSandro MJ, Hayward SW, Lipschutz J, Cunha GR (1997) The effect of testosterone on androgen receptors and human penile growth. J Urol 158:1113–1118

Becker AJ, Uckert S, Stief CG, Truss MC, Machtens S, Scheller F, Knapp WH, Hartmann U, Jonas U (2000) Cavernous and systemic testosterone levels in different phases of human penile erection. Urology 56:125–129

Becker AJ, Uckert S, Stief CG, Scheller F, Knapp WH, Hartmann U, Jonas U (2001) Cavernous and systemic testosterone plasma levels during different penile conditions in healthy males and patients with erectile dysfunction. Urology 58:435–440

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim, Shen R, Storer TW (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:E1172–1181

Bodie J, Lewis J, Schow D, Monga M (2003) Laboratory evaluations of erectile dysfunction: an evidence based approach. J Urol 169:2262–2264

Braun M, Sommer F, Jawadi N, Jawadi R, Engelmann U (2003) Die "Kölner 20.000er-Umfrage" – Prävalenz und Therapiebedarf von Männern mit erektiler Dysfunktion [abstract]. Reproduktionsmedizin 19:234

Buena F, Swerdloff RS, Steiner BS, Lutchmansingh P, Peterson MA, Pandian MR, Galmarini M, Bhasin S (1993) Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. Fertil Steril 59:1118–1123

Buvat J, Lemaire A (1997) Endocrine screening in 1,022 men with erectile dysfunction: clinical significance and cost-effective strategy. J Urol 158:1764–1767

Carani C, Scuteri A, Marrama P, Bancroft J (1990) The effects of testosterone administration and visual erotic stimuli on nocturnal penile tumescence in normal men. Horm Behav 24:435–441

Carani C, Granata AR, Bancroft J, Marrama P (1995) The effects of testosterone replacement on nocturnal penile tumescence and rigidity and erectile response to visual erotic stimuli in hypogonadal men. Psychoneuroendocrinology 20:743–753

Carosa E, Benvenga S, Trimarchi F, Lenzi A, Pepe M, Simonelli C, Jannini EA (2002) Sexual inactivity results in reversible reduction of LH bioavailability. Int J Impot Res 14:93–99

Chamness SL, Ricker DD, Crone JK, Dembeck CL, Maguire MP, Burnett AL, Chang TS (1995) The effect of androgen on nitric oxide synthase in the male reproductive tract of the rat. Fertil Steril 63:1101–1107

Garban H, Marquez D, Cai L, Rajfer J, Gonzalez-Cadavid NF (1995) Restoration of normal adult penile erectile response in aged rats by long-term treatment with androgens. Biol Reprod 53:1365–1372

Granata AR, Rochira V, Lerchl A, Marrama P, Carani C (1997) Relationship between sleep-related erections and testosterone levels in men. J Androl 18:522–527

Guay AT, Perez JB, Jacobson J, Newton RA (2001) Efficacy and safety of sildenafil citrate for treatment of erectile dysfunctionin a population with associated organic risk factors. J Androl 22:793–797

Hirshkowitz M, Moore CA, O'Connor S, Bellamy M, Cunningham GR (1997) Androgen and sleep-related erections. J Psychosom Res 42:541–546

Jain P, Rademaker AW, McVary KT (2000) Testosterone supplementation for erectile dysfunction: results of a meta-analysis. J Urol 164:371–375

Jannini EA, Screponi E, Carosa E, Pepe M, Lo Giudice F, Trimarchi F, Benvenga S (1999) Lack of sexual activity from erectile dysfunction is associated with a reversible reduction in serum testosterone. Int J Androl 22:385–392

Johnson AR, Jarow JP (1992) Is routine endocrine testing of impotent men necessary? J Urol 147:1542–1544

Kalinchenko SY, Kozlov GI, Gontcharov NP, Katsiya GV (2003) Oral testosterone undecanoate reverses erectile dysfunction associated with diabetes mellitus in patients failing on sildenafil citrate therapy alone. Aging Male 6:94–99

Kandeel FR, Koussa VKT, Swerdloff RS (2001) Male sexual function and its disorders: physiology, pathophysiology, clinical investigation, and treatment. Endocr Rev 22:342–388

Kwan M, Greenleaf WJ, Mann J, Crapo L, Davidson JM (1983) The nature of androgen action on male sexuality: a combined laboratory-self-report study on hypogonadal men. J Clin Endocrinol Metab 57:557–562

Lee KK, Berman N, Alexander GM, Hull L, Swerdloff RS, Wang C (2003) A simple self-report diary for assessing psychosexual function in hypogonadal men. J Androl 24:688–698

Levy JB, Seay TM, Tindall DJ, Husman DA (1996) The effects of androgen administration on phallic androgen receptor expression. J Urol 156:775–779

Lugg JA, Rajfer J, Gonzalez-Cadavid NF (1995) Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. Endocrinology 136:1495–1501

Maatman TJ, Montague DK (1986) Routine endocrine screening in impotence. Urology 27:499–502

Marin R, Escrig A, Abreu P, Mas M (1999) Androgen-dependent nitric oxide release in rat penis correlates with levels of constitutive nitric oxide synthase isoenzymes. Biol Reprod 61:1012–1016

Mills TM, Lewis RW, Stopper VS (1998) Androgenic maintenance of inflow and veno-occlusion during erection in the rat. Biol Reprod 59:1413–1418

Mills TM, Stopper VS, Wiedmeier VT (1994) Effects of castration and androgen replacement on the hemodynamics of penile erection in the rat. Biol Reprod 51:234–238

Nehra A, Steers WD, Althof SE, Andersson KE, Burnett AL 2nd, Costabile RA, Goldstein I, Kloner RA, Lue TF, Morales A, Rosen RC, Shabsigh R, Siroky MB, King L (2003) Third international conference on the management of erectile dysfunction: linking pathophysiology and therapeutic response. J Urol 170:S3–5

Nickel JC, Morales A, Condra M, Fenemore J, Surridge DH (1984) Endocrine dysfunction in impotence: incidence, significance and cost-effective screening. J Urol 132:40–43

NIH Consensus Development Panel on Impotence (1993) Impotence. JAMA 270:83–90

Palese MA, Crone JK, Burnett AL (2003) A castrated mouse model of erectile dysfunction. J Androl 24:699–703

Park KH, Kim SW, Kim KD, Paick JS (1999) Effects of androgens on the expression of nitric oxide synthase mRNAs in rat corpus cavernosum. BJU Int 83:327–333

Penson DF, Ng C, Cai L, Rajfer J, Gonzalez-Cadavid NF (1996) Androgen and pituitary control of penile nitric oxide synthase and erectile function in the rat. Biol Reprod 55:567–574

Reilly CM, Zamorano P, Stopper VS, Mills TM (1997) Androgenic regulation of NO availability in rat penile erection. J Androl 18:110–115

Rhoden EL, Teloken C, Mafessoni R, Souto CA (2002) Is there any relation between serum levels of total testosterone and the severity of erectile dysfunction? Int J Impot Res 14:167–171

Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A (1997) The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology 49:822–830

Schirar A, Bonnefond C, Meusnier C, Devinoy E (1997) Androgens modulate nitric oxide synthase messenger ribonucleic acid expression in neurons of the major pelvic ganglion in the rat. Endocrinology 138:3093–3102

Seftel AD (1997) The positive trophic effects of testosterone on neuronal nitric oxide synthase (nNOS) expression. J Androl 18:745–745

Shabsigh R (1997) The effects of testosterone on the cavernous tissue and erectile function. World J Urol 15:21–26

Shabsigh R (2003) Hypogonadism and erectile dysfunction: the role for testosterone therapy. Int J Impot Res 15 Suppl 4:S9-S13

Shabsigh R, Anastasiadis AG (2003) Erectile dysfunction. Annu Rev Med 54:153–168

Shabsigh R, Kaufman JM, Steidle C, Padma-Nathan H (2003) Testosterone replacement therapy with testosterone-gel 1% converts sildenafil non-responders to responders in men with hypogonadism and erectile dysfunction who failed prior sildenafil therapy [abstract]. J Urol 169 Suppl 4:247

Traish AM, Park K, Dhir V, Kim NN, Moreland RB, Goldstein I (1999) Effects of castration and androgen replacement on erectile function in a rabbit model. Endocrinology 140:1861–1868

Traish AM, Munarriz R, O'Connell L, Choi S, Kim SW, Kim NN, Huang YH, Goldstein I (2003) Effects of medical or surgical castration on erectile function in an animal model. J Androl 24:381–387

van Ahlen H, Hertle L (2000) Disorders of sperm disposition. In: Nieschlag E, Behre HM, eds. Andrology – Male reproductive health and dysfunction. 2nd edition. Springer, Heidelberg, pp. 191–222

Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–2853

Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weger T (2002) Long term efficacy and safety of transdermal testosterone gel (Androgel) in hypogonadal men [abstract]. 84th Annual Meeting of the U.S. Endocrine Society, abstract P2–646

Wingard CJ, Johnson JA, Holmes A, Prikosh A (2003) Improved erectile function after Rho-kinase inhibition in a rat castrate model of erectile dysfunction. Am J Physiol Regul Integr Comp Physiol 284:R1572–R1579

Zvara P, Sioufi R, Schipper HM, Begin LR, Brock GB (1995) Nitric oxide mediated erectile activity is a testosterone dependent event: a rat erection model. Int J Impot Res 7:209–219

12

Testosterone and the prostate

J.T. Isaacs

Contents

12.1 Introduction

Despite major progress in the biological sciences during the last 50 years, it is rather remarkable that we have entered the twenty-first century and still the specific function of the prostate gland remains unknown. Indeed, the prostate is the largest organ of unknown specific function in the human body. Although it is believed that the prostate is important in protecting the lower urinary tract from infection and for fertility, it is frequently the site of infection and inflammation, and sperm harvested from the epididymis without exposure to seminal or prostatic fluid can produce fertilization and successful birth (Silver *et al.* 1988). The fact that the specific in vivo function of prostate is not fully understood might not be so problematic if it were not the case that the prostate is the most common site of neoplastic transformation in men, with approximately one in six men in western industrialized

nations eventually developing clinically detected prostatic cancer during their lifetime (Jamal *et al.* 2003). Furthermore, the prostate is the most common site of benign neoplastic disease in males (Berry *et al.* 1984). More than 50% of all men above the age of 50 have benign prostatic hyperplasia (BPH) with ≈25% of men eventually requiring treatment for this condition (Berry *et al.* 1984). Thus, it is remarkable that despite the high prevalence of prostatic diseases, the etiologies of neither prostatic cancer nor BPH are known.

A major reason why both the specific function of the prostate and etiology of the prostatic neoplasms have been difficult to elucidate is that the gross structure and histological appearance of this gland vary widely in the animal kingdom and thus comparative animal studies have been problematic. All placental (i.e., eutherial) mammals have male sex accessory tissues that minimally include the prostate gland (Price and Williams-Ashman 1961). The term prostate is derived from the Latin word "to stand before." Thus, the gland that in males of placental mammals "stands before" the base of the bladder and produces and releases secretion into the male ejaculate is defined as the prostate. In males of most placental mammals, there are additional glands that likewise release excretion into the ejaculate and these glands are given a variety of names depending on the species (e.g., seminal vesicles, bulbourethral glands, periurethral glands, preputial glands, etc.). Along with the prostate these glands are called male accessory sex tissues. No organ system varies so widely among the animal species as the male sex accessory tissues (Price and Williams-Ashman 1961). In humans, these include the prostate, seminal vesicles, bulbourethral gland, Cowper's glands, and glands of Littre. The dog is the only species other than man which spontaneously develops both BPH and prostatic cancer with aging (Isaacs 1984a). The dog has a well-developed prostate but completely lacks seminal vesicles. In contrast, the rat has a prostate that is composed of four anatomically and biochemically distinct prostatic lobes (i.e., the ventral, dorsal, lateral, and anterior lobes, the latter lobe also called the coagulating gland). In addition, the rat has seminal vesicles and preputial glands. Besides this anatomical variation, there is a large variation among the different species in the secretory products produced and released by the prostate into the ejaculate (Mann and Mann 1981).

For example, the human prostatic epithelial cells synthesize and secrete a series of unique proteins into the ejaculate (Coffey 1992). These include serine protease, prostate-specific antigen (PSA), human glandular kallikrein-2 (hK-2) and prostatic-specific acid phosphatase (PAP). The essentially exclusive production of these proteins by normal and malignant prostatic cells has allowed the abnormal detection of these proteins in the serum of men to be useful as a means of (1) initially detecting prostatic cancer in asymptomatic men, 2) monitoring residual presence of systemic micrometastatic disease in men who have undergone radical

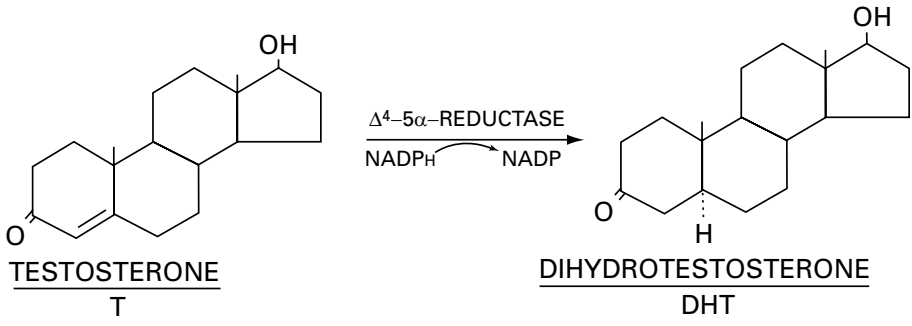


Fig. 12.1 Irreversible conversion of testosterone to DHT catalyzed by the NADPH-dependent type I or type II 5α-reductase enzyme.

prostatectomy for presumed localized disease, and 3) monitoring the response of clinical detected metastatic disease to systemic therapy. Although other animal species secrete prostate-specific proteins (e.g., prostatein secreted by rat ventral prostate and the arginines esterase secret by the dog prostate), there are no genes directly homologous to PSA or hK-2, based on DNA sequence, in the dog or rat genome. These is a homologous PAP gene in the rat, however, the level of expression is nearly 1000-fold lower in rat versus human prostate epithelial cells (Coffey 1992).

The human prostate is also unique in that it synthesizes and secretes large amounts of citrate (Coffey 1992). Indeed the concentration of citrate in prostatic secretory fluid (i.e., 75 mM) is 615 times higher than that of blood serum. Likewise the human prostatic epithelial cells concentrate Zn^{2+} from the blood and transport it into the prostate secretion. As a result of this activity, the prostate has one of the highest tissue concentrations of zinc in the human body. It is believed that the role of such a high Zn^{2+} concentration in the prostate and its secretion is to function as a natural bactericidal compound (Coffey 1992). Similarly the prostate is one of the richest sources of the highly charged, basic aliphatic polyamines (e.g., spermine). The biological role of polyamines has not been fully resolved although it is definitively known that polyamine metabolism is correlated with growth and that polyamines bind tightly to DNA and effect its confirmation and template ability for DNA replication and transcription.

Based on such varied anatomy and biochemistry, it has been difficult to define the etiology of either BPH or prostatic cancer. It is known, however, that testosterone and particularly its 5α-reductase metabolite, 5α-dihydrotestosterone (DHT), has at least a permissive role, if not an inductive one, in both of these prostatic neoplasms (Fig. 12.1). To appreciate the role of testosterone and DHT in these neoplastic diseases, an understanding of how testosterone functions in the normal development and physiology of the prostate is required.

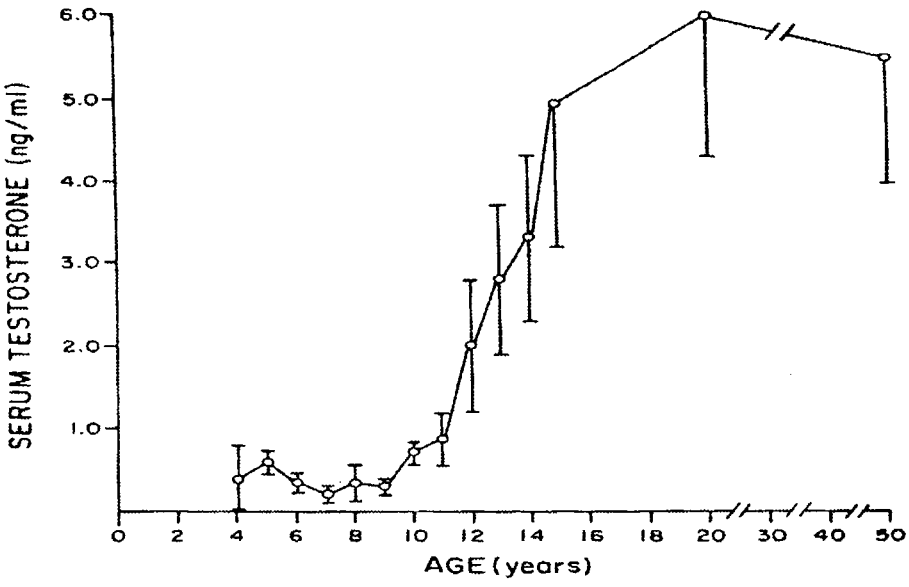


Fig. 12.2 Serum testosterone levels during aging in men (Data from Frasier *et al.* 1969).

12.2 Role of testosterone in the development and maintenance of the prostate

The urogenital sinus is the embryonic anlagen from which the prostate develops *in utero*. For the prostate to develop normally, a critical level of androgenic stimulation is required at specific times during its development *in utero* (Wilson 1984). In the developing male, the fetal testis secretes testosterone into the fetal circulation at sufficient levels to stimulate the differentiation and growth of a portion of the urogenital sinus tissue, producing the definitive prostate gland. This usually begins during the first three months of fetal growth. If sufficient serum testosterone is not present at this critical state of intrauterine development, the prostate does not develop (Wilson 1984).

After birth, serum testosterone levels decrease to a low baseline value until puberty, when they rise to the adult range (Frasier *et al.* 1969) (Fig. 12.2). Until puberty, the prostate remains small (approximately 1–2 g) (Isaacs 1984a). During puberty, the prostate grows to its adult size of approximately 20 g (Isaacs 1984a). Between the age of 10 and 20 years, the rate of prostatic growth is exponential with a prostatic weight-doubling time of 2.78 years (Isaacs 1984a) (Fig. 12.3). This period of exponential growth corresponds to the time period when the serum testosterone levels are rising from initially low levels seen before the age of 10 to the high levels seen in an adult male (Frasier *et al.* 1969) (Fig.12.2). If a boy is castrated

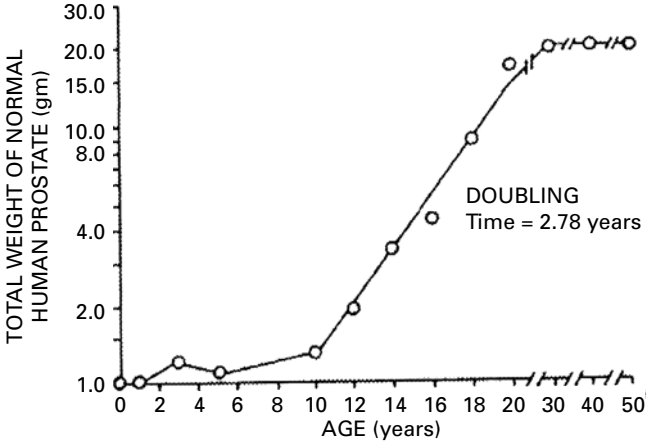


Fig. 12.3 Growth of the normal human prostate from birth to adulthood (Data from Boyd 1962).

before the age of ten, the serum testosterone levels do not rise to their normal adult level and the proliferative growth of the human prostate between 10 and 20 years of life is completely blocked (Moore 1944; Huggins and Johnson 1947). These results demonstrate that a physiological level of testosterone is chronically required for the normal growth of the human prostate. This chronic requirement for testosterone derives from the necessity for androgens to regulate the total prostatic cell number by affecting both the rate of cell proliferation and cell death. Androgen does this by stimulating the rate of cell proliferation (i.e., agonistic ability of androgen) while simultaneously inhibiting the rate of cell death (antagonistic ability of androgen) (Isaacs 1984b). Because of this dual agonist/antagonist effect of androgen on the prostate, the rate of cell proliferation is greater than the rate of cell death during the normal prostatic growth period occurring between 10 and 20 years of age. Having reached its maximum adult size by 20 years of age, the prostate normally ceases its continuous net growth (Isaacs 1984a). This does not mean, however, that the cells of the adult prostate in men over 20 years of age do not continuously turn over with time, but that the rate of prostatic cell proliferation is balanced by an equal rate of prostatic cell death, such that neither involution nor overgrowth of the gland normally occurs with time. Thus the adult prostate in men over 20 is an example of a steady-state self-renewing tissue. If an adult male whose prostate is in this steady-state maintenance condition is castrated, serum testosterone levels rapidly decrease to low values comparable to those seen in males younger than 10 years of age. As a result, the prostate rapidly involutes. Such involution demonstrates that a physiological level of testosterone is chronically required, not only for initial development, but also for maintenance of the normal prostate. In order to define the molecular mechanism[s] responsible for how testosterone maintains

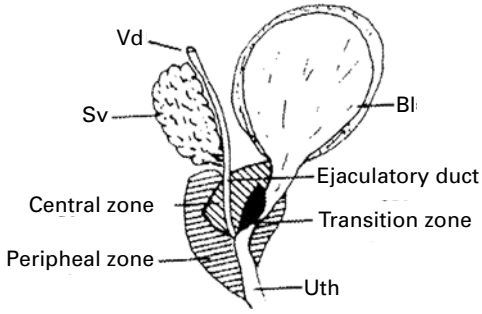


Fig. 12.4 Anatomy of the prostate, B1, bladder; Sv, seminal vesicle; Vd, vas deferens; Uth, urethra.

the normal prostate, an understanding of the cellular organization of the gland is required.

12.3 Zonal and cellular organization of the prostate

The normal human prostate is not composed of anatomically separate lobes as in many animals, but is instead divided into four zones (Fig. 12.4). The peripheral zone comprises 70 to 75% of the gland, the central zone 20 to 25%, and the transitional zone 5%, while the anterior surface consists of the fibromuscular stroma (McNeal *et al.* 1988). Most cancers develop in the peripheral zone. Benign prostatic hyperplasia (BPH) develops in the transition zone as a part of the aging process. Although hyperplastic changes develop, the mass of the peripheral zone remains constant at 20 to 25 g (McNeal *et al.* 1988). Within the peripheral, central, and transition zones, the tissue is organized as tubular-alveolar glands composed of a well-developed stromal compartment containing nerves, fibroblasts, infiltrating lymphocytes and macrophages, endothelial cell capillaries, and smooth muscle cells surrounding glandular acini composed of a two-layered (i.e., basal and secretory luminal) epithelium (Fig. 12.5). Scattered throughout this epithelial compartment are occasional neuroendocrine (NE) cells which are characterized by expression of chromogranin A, serotonin, and neuron-specific enolase, but not the androgen receptor (Bonkhoff 1998). Functionally, the epithelium is composed of multiple stem cell units supported by paracrine interaction from the stromal compartment (Bonkhoff and Remberger 1996; Bonkhoff *et al.* 1994; Hudson *et al.* 2000; Isaacs 1987; Isaacs and Coffey 1989; Robinson *et al.* 1998; Van Leenders *et al.* 2000) (Fig. 12.5). In an individual stem cell unit, the stem cell which has the capacity for unlimited self-renewal characteristically expresses $\alpha_2\beta_1$-integrins (Collins *et al.* 2001), but only rarely proliferates to provide progeny which differentiate to become either transit amplifying or NE cells (Bonkhoff *et al.* 1994). The stem and the majority

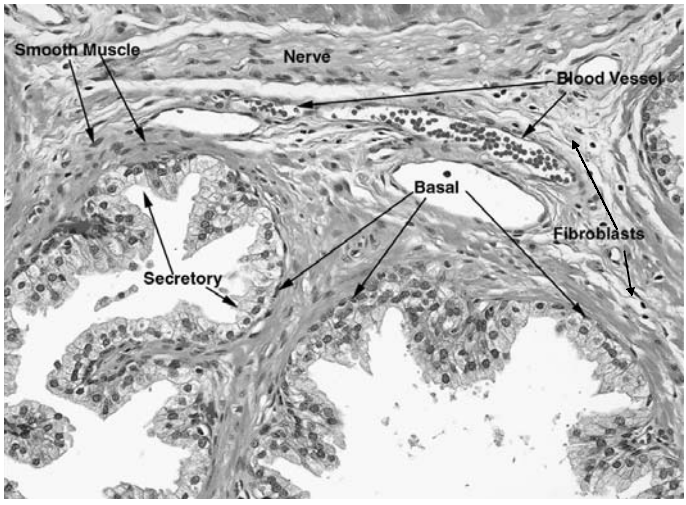
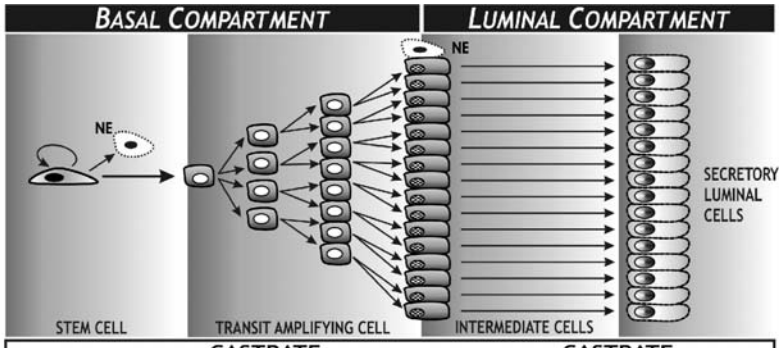


Fig. 12.5 Cellular heterogeneity within the normal prostate
Histological architecture of the prostate is comprised of blood vessels that provide nutrients, including androgen, to the fibrous stromal layer which consists primarily of fibroblasts and smooth muscle cells, and to the epithelial layer. Epithelium can be subdivided into a basal epithelium, which contains AR negative proliferating cells, and secretory luminal epithelium, which consists of fully differentiated AR and $p27^{Kip1}$ positive, nonproliferating cells.

of the transit amplifying cells are believed to be located in the basal epithelial layer (Figs. 12.5 and 12.6).

Basal cells express the p53 related p63 protein, the plasma membrane receptor for hepatocyte growth factor (known as c-MET), and pro-survival protein, bcl-2 (Gmyrek *et al.* 2001; McDonnell *et al.* 1992; Signoretti *et al.* 2000; Watabe *et al.* 2002). A minority of stem cell progeny differentiate into NE cells which secrete neuroendocrine peptides such as bombesin, calcitonin, and parathyroid hormone-related peptide (Rumpold *et al.* 2002). A subset of these basal cells shows high proliferative activity as evidenced by positive staining for Ki-67 and are termed transit amplifying cells, which initially do not express the androgen receptor (Bonkhoff *et al.* 1994; 1998). During this hierarchical expansion, these transit amplifying cells undergo a maturation process in which they progress to "intermediate-like" cells expressing prostate specific stem cell antigen (PSCA), and begin expressing the androgen receptor (AR). As these intermediate cells mature, they stop proliferating and terminally differentiate into mature secretory luminal cells which are non-proliferative and positive for AR and $p27^{Kip1}$ cyclin-dependent kinase inhibitor (Bonkhoff *et al.* 1994; 1998; De Marzo *et al.* 1998) (Fig. 12.5). Because of hierarchical expansion, these non-proliferating AR /$p27^{Kip1}$ positive secretory luminal cells are quantitatively the major subtype of epithelial cells present in the normal prostate. They also express



	CASTRATE MAJORITY OF BASAL CELLS REMAIN		CASTRATE MAJORITY OF SECRETORY CELLS DIE	
ANDROGEN RESPONSE	INDEPENDENT			DEPENDENT
ANDROMEDIN RESPONSE	INDEPENDENT	SENSITIVE		DEPENDENT
SELECTED PHENOTYPIC AND PROTEIN MARKER EXPRESSION				
Self Renewal Capacity	Extensive	Limited	Very Limited	None
Proliferative Index	Very Low	Much higher	Low	None
Keratin 5	+		+	−
Keratin 14	+		+	−
p63	+		−	−
Bcl-2	+		+	−
P cadherin	+		?	+
GST-pi	+		+/−	−
C-MET	+/−		+/−	−
p27 ^{kip1}	+	−	−/+	+
Keratins 8,18	−		+	+
NKX 3.1	−		−/+	+
PSA	−		−/+	+
PSAP	−		−/+	+
AR	−		−/+	+

Fig. 12.6 Stem cell model of prostatic epithelial cell compartmentalization
The prostate gland consists of a number of stem cell units which arise from one stem cell. Such a stem cell is located in the basal epithelial layer of the prostate and, upon division, gives rise to a population of transit amplifying cells. The latter divide in the basal layer and mature into an intermediate cell type. These intermediate cells migrate into the luminal layer where they differentiate into the secretory luminal cells. Expression of a number of genetic markers characteristic of each cell subtype is as indicated. NE denotes neuroendocrine cells; + denotes expression of marker; − denotes lack of detectable expression of marker.

the prostate-specific differentiation markers, prostatic specific acid phosphatase (PSAP), prostate specific antigen (PSA), NKX 3.1, human glandular kallikrein-2 (hK_2), prostate specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA), as well as vascular endothelial growth factor (VEGF) (Jain *et al.* 2002; Joseph *et al.* 1997; Liu *et al.* 1997; Ornstein *et al.* 2001; Schuur *et al.* 1996). The transcriptional expression of these prostate-specific differentiation marker genes

is enhanced by occupancy of the AR by physiologic androgen and the subsequent binding of the occupied AR at androgen response elements in the promoter and enhancer sequence of these genes within the nuclei of these secretory luminal cells (Jain *et al.* 2002; Schuur *et al.* 1996; Watt *et al.* 2001; Zelivianski *et al.* 2002; Mitchell *et al.* 2000).

12.4 Testosterone metabolism in the prostate

Quantitatively, the major circulating androgen in the blood is testosterone. Within the prostate, however, testosterone is enzymatically converted to 5α-dihydrotestosterone (DHT) (Wilson 1984). The class of enzymes responsible for the irreversible conversion of testosterone to DHT are the membrane-bound NADPH-dependent Δ^4-3-ketosteroid 5α-oxidoreductases (i.e., 5α-reductases) (Bruchovsky and Wilson 1968). Biochemical studies have demonstrated that the irreversible conversion of testosterone to DHT by 5α-reductase (Fig. 12.1), involves a sequential series of steps (Levy *et al.* 1990). Initially, reduced nicotinamide-adenine dinucleotide phosphate (NADPH) cofactor binds to the 5α-reductase enzyme to form a 5α-reductase-NADPH complex. Once formed, testosterone binds to this 5α-reductase-NADPH complex. Electrons are stereospecifically transferred from NADPH to reduce the Δ^4 double bond of testosterone, producing a 5α-reductase-oxidized $NADP^+$-5α-DHT complex. After 5α-DHT is produced, it must leave this complex before the bound $NADP^+$ is able to leave, thus regenerating active 5α-reductase enzyme for another catalytic cycle (Levy *et al.* 1990).

There are two distinct 5α-reductase genes in man, each encoding a biochemically distinct isozyme. Both isozymes have been cloned and the complete DNA-based sequence and amino acid composition are now known (Andersson and Russell 1990; Jenkins *et al.* 1991; Labrie *et al.* 1992; Thigpen *et al.* 1992). The genes encoding the proteins for both 5α-reductase type 1 and 2 isozymes have a similar structure containing five exons separated by four introns. The two genes share ≈46% DNA sequence homology and encode for a protein of ≈29,000 molecular weight. The type 1 isozyme is encoded by a gene on human chromosome 5p15 (Jenkins *et al.* 1991). It has a neutral pH optimum, a requirement for high concentration of testosterone to saturate the enzyme (high $K_m = 3\ \mu M$), and is rather insensitive to finasteride inhibition ($K_i \sim 300$ nM) (Andersson and Russell 1990; Jenkins *et al.* 1991). The type 1 isozyme is present at low levels in the prostate but is the predominant 5α-reductase isozyme in skin; it is also present in the liver (Jenkins *et al.* 1992; Normington and Russell 1992).

The type 2 isozyme is encoded by a gene on human chromosome 2p23 (Thigpen *et al.* 1992). It has an acidic (pH 5.0) optimum, has a lower K_m (0.5 μM) for testosterone, and is sensitive to finasteride inhibitor ($K_i = 23$ nM). The type 2 isozyme is the predominant 5α-reductase in androgen target tissue, including the

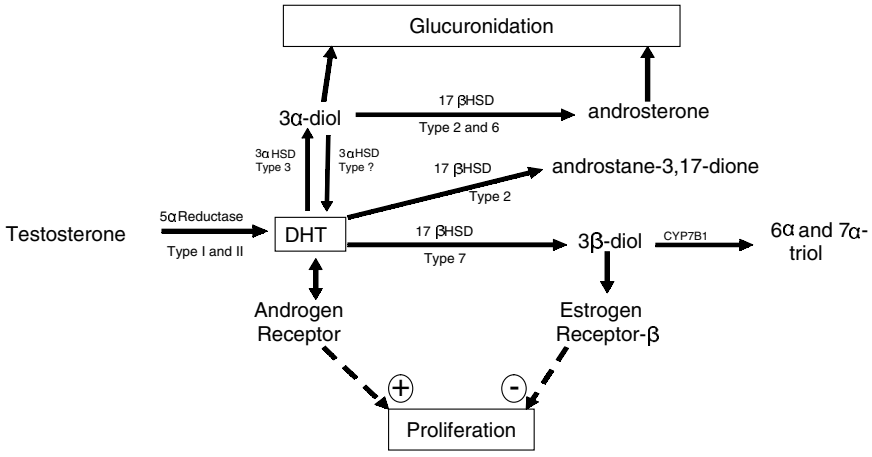


Fig. 12.7 Summary of the enzymatic pathway for androgen metabolism within the prostate.

prostate. Analysis of individuals with male pseudohermaphroditism caused by 5α-reductase deficiency has revealed no mutation in the type 1 isozyme gene (Jenkins *et al.* 1992). In contrast, molecular analysis demonstrated that mutation in the 5α-reductase type 2 gene accounts for this disorder (Andersson *et al.* 1991; Thigpen *et al.* 1992). Based on these results, it has been suggested that the type 1 isozyme functions in a catabolic manner in the metabolic removal of androgens by nontarget tissue, whereas the type 2 isozyme functions in an anabolic role to amplify the androgenicity of testosterone by effectively converting it to DHT within androgen target tissue (Normington and Russell 1992).

Once formed via the 5α-reductase type 1 or 2, DHT can reversibly bind to the androgen receptor to regulate prostatic cellular proliferation and survival. (Fig. 12.7). Alternatively, DHT can be further reductively metabolized to 5α-androstane-3α,17β-diol (3α-diol) by the 3αHSD type 3 enzyme (i.e., also known as AKRIC2) (Rizner *et al.* 2003) (Fig. 12.7). Once formed, 3α-diol can be re-oxidized back to DHT via an oxidative 3α-HSD enzyme not fully characterized in the normal prostate or glucuronidated at position 3 and excreted by the prostate (Rizner *et al.* 2003). 3α-diol can also be oxidized at its 17β-hydroxy position by 17β-HSD type 2 or type 6 enzymes to form 5α-androsterone which can also be glucuronidated at position 3 and excreted (Biswas and Russell 1997; Rizner *et al.* 2003) (Fig. 12.7). DHT can also be either oxidatively metabolized at its 17β-hydroxy group by the 17β-HSD type 2 enzyme to form 5α-androstane-3,17 dione (Rizner *et al.* 2003) or reductively metabolized at its 3 keto group to produce 5α-androstane-3β,17β-diol (3β-diol) by 17β HSD type 7 enzyme (Torn *et al.* 2003) (Fig. 12.7). Interestingly, it has been documented that the endogenous estrogen in the prostate is not 17β-estradiol but 3β-diol (Weihua *et al.* 2001). Also it has been documented that

within the normal prostate both the isotypes of the estrogen receptor (i.e., ERα and ERβ) are expressed and both can bind 3β-diol (Weihua *et al.* 2001). The ERα is expressed predominately in the prostatic stromal cells while ERβ is expressed in the epithelial cells (Fixemer *et al.* 2003). The Gustafsson group which initially discovered ERβ has postulated that 3β-diol binding to the ERβ within the prostatic epithelial cells results in antagonism of the AR signaling for proliferation (Weihua *et al.* 2002). The level of such an ERβ dependent anti-proliferative effect is thus dependent upon the level of 3β-diol. This 3β-diol level is itself regulated by the activity of the CYP7B1 enzyme which hydroxylates 3β-diol to 5α-androstane-3β, 6α, 17β-triol (6α-triol) and 5α-androstane-3β, 7α, 17β-triol (7α-triol) (Isaacs *et al.* 1979; Weihua *et al.* 2002) (Fig. 12.7).

The extensive metabolic pathway for androgen within the prostate functions as a means for autoregulation so that the prostatic level of DHT remains constant during the episodic and diurnal variations in both total and free serum testosterone levels (Plymate *et al.* 1989). Because growth versus regression (i.e. death) of the prostate is determined by the specific level of prostatic DHT (Kyprianou and Isaacs 1987), a constant prostatic DHT level is critical and is in turn required for the dose-dependent ability of DHT to bind to and regulate the function of the androgen receptors (Liao *et al.* 1972).

Androgen receptors are ligand-dependent zinc finger DNA binding proteins whose genomic binding co-ordinates formation of transcriptional complexes at the regulatory elements of targeted genes. The AR gene is located on the long arm of the X chromosome (i.e. Xq11.2), and encodes a protein with three critical domains: 1) an N-terminal domain (NTD) involved in homotypic dimerization and binding with other transcriptional co-activator or co-repressor proteins; 2) a DNA binding domain with two zinc finger binding motifs and hinge region, and 3) a C-terminal steroid ligand binding domain (LBD), which is also involved in homotypic dimerization and co-activation binding. This latter C-terminal LBD domain is also where 90-Kda heat shock protein (i.e., Hsp-90) dimers bind to stabilize the AR protein during folding subsequent to its synthesis (Chadli *et al.* 2000). Specific interaction with androgenic ligands results in the conformational activation of the androgen receptor. This allows the dissociation of the Hsp-90 dimer proteins and thus the binding and dimerization of the occupied androgen receptor (Langley *et al.* 1995) to androgen-response elements present in the promoter and enhancer regions in AR-regulated genes (Jain *et al.* 2002; Mitchell *et al.* 2000; Schuur *et al.* 1996; Watt *et al.* 2001; Zelivianski *et al.* 2002).

This initial genomic AR binding allows further binding to specific regions of the bound AR by additional nuclear proteins (i.e., transcriptional coactivator proteins like SRC-1, ARA 70, etc., and general transcription factors [GTF] like TFIIF and H) to produce transcriptional complexes which can activate or repress specific

gene expression (Sampson *et al.* 2001). For activation, formation of an active transcriptional complex is required, resulting in site-directed chromatin remodeling via histone acetylation and methylation which enhances target gene expression (He *et al.* 2001; Kang *et al.* 2002; Sampson *et al.* 2001; Shang *et al.* 2002; Xu *et al.* 1998). SRC-1 is a member of the p160 transcriptional coactivator gene family that includes SRC-1, TIF2 (also termed *GRIP-1* and *SRC-2*), and p/CIP (also termed *RAC3, ACTR, AIBI*, and *SRC-3*) (89). Cell-free in vitro transcription and in vivo experiments have indicated that the SRC-1 family members enhance androgen receptor-dependent transactivation of nuclear genes. The mechanism for such enhancement involves binding of p160 proteins to the DNA-bound AR. This allows the p160 to acetylate histones via its histone acetyltransferase (HAT) activity. Additional coactivators with HAT activity such as CBP, p300, or p/CAF also bind to the p160/AR complex. This results in chromatin remodeling and additional binding of GTFs such as TBp and TIFIIB with the AR coactivation complexes (He *et al.* 2001; Kang *et al.* 2002; Sampson *et al.* 2001; Shang *et al.* 2002; Xu *et al.*). These AR-coordinated complexes regulate the expression of a series of genes resulting in the complex differentiation and growth of the prostate (Coffey 1992). The critical importance of DHT and its receptor in this developmental process has been demonstrated by the fact that the prostate does not develop in males who have inherited either a stop mutation that prevents AR expression (Gottlieb *et al.* 1999) or an inactivating mutation in the type II 5α-reductase gene, thus preventing high prostatic DHT formation (Imperato-McGinley *et al.* 1980), even though serum testosterone levels are normal in individuals with either type of mutation.

12.5 Paracrine androgen axis in the normal prostate

In contrast to the regulation of transcription of the prostate differentiation marker proteins, AR in the nuclei of the secretory luminal cells does not directly regulate the survival of these cells nor does it positively regulate the proliferation and survival of the prostatic epithelial stem and transit amplifying cells. Instead, survival of the secretory luminal cells and the proliferation of the transit amplifying cells requires the androgen-dependent production of peptide growth factors by the prostatic stromal cells (Cunha *et al.* 1987; Hayward *et al.* 1992; Kurita *et al.* 2001). These processes are initiated by testosterone diffusing from the capillary bed in the stromal compartment of the normal prostate across the basement membrane (BM) to enter the basal epithelial cells. These basal cells express 5α-reductase type I and II proteins which enzymatically convert testosterone to 5α-dihydrotestosterone (Bonkhoff *et al.* 1996). Once formed, DHT diffuses both into the secretory luminal cells in the epithelial compartment and also back across the BM to the smooth muscle cells

and fibroblasts in the stromal compartment. Secretory luminal cells also express 5α-reductase type I activity (Bonkhoff *et al.* 1996), thus further increasing their cellular level of DHT above that provided by the basal cells. Within these secretory luminal epithelial cell nuclei, this enhanced level of DHT binds to the AR and directly transcriptionally upregulates the expression of the prostate-specific differentiation markers (PSAP, PSA, hKh2, PSCA, NKX3.1 and PSMA) (Jain *et al.* 2002; Mitchell *et al.* 2000; Ornstein *et al.* 2001; Schuur *et al.* 1996; Watabe *et al.* 2002; Watt *et al.* 2001; Zelivianski *et al.* 2002) and indirectly also vascular endothelial growth factor (VEGF) (Joseph *et al.* 1997). These secretory luminal cells also express transforming growth factor β_1 ($TGFB_1$) (Gerdes *et al.* 1998). These growth factors diffuse across the BM to affect stromal cells. Specifically, VEGF effects the survival of the stromal endothelial cells (Joseph *et al.* 1997) and TGF-ß$_1$ inhibits stromal cell proliferation and induces smooth muscle differentiation and neuronal trophism (Peehl *et al.* 1997; Yang *et al.* 1997).

Binding of DHT to the AR within the nuclei of these stromal smooth muscle cells inhibits their expression of certain cytokines such as $TGF\beta_1$ (Kyprianou and Isaacs 1989; Wikstrom *et al.* 1999) while enhancing their secretion of "andromedins" i.e., androgen-induced stromal peptide growth factors (Lu *et al.* 1999; Planz *et al.* 1999). These andromedins diffuse back across the BM into the epithelial compartment where they interact with their specific cognate plasma membrane receptors of the secretory luminal cells generating intracellular signaling e.g., downregulation of TGβ receptors needed to repress the apoptotic death pathway in the secretory luminal cells (Martikainen *et al.* 1990). Binding of the andromedins to the plasma membrane receptors of the transit amplifying epithelial cells can recruit them into the cell cycle. If a sufficient systemic androgen level is not chronically maintained (e.g., following androgen ablation), then the level of DHT-occupied AR within prostatic stromal cells decreases to a level unable to maintain adequate expression of the stromally derived "andromedins" and unable to repress expression of $TGF\beta_1$ (Kyprianou and Isaacs 1989; Wikstrom *et al.* 1999). Without adequate "andromedins," prostatic transit amplifying epithelial cells remain proliferatively quiescent in G_o and do not enter the cell cycle, while in the prostatic secretory luminal epithelial cells, lack of sufficient andromedins results in the upregulation of expression of type I and II $TGF\beta_1$ receptors (Wikstrom *et al.* 1999). The enhanced levels of $TGF\beta_1$ receptors in these secretory luminal cells are activated by the enhanced levels of $TGF\beta_1$ ligand produced by stromal cells following androgen ablation (Kyprianou and Isaacs 1989; Wikstrom *et al.* 1999). This enhanced $TGF\beta_1$ receptor signaling activates the energy-dependent apoptotic cascade within the secretory luminal cells, inducing their death (Denmeade *et al.* 1996; Kyprianou and Isaacs 1989; Martikainen *et al.* 1990). This apoptotic cascade involves changes

in the intracellular free calcium level, caspase and nuclease activation, and degradation of the secretory luminal cells into apoptotic fragments (Denmeade *et al.* 1996; Kyprianou and Isaacs 1988; Kyprianou *et al.* 1988). Since secretory luminal cells are the source of VEGF production in the prostate, their death results in a lowering of the prostatic VEGF levels (Joseph *et al.* 1997). This lowering of the tissue VEGF level results in the activation of the apoptotic death of a subset of stromal endothelial cells, reducing tissue blood flow (Lissbrant *et al.* 2001).

While secretory luminal cells undergo apoptosis following androgen ablation, the basal stem and transit amplifying cells do not (English *et al.* 1987). A possible explanation for this observation is that prostatic stromal cells express hepatocyte growth factor (HGF) (Gmyrek *et al.* 2001). HGF expression by these stromal cells is not regulated by androgen occupancy of the AR in these stromal cells (Kasai *et al.* 1996). Basal stem and transit amplifying cells constitutively express c-MET, the plasma membrane cognate receptor for HGF, while secretory luminal cells do not (Gmyrek *et al.* 2001). Such c-MET signaling is inhibitory for basal cell apoptosis and proliferation (Gmyrek *et al.* 2001). Thus, following androgen ablation, the prostatic stromal cells continue to supply adequate levels of HGF to bind to and induce signaling by the basal cells' c-MET receptors, thus blocking both activation of apoptosis and inhibiting proliferation of these basal cells (Gmyrek *et al.* 2001).

12.6 Androgen in benign prostatic hyperplasia

When normal prostatic tissue is used to establish in vitro cultures, only the transit amplifying cells (i.e., intermediate cell type) continue to proliferate during the subsequent several passages (Liu and Peehl 2001). Low passage cultures of these transit amplifying cells have a high rate of proliferation (i.e., $\geq$50% proliferation per day) when grown in vitro in serum-free defined media (Chopra *et al.* 1996). Cells in such low passage cultures do not express AR and thus are not affected by adding androgen to the culture media. These cells are dependent, however, upon a critical mixture of peptide growth factor andromedins in the media for their survival and high rate of proliferation (Chopra *et al.* 1996). In contrast to the high proliferation rate in in vitro cultures, only 0.2% of the epithelial cells proliferate per day in normal prostatic tissue in vivo, even though these cells are exposed continuously to maximal physiological levels of andromedins present in non-androgen-ablated hosts (Berges *et al.* 1995). These observations raise the issue of how the in vivo proliferation of the transit amplifying cells becomes restricted to allow only homeostatic renewal and not net continuous prostatic epithelial growth, even though the level of the stromally produced andromedins remains constantly high in the presence of physiologic androgen levels.

One explanation is that AR signaling in the nuclei of the prostatic secretory luminal cells and the subset of AR expressing transit amplifying cells actively inhibits proliferation of these cells even in the presence of continuous andromedin stimulation (Geck *et al.* 1997; Ling *et al.* 2001; Whitacre *et al.* 2002). This mechanism has been documented experimentally using both human (Ling *et al.* 2001) and rodent (Whitacre *et al.* 2002) prostate epithelial cells. These latter studies have demonstrated that when AR negative prostatic epithelial cells are transgenically induced to express AR, and are then exposed to physiological levels of androgen, their in vitro proliferation is profoundly inhibited even in the presence of andromedins with no effect upon cell survival (Ling *et al.* 2001; Whitacre *et al.* 2002). These results demonstrate that for non-malignant prostatic epithelial cells, the ligand-occupied AR functions as a growth suppressor via its ability to inhibit andromedin-induced proliferation. While functioning as a growth suppressor, such AR signaling also induces differentiation of these transit amplifying cells from an intermediate to a secretory luminal cell phenotype (Ling *et al.* 2001; Whitacre *et al.* 2002). This AR-mediated inhibition of andromedin-induced proliferation appears to be related to AR-induced upregulation of the $p27^{Kip1}$ cyclin dependent kinase inhibition protein (Chen *et al.* 1996; Tsihlias *et al.* 2000; Waltregny *et al.* 2001). The mechanism for this upregulation in normal prostatic epithelial cells involves enhanced stability of the $p27^{Kip1}$ protein secondary to AR-induced transcriptional repression of expression of the E3 ubiquitin ligase, Skp2 involved in $p27^{Kip1}$ degradation (Lu *et al.* 2002; Waltregny *et al.* 2001).

In BPH, there is an increase in the cellular content of the transition zone of the prostate. This neoplastic growth could involve: 1) enhanced number of epithelial stem cell units, 2) enhanced number of proliferations by transit amplifying cells before these mature into non-proliferating luminal secretory cells, and/or 3) decreased ability of AR to limit the proliferation of luminal secretory cells. BPH characteristically is also associated with an enhanced number of stromal cells. Since at least a subset of these stromal cells express AR and thus andromedins, androgen regulation within these stromal cells may be abnormal, leading to enhanced andromedin production. Theoretically, in order to inhibit such enhanced andromedin production, androgen ablation could be utilized to treat BPH. Unfortunately, such systemic androgen ablation has other unacceptable side effects on bone density, muscle mass, and libido. For these reasons, BPH is often treated medically with 5α-reductase inhibitors like the 5α-reductase type II inhibitor, finasteride or the dual type I and II inhibitor, dutasteride (Foley and Kirby 2003). In this way, testosterone levels are not lowered even though prostatic DHT is lowered and the stromal andromedin production is also lowered but without the other side effects. Indeed, such 5α-reductase inhibition does reduce the size of the prostate by ≈25%, even though systemic androgen levels are not decreased (Foley and Kirby 2003).

12.7 Conversion of paracrine to autocrine mechanism of androgen action during prostatic carcinogenesis

While it is clear that prostate cancer arises from the epithelial compartment, the identification of the specific epithelial cell subtype which the carcinogenic process initiates has only recently been the focus of study. Currently, the precursor for most peripheral zone prostatic carcinomas is thought to be high-grade prostatic intraepithelial neoplasia (HGPIN) (McNeal and Bostwick 1986). It is believed that HGPIN arises from low-grade PIN, which in turn is thought to stem from normal prostate epithelium. The cell type of origin for HGPIN, however, is still incompletely understood. A widely held view of carcinogenesis is that the common carcinomas generally arise in self-renewing tissues in which dividing cells acquire somatic genetic alterations in growth regulatory genes. In normal human prostate epithelium, most cell divisions take place in the basal cell compartment where the tissue stem and presumably the transit amplifying cells reside (Bonkhoff *et al.* 1994; 1998). The majority of secretory luminal cells do not normally proliferate and are the terminally differentiated cells that perform the androgen-regulated differentiated functions of the prostate, such as prostate-specific antigen (PSA) production and secretion. Both prostate cancer and HGPIN cells possess many phenotypic and morphological features of secretory luminal cells (i.e., cytokeratin 8 and 18, PSA, hK_2, PSMA, and AR expression), yet they also contain features of the basal transit amplifying cell compartment such as c-MET expression, DNA replication and extensive self renewal (De Marzo *et al.* 1998; Meeker *et al.* 2002; van Leenders *et al.* 2002; Verhagen *et al.* 1992) (Fig.12.2). Thus, in carcinoma these stem-cell and transit amplifying cell-like features have been shifted up from the basal into the secretory luminal compartment (De Marzo *et al.* 1998a; Meeker *et al.* 2002). It has been postulated that the cell of origin for prostate cancer is an intermediate, prostatic epithelial cell, presumably derived from the basal transit amplifying population which undergoes the initial malignant molecular changes allowing gene expression and morphologic features of both basal and secretory luminal cells (De Marzo *et al.* 1998a; De Marzo *et al.* 1998b; Meeker *et al.* 2002; van Leenders *et al.* 2002; Verhagen *et al.* 1992).

The site of these phenotypically intermediate, initiated cells appears not to be random within the prostate. Instead, they are enriched in sites of focal glandular atrophy where the luminal epithelial cells, atrophic in appearance, are quite proliferative and often surrounded by inflammation within the gland. Therefore, these sites have been termed "proliferative inflammatory atrophy" (PIA)(De Marzo *et al.* 1999). Based upon the following lines of evidence, these PIA lesions are proposed to be an intermediate transition stage to HGPIN and/or early prostatic carcinoma: 1.) Compared with normal-appearing epithelium, PIA is highly proliferative. 2.) PIA

contains many proliferating cells in the luminal layer, which is similar to PIN. 3.) Many of the luminal cells in PIA have decreased expression of the $p27^{Kip1}$ cyclin-dependent kinase inhibitor even though they express AR. 4.) PIA contains many cells with phenotypic features of "intermediate cells," which have been proposed as the target cells for carcinogenesis in the prostate. 5.) PIA contains very few cells undergoing apoptosis, with many cells in the luminal layer expressing bcl-2. 6.) PIA shows increased expression in the carcinogen-detoxifying enzyme, glutathione-S-transferase Pi (GSTP1), and GST alpha in many of the cells, consistent with a stress response to an increased oxidative burden. 7.) Finally, PIA shows frequent morphologic transitions to PIN and frequently occurs adjacent to small cancers (De Marzo *et al.* 2001).

Based on these findings, a new model of prostate carcinogenesis has been proposed whereby chronic and acute inflammation, in conjunction with dietary and other environmental factors, targets prostate epithelial cells for injury and destruction. Increased proliferation occurs as a regenerative response to lost epithelial cells; it occurs in cells with a transit amplifying or intermediate phenotype (Meeker *et al.* 2002; van Leenders *et al.* 2002). In this process, GSTP1 expression is elevated in many of the cells in PIA as a genome protective measure. Although elevated in many of the cells in PIA, GSTP1 expression is eventually lost in some cells as the result of aberrant methylation of the CpG island of the GSTP1 gene promoter (Lin *et al.* 2001). Indeed, such aberrant methylation of the GSTP1 promoter is one of the earliest molecular abnormalities characteristic of prostate cancer cells. This heritable epigenetic alteration places these cells at increased risk for the accumulation of additional genetic damage, with acceleration of the neoplastic process toward PIN (Lin *et al.* 2001). One of these additional genetic changes involves telomerase shortening by PIN cells. This appears to increase their genetic instability, driving further genetic damage and producing invasive cancers (Meeker *et al.* 2002).

During the initiation of prostate carcinogenesis, there are distinct "hard wiring" changes in the AR signaling pathways. Normally the proliferating transit amplifying cells in the basal epithelial layer do not express the androgen receptor or express only low levels of AR. As discussed, during their maturation, these cells eventually express higher levels of AR. Once a critical AR level is reached, the occupancy of AR by its ligand inhibits proliferation of these cells and induces their differentiation into secretory luminal cells. In contrast, the intermediate type of proliferating cells in PIA variably express higher levels of AR and such AR expression is further enhanced in proliferating cells in HGPIN (De Marzo *et al.* 2001). Associated with this enhanced expression of the AR is the decreased expression of ERβ by HGPIN cells (Fixemer *et al.* 2003). This indicated that "hard wiring" changes occur in the AR/ERβ signaling pathways even at this early stage of cancer development since now AR expressing/ERβ negative cells are proliferating and not growth arrested.

These changes produce a "gain of function" ability by AR so that it now engages the molecular signaling pathways directly, stimulating the proliferation and survival of these initiated prostatic cells. Unlike the paracrine situation in the normal prostate in which such growth regulation is initiated by AR binding to genomic sequences in the nuclei of stromal cells, during prostatic carcinogenesis genomic AR binding within the transformed cells itself activates this growth regulation. Due to these "hard wiring" changes, there is a conversion from paracrine to autocrine AR signaling pathways in invasive prostate cancer (Gao and Isaacs 1998; Gao *et al.* 2001). These "gain of function" hard wiring changes pathologically allow androgen/AR complexes to bind to and enhance expression of survival and proliferation genes which physiologically are not affected by these complexes in either normal transit amplifying or secretory luminal cells (Gao and Isaacs 1998; Gao *et al.* 2001). In addition, such gain of function AR oncogenic signaling no longer represses but instead stimulates Skp2 expression. Such Skp2 enhanced expression results in downregulation of $p27^{Kip1}$ protein, enhancing proliferation of these cancer cells (Yang *et al.* 2002).

12.8 Role of androgen in prostate cancer

Even with these "hard wiring" changes, activation of these pathological growth-promoting (i.e., oncogenic) pathways can still be dependent upon the binding of androgen to its receptor in the nuclei of these neoplastic cells themselves (i.e., androgen and AR-dependent), or they can be constitutive (i.e., independent of the binding of physiological androgens to the receptors), but still requiring AR functioning in the nuclei of these malignant cells to enhance the transcription of both secretory markers and also growth-promoting genes, (i.e., androgen independent but still AR dependent).

In order to appreciate the therapeutic relevance of these mechanistic distinctions, an understanding of the cellular heterogeneity and responsiveness of prostate cancer cellular subtypes is required. Androgen ablation therapy, whether by surgical or medical means, induces the elimination of only testosterone-dependent prostate cancer cells since these cells require a critical level of physiological androgen for their continuous proliferation and survival (Gao and Isaacs 1998; Gao *et al.* 2001; Kyprianou *et al.* 1990). Unfortunately, androgen ablation is not curative because, once clinically detected, prostate cancers are heterogeneously composed of clones of androgen-dependent cancer cells and also malignant clones which are androgen-independent (Isaacs 1999). These latter cells are androgen-independent since androgen occupancy of their nuclear AR is not required for their survival (Isaacs 1999). There are two basic subtypes of such androgen-independent prostate cancer cells. One subtype retains a sensitivity to androgen occupancy of its nuclear

AR to enhance its rate of cell proliferation even though such occupancy is not required for its survival. Thus, these cells are androgen-independent/sensitive since their rate of growth is inhibited but not prevented by androgen ablation. The other subtype is termed androgen independent/insensitive since androgen ablation decreases neither their rate of proliferation nor survival (Isaacs 1999).

These last two subtypes of malignant clones are not eliminated by standard androgen ablation and thus these are the malignant cells that eventually kill the patient (Isaacs 1999). It had been assumed that following androgen ablation, such androgen-independent/insensitive prostate cancer cells no longer express androgen receptor and that in such androgen-independent/sensitive cells, the expressed AR had no function in regulating survival. This assumption was based upon earlier observations that the majority of serially passaged rodent and human (i.e., PC-3, DU-145) in vitro cell lines established from androgen ablation-failing hosts consistently did not express AR. In contrast to this experimental situation, more than 90% of prostatic cancers obtained directly from patients failing androgen ablation actually overexpress AR (Hobisch *et al.* 1996; Linja *et al.* 2001; Taplin *et al.* 1999). In approximately 30% of such progressing prostatic cancer, this overexpression is associated with genetic amplification (Brown *et al.* 2002; Hyytinen *et al.* 2002) and, in 10–40%, with AR mutations (Buchanan *et al.* 2001; Hyytinen *et al.* 2002; Taplin *et al.* 2003). These clinical results strongly implicate continual involvement of AR in stimulation of proliferation and/or inhibition of death even in ligand (i.e., androgen) independent prostate cancer cells resistant to androgen ablation. This is supported by a growing body of experimental studies using prostate cancer model systems which have documented that manipulations which interfere with AR expression, nuclear translocation, and/or appropriate genomic binding inhibit proliferation and induce apoptosis of ligand-independent (i.e., androgen ablation resistant) AR expressing prostatic cancer cells (Chen *et al.* 1998; Eder *et al.* 2002; Zegarra-Moro *et al.* 2002). Thus, targeted inhibition of these ligand-independent-AR signaling pathways should provide rational drug development for androgen ablation resistant prostatic cancers (Litvinov *et al.* 2003).

12.9 Side effects of androgen replacement/ablation in the aging male

Besides its effects upon normal and abnormal growth and physiology of the prostate, testosterone is also critical for maintenance of bone and muscle metabolism, as well as libido. For this reason, aging males who have an insufficient level of serum testosterone (i.e. hypogonadal males) suffer clinically from loss of bone and muscle mass as well as a decreased libido. Such aging hypogonadal males are candidates for exogenous testosterone replacement. Due to its growth-promoting effects on the prostate, however, such hormonal replacement therapy could enhance the

development of BPH and/or prostate cancer. Thus, the decision to initiate such replacement hormonal therapy must be evaluated on risk vs. benefit analysis for each patient individually.

The side effects observed in naturally developed hypogonadal males are also a problem in males either at high risk of developing prostate cancer who are treated with androgen ablation therapy as a preventative modality or in patients with clinically established prostate cancer who are being given androgen ablation as therapy. In order to allow replacement hormonal therapy in hypogonadal patients and lessen side effects of such testosterone ablative therapies in patients with established prostate cancer, small molecule selective androgen response modifiers (SARMs) are being developed which retain the positive androgenic effects on bone, muscle, and libido, but which have little or no growth-stimulatory effects on the prostate. This approach is possible due to the increasing basic knowledge about the mechanism(s) of such androgenic effects at the molecular level (Litvinov and Isaacs 2003). These molecular studies have documented that the binding of natural and synthetic SARMs induces a spectrum of conformational changes in the androgen receptor. This spectrum of conformations results in differential ability of the SARM-occupied AR to dimerize and bind to specific target genes and specific transcriptional cofactors inducing either stimulation or repression of transcription. Thus, the development of such SARMs will usher in an exciting time during which clinical testing will determine whether modulating testosterone's effects will have an impact on the prevention and treatment of multiple diseases of the aging male.

12.10 Key messages

- Testosterone is the major growth and functional regulator of the prostate.
- The prostate is organized functionally in stem cell units composed of stem cells, transit amplifying (TA) cells, intermediate cells and secretory luminal cells.
- Testosterone is metabolized within the prostate to both more potent androgens (i.e., DHT) and to an estrogenic metabolite (i.e., *3β diol*).
- DHT binds to the androgen receptor within prostatic stromal cells to induce the production and secretion of growth factors known as andromedins.
- Andromedins stimulate the proliferation of TA cells and the survival of TA, intermediate and secretory luminal cells (i.e., paracrine androgen axis).
- DHT binds to the androgen receptor in secretory luminal cells and directly induces the transcription of prostate differentiation markers, such as PSA, hK_2 and PAP.
- Prostate cancers are derived from TA/intermediate cells. During prostatic carcinogenesis, molecular changes induce a conversion from a paracrine to an autocrine pathway so that the AR then directly stimulates the proliferation and survival of prostate cancer cells.
- Approaches to lower testosterone's stimulating abilities should have both preventative and treatment effect on prostatic cancer and BPH.

- Besides its effects upon normal and abnormal growth and physiology of the prostate, testosterone is also a critical regulator of bone and muscle metabolism, as well as libido. Therefore, therapies which reduce testosterone's effects on the development and clinical progression of either BPH or prostate cancer have major side effects upon quality of life.
- In addition, there are aging males who suffer from abnormally low serum testosterone levels with similar quality-of-life side effects. These patients can be supplemented with exogenous testosterone, but this can enhance the risk of developing BPH and prostate cancer.
- To allow replacement therapy in patients with low serum testosterone and lessen side effects of testosterone, ablative therapies in patients with established prostate cancer, small molecule selective androgen response modifiers (SARMs) are being developed which retain the positive androgenic effects on bone, muscle, and libido, but which have little or no growth stimulatory effects on the prostate.

12.11 REFERENCES

Andersson S, Russell DW (1990) Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. Proc Natl Acad Sci USA 87:3640–3644

Berges RR, Vukanovicm, J Epstein, JI CarMichel M, Cisek L, Johnson DE, Veltri RW, Walsh PC, Isaacs JT (1995) Implication of the cell kinetic changes during the progression of human prostatic cancer. Clin Can Res 1:473–480

Biswas MG, Russell DW (1997) Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate. J Biol Chem 272:15959–15966

Berry SJ, Coffey DS, Walsh PC, Ewing LL (1984) The development of human benign prostatic hyperplasia with age. J Urol 132:474–479

Bonkhoff H (1998) Neuroendocrine cells in benign and malignant prostate tissue: morphogenesis proliferation and androgen receptor status. Prostate Suppl 8:18–22

Bonkhoff H, Remberger K (1996) Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth – a stem cell model. Prostate 28:98–106

Bonkhoff H, Stein U, Remberger K (1994a) Multidirectional differentiation in the normal hyperplastic and neoplastic human prostate: simultaneous demonstration of cell-specific epthelial markers. Hum Pathol 25:42–46

Bonkhoff H, Stein U, Remberger K (1994b) The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate 24:114–118

Bonkhoff H, Stein U, Aumüller G, Remberger K (1996) Differential expression of 5 alpha-reductase isoenzymes in the human prostate and prostatic carcinomas. Prostate 29:261–267

Bonkhoff H, Fixemer T, Remberger K (1998) Relation between Bcl-2 cell proliferation and the androgen receptor status in prostate tissue and precursors of prostate cancer. Prostate 34: 251–258

Boyd E (1962) Growth including reproduction and morphological development. In: Altman and Dittmer (eds) Biological Handbooks Federation of American Societies for Experimental Biology, Washington DC, pp 346–348

Brown RS, Edwards J, Dogan A, Payne H, Harland SJ, Bartlett JM, Masters JR (2002) Amplification of the androgen receptor gene in bone metastases from hormone-refractory prostate cancer: J Pathol 198:237–244

Bruchovsky N, Wilson JD (1968) The conversion of testosterone in 5 α-androstan-17-β-ol-3-one by the rat prostate in vivo and in vitro. J Biol Chem 243:2012–2021

Buchanan G, Greenberg NM, Scher HI, Harris JM, Marshall VR, Tilley WD (2001) Collocation of androgen receptor gene mutations in prostate cancer. Clin Cancer Res 7:1273–1281

Chadli A, Bouhouche I, Sullivan W, Stensgard B, McMahon N, Catelli MG, Toft DO (2000) Dimerization and N-terminal domain proximity underlie the function of the molecular chaperone heat shock protein 90. Proc Natl Acad Sci USA 97:12524–12529

Chen Y, Robles AI, Martinez LA, Liu F, Gimenez-Conti IB, Conti CJ (1996) Expression of G1 cyclins cyclin-dependent kinases and cyclin-dependent kinase inhibitors in androgen-induced prostate proliferation in castrated rats. Cell Growth Differ 7:1571–1578

Chen S, Song CS, Lavrovsky Y, Bi B, Vellanoweth R, Chatterjee B, Roy AK (1998) Catalytic cleavage of the androgen receptor messenger RNA and functional inhibition of androgen receptor activity by a hammerhead ribozyme. Mol Endocrinol 12:1558–1566

Chopra DP, Grignon DJ, Joiakim A, Mathieu PA, Mohamed A, Sakr WA, Powell IJ, Sarkar FH (1996) Differential growth factor responses of epithelial cell cultures derived from normal human prostate benign prostatic hyperplasia and primary prostate carcinoma. J Cell Physiol 169:269–280

Coffey DS (1992) The molecular biology endocrinology and physiology of the prostate and seminal vesicles. In: Walsh PC, Retik AB, Stamey TA, Vaughan ED (eds) Campbell's Textbook of Urology. Saunders, Philadelphia, pp 221–266

Collins AT, Habib FK, Maitland NJ, Neal DE (2001) Identification and isolation of human prostate epithelial stem cells based on $\alpha_2\beta_1$-integrin expression. J Cell Sci 114:3865–3872

Cunha GR, Donjacour AA, Cooke PS, Mee S, Bigsby RM, Higgins SJ, Sugimura Y (1987) The endocrinology and developmental biology of the prostate. Endocr Rev 8:338–362

De Marzo AM, Meeker AK, Epstein JI, Coffey DS (1998a) Prostate stem cell compartments: expression of the cell cycle inhibitor p27kip1 in normal hyperplastic and neoplastic cells. Am J Pathol 153:911–919

De Marzo AM, Nelson WG, Meeker AK, Coffey DS (1998b) Stem cell features of benign and malignant prostate epithelial cells. J Urol 160:2381–2392

De Marzo AM, Marchi VL, Epstein JI, Nelson WG (1999) Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am J Pathol 155:1985–1992

De Marzo AM, Putzi MJ, Nelson WG (2001) New concepts in the pathology of prostatic epithelial carcinogenesis. Urology 57(Suppl 1):103–114

Denmeade SR, Lin XS, Isaacs JT (1996) Role of programmed (apoptotic) cell death during the progression and therapy of prostate cancer. Prostate 28:251–265

Eder IE, Hoffmann J, Rogatsch H, Schafer G, Zopf D, Bartsch G, Klocker H (2002) Inhibition of LNCaP prostate tumor growth in vivo by an antisense oligonucleotide directed against the human androgen receptor. Cancer Gene Ther 9:117–125

English HF, Santen RJ, Isaacs JT (1987) Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. Prostate 11:229–242

Fixemer T, Remberger K, Bonkhoff H (2003) Differential expression of the estrogen receptor beta (ERbeta) in human prostate tissue premalignant changes and in primary metastatic and recurrent prostatic adenocarcinoma. Prostate 54:79–87

Foley CL, Kirby RS (2003) 5 alpha-reductase inhibitors: what's new? Curr Opin Urol 13:31–37

Frasier SD, Gafford F, Horton RD (1969) Plasma androgens in childhood and adolescence. J Clin Endocrinol Metab 29:1404–1408

Gao J, Isaacs JT (1998) Development of an androgen receptor null model for identifying the site of initiation for androgen stimulation of proliferation and suppression of programmed (apoptotic) death of PC-82 human prostate cancer cells. Cancer Res 58:3299–3306

Gao J, Arnold JT, Isaacs JT (2001) Conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth during malignant transformation of prostatic epithelial cells. Cancer Res 61:5038–5044

Geck P, Szelei J, Jimenez J, Lin TM, Sonnenschein C, Soto AM (1997) Expression of novel genes linked to the androgen-induced proliferative shutoff in prostate cancer cells. J Steroid Biochem Mol Biol 63:211–218

Gerdes MJ, Larsen M, McBride L, Dang TD, Lu B, Rowley DR (1998) Localization of transforming growth factor-beta1 and type II receptor in developing normal human prostate and carcinoma tissues. J Histochem Cytochem 46:379–388

Gmyrek GA, Walburg M, Webb CP, Yu HM, You X, Vaughan ED, Van Woude GF, Knudsen BS (2001) Normal and malignant prostate epithelial cells differ in their response to hepatocyte growth factor/scatter factor. Am J Pathol 159:579–590

Gottlieb B, Vasiliou DM, Lumbroso R, Beitel LK, Pinsky L, Trifiro MA (1999) Analysis of exon 1 mutations in the androgen receptor gene. Hum Mutat 14:527–539

Hayward SW, Del Buono R, Deshpande N, Hall PA (1992) A functional model of adult human prostate epithelium: The role of androgens and stroma in architectural organization and the maintenance of differentiated secretory function. J Cell Sci 102:361–372

He B, Bowen NT, Minges JT, Wilson EM (2001) Androgen-induced NH2- and COOH-terminal interaction inhibits p160 coactivator recruitment by activation function 2. J Biol Chem 276:42293–42301

Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A (1996) Androgen receptor status of lymph node metastases from prostate cancer: Prostate 28:129–135

Hudson DL, O'Hare M, Watt FM, Master JR (2000) Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. Lab Invest 80:1243–1250

Huggins C, Johnson MA (1947) Carcinoma of the bladder and prostate. JAMA. 135:1146–1152

Hyytinen ER, Haapala K, Thompson J, Lappalainen I, Roiha M, Rantala I, Helin HJ, Janne OA, Vihinen M, Palvimo JJ, Koivisto PA (2002) Pattern of somatic androgen receptor gene mutations in patients with hormone-refractory prostate cancer. Lab Invest 82:1591–1598

Imperato-McGinley J, Peterson RE, Leshin M, Griffen JE, Cooper G, Draghi S, Berenyi M, Wilson JD (1980) Steroid 5α-reductase deficiency in a 65-year-old male pseudohermaphrodite: The natural history ultrastructure of the testes and evidence for inherited enzyme heterogeneity. J Clin Endocrinol Metab 50:15–21

Isaacs JT (1987) Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. In: Rogers CH, Coffey DS, Cunha G, Grayhack J, Hinman F, Horton R (eds) Benign prostatic hyperplasia Report No INH 87-2881. Department of Health and Human Services, National Institutes of Health Bethesda MD pp 85–94

Isaacs JT (1994) Role of androgens in prostatic cancer. Vitam Horm 49:433–502

Isaacs JT (1999) The biology of hormone refractory prostate cancer: Why does it develop? In: Urologic Clinics of North America: WB Saunders Company, Philadelphia PA, Vol 26 pp 263–273

Isaacs JT, Coffey DS (1989) Etiology of BPH. Prostate, Supplement 2:33–50

Isaacs JT (1984a) Common characteristics of human and canine benign prostatic hyperplasia. In: Kimball FA (ed), New approaches to the study of benign prostatic hyperplasia. AR Liss Inc New York, pp 217–234

Isaacs JT (1984b) Antagonistic effect of androgens on prostatic cell death. Prostate 5:545–557

Isaacs JT, Brendler CB, Walsh PC (1983) Changes in the metabolism of dihydrotestosterone in the hyperplastic human prostate. J Clin Endocrinol Metab 56:139–146

Isaacs JT, McDermott IR, Coffey DS (1979) The identification and characterization of a new $C_{19}O_3$ steroid metabolite in the rat ventral prostate: 5α-androstane-3β 6α-17β-trial. Steroids 33:639–675

Jain A, Lam A, Vivanco I, Carey MF, Reiter RE (2002) Identification of an androgen-dependent enhancer within the prostate stem cell antigen gene. Mol Endocrinol 16:2323–2337

Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ (2003) Cancer statistics 2003. CA Cancer J Clin 53:5–26

Jenkins EP, Andersson S, Imperato-McGinley J, Wilson JD, Russell DW (1992) Genetic and pharmacologic evidence for more than one human steroid 5α-reductase. J Clin Invest 89:293–300

Jenkins EP, Hsieh C-L, Milatovich A, Normington K, Berman DM, Franke U, Russel PW (1991) Characterization and chromosomal mapping of a human steroid 5 α-reductase gene and pseudogene and mapping of the mouse homologue. Genomics 11:1102–1112

Joseph IB, Nelson JB, Denmeade SR, Isaacs JT(1997) Androgens regulate vascular endothelial growth factor content in normal and malignant prostatic tissue. Clin Cancer Res 3:2507–2511

Kang Z, Pirskanen A, Janne OA, Palvimo JJ (2002) Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277:48366–48371

Kasai S, Sugimura K, Matsumoto K, Nozomu N, Kishimoto T, Nakamura T (1996) Hepatocyte growth factor is a paracrine regulator of rat prostate epithelial growth. Biochem Biophys Res Communications 228:646–652

Kurita T, Wany YZ, Donjacour AA, Zhao C, Lydon JP, O'Malley BW, Isaacs JT, Dahiya R, Cunha GR (2001) Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system. Cell Death Differ 8:192–200

Kyprianou N, English H, Isaacs JT (1990) Programmed cell death during regression of the PC-82 human prostate cancer following androgen ablation. Cancer Res 50:3748–3752

Kyprianou N, English HF, Isaacs JT (1988) Activation of a Ca^2+-Mg^{2+}-dependent endonuclease as an early event in castration-induced prostatic cell death. Prostate 13:103–117

Kyprianou N, Isaacs JT (1987) Quantal relationship between prostatic dihydrotestosterone and prostatic cell content: Critical threshold concept. Prostate 11:41–50

Kyprianou N, Isaacs JT (1988) Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 122:552–562

Kyprianou N, Isaacs JT (1989) Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Mol Endocrinol 3:1515–1522

Labrie F, Sugimoto Y, Luu-The Simand J, Lachance YI, Bachuarov D, Leblanc J, Durocher F, Paquet N (1992) Structure of human type II 5α-reductase gene. Endocrinology 131:1571–1573

Levy MA, Brandt M, Heys JR, Holt DA, Metcalf BW (1990) Inhibition of rat liver steroid 5α-reductase by 3-androstene-3-carboxylic acids: Mechanism of enzyme-inhibitor interaction. Biochemistry 29:2815–2824

Liao S, Leong T, Tymocyko JL (1972) Structural recognition in interactions of androgens and receptor proteins and in their association with nuclear components. J Steroid Biochem 3:401–407

Lin X, Tascilar M, Lee WH, Vles WJ, Lee BH, Veeraswamy R, Asgari K, Freije D, van Rees B, Gage WR, Bova GS, Isaacs WB, Brooks JD, de Weese TL, De Marzo AM, Nelson WG (2001) GSTP1 CpG island hypermethylation is responsible for the absence of GSTP1 expression in human prostate cancer cells. Am J Pathol 159:1815–1826

Ling MT, Chan KW, Choo CK (2001) Androgen induces differentiation of a human papillomavirus 16 E6/E7 immortalized prostate epithelial cell line. J Endocrinol 170:287–296

Linja MJ, Savinainen KJ, Saramaki OR, Tammela TLJ, Vessella RL, Visakorpi T (2001) Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 61:3550–3555

Lissbrant IF, Lissbrant E, Damber JE, Bergh A (2001) Blood vessels are regulators of growth diagnostic markers and therapeutic targets in prostate cancer. Scand J Urol Nephrol 35:437–452

Litvinov IV, DeMarzo AM, Isaacs JT (2003) Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 88:2972–2982

Liu AY, Peehl DM (2001) Characterization of cultured human prostatic epithelial cells by cluster designation antigen expression. Cell Tissue Res 305:389–397

Liu H, Moy P, Kim S, Xia Y, Rajasekaran A, Navarro V, Knudsen B, Bander NH (1997) Monoclonal antibodies to the extracellular domain of prostate-specific membrane antigen also react with tumor vascular endothelium. Cancer Res 57:3629–3634

Lu L, Schulz H, Wolf DA (2002) The F-box protein SKP2 mediates androgen control of p27 stability in LNCaP human prostate cancer cells. BMC Cell Biology 3:22

Lu W, Luo Y, Kan M, McKeehan WL (1999) Fibroblast growth factor-10: second candidate stromal to epithelial cell andromedin in prostate. J Biol Chem 274:12827–12834

Mann T, Mann CL (1981) Male reproductive function and semen. Springer-Verlag New York

Martikainen P, Kyprianou N, Isaacs JT (1990) Effect of transforming growth factor-β_1 on proliferation and death of rat prostatic cells. Endocrinology 127:2963–2968

McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LWK, Hsieh JT, Tu SM, Campbell ML (1992) Expression of the protooncogene Bcl-2 in the prostate and association with emergence of androgen-independent prostate cancer. Cancer Res 52:6940–6944

McNeal JE, Bostwick DG (1986) Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 17:64–71

McNeal JE, Redwine EA, Freiha FS, Stamey TA (1988) Zonal distribution of prostatic adenocarcinoma. Am J Surg Pathol 12:897

Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ, DeMarzo AM (2002) Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. Cancer Res 62:6406–6409

Mitchell SH, Murtha PE, Zhang S, Zhu W, Young CYF (2000) An androgen response element mediates LNCaP cell dependent androgen induction of the hK2 gene. Mol Cell Endocrinol 168:89–99

Moore RA (1944) Benign hypertrophy and carcinoma of the prostate: Occurrence and experimental production in animals. Surgery 16:152–167

Normington K, Russell DW (1992) Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozymes. J Biol Chem 267:19548–19554

Ornstein DK, Cinquanta M, Weiler S, Duray PH, Emmert-Buck MR, Vocke CD, Linehan WM, Ferretti JA (2001) Expression studies and mutational analysis of the androgen regulated homeobox gene NKX31 in benign and malignant prostate epithelium. J Urol 165:1329–1334

Peehl Dmand, Sellers RG (1997) Induction of smooth muscle cell phenotype in cultured human prostatic stromal cells. Experimental Cell Res 232:208–215

Planz B, Aretz HT, Wang Q, Tabatabaei S, Kirley SD, Lin CW, McDougal WS (1999) Immunolocalization of the keratinocyte growth factor in benign and neoplastic human prostate and is relation to androgen receptor. Prostate 41:233–242

Plymate SR, Tenover JS, Brenner WJ (1989) Circadian variation in testosterone sex hormone-binding globulin and calculated non-sex hormone-binding globulin bound testosterone in healthy young and elderly man. J Androl 10:366–371

Price D, Williams-Ashman GH (1961) The accessory reproductive glands of mammals In: Young WC (ed) Sex and internal secretions 3rd ed. Williams & Wilkins, Baltimore MD, pp 366–488

Rizner TL, Lin HK, Peehl DM, Steckelbroeck S, Bauman DR, Penning TM (2003) Human type 3 3α-hydroxysteroid dehydrogenase (aldo-keto reductase 1C2) and androgen metabolism in prostate cells. Endocrinology 144:2922–2932

Robinson EJ, Neal DE, Collins AT (1998) Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostate epithelium. Prostate 37:149–160

Rumpold H, Heinrich E, Untergasser G, Hermann M, Pfister G, Plas E, Berger P (2002) Neuroendocrine differentiation of human prostatic primary epithelial cells in vitro. Prostate 53:101–108

Sampson ER, Yeh SY, Chang HC, Tsai MY, Wang X, Ting HJ, Chang C (2001) Identification and characterization of androgen receptor associated coregulators in prostate cancer cells. J Biol Regul Homeost Agents 15:123–129

Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR (1996) Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 271:7043–7051

Shang Y, Myers M, Brown M (2002) Formation of the androgen receptor transcription complex. Mol Cell 9:601–610

Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, Yang A, Montironi R, McKeon F, Loda M (2000) p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 157:1769–1775

Silver SJ, Balmacoda J, Borrero C (1988) Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. Fertil Steril 50:525–530

Taplin ME, Bubley GJ, Ko YJ, Small EJ, Upton M, Rajeshkuma B, Balk SP (1999) Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 59:2511–2515

Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J, Stadler W, Hayes DF, Kantoff PW, Vogelzang NJ, Small EJ; Cancer and Leukemia Group B Study 9663 (2003) Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol 21:2673–2678

Thigpen AE, Davis DL, Milatovich A, Mendonca BD, Imperato-McGinley J, Griffin J, Franke U, Wilson JD, Russel DW (1992) Molecular genetics of steroid 5α-reductase 2 deficiency. J Clin Invest 90:799–809

Torn S, Nokelainen P, Kurlela R, Pulkka A, Menjivar M, Ghosh S, Coca-Prados M, Peltoketo H, Isomaa V, Vihko P (2003) Production purification and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. Biochem and Biophys Res Commun 305:37–45

Tsihlias J, Zhang W, Bhattacharya N, Flanagan M, Klotz L, Slingerland J (2000) Involvement of p27Kip1 in G1 arrest by high dose 5α-dihydrotestosterone in LNCaP human prostate cancer cells. Oncogene 19:670–679

Van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiter D, Schalken J (2000) Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy. Lab Invest 80:1251–1258

Van Leenders G, van Balken B, Aalders T, Hulsbergen-van de Kaa C, Ruiter D, Schalken J (2002) Intermediate cells in normal and malignant prostate epithelium express c-MET: Implications for prostate cancer invasion. Prostate 51:98–107

Verhagen AP, Ramaekers FC, Aalders TW, Schaafsma HE, Debruyne FM, Schalken JA (1992) Colocalization of basal and luminal cell-type cytokeratins in human prostate cancer. Cancer Res 52:6182–6187

Waltregny D, Leav I, Signoretti S, Soung P, Lin D, Merk F, Adams JY, Bhattacharya N, Cirenei N, Loda M (2001) Androgen-driven prostate epithelial cell proliferation and differentiation in vivo involve the regulation of p27. Mol Endocrinol 15:765–782

Watabe T, Lin M, Ide H, Donjacour AA, Cunha GR, Witte ON, Reiter RE (2002) Growth regeneration and tumorigenesis of the prostate activates the PSCA promoter. Proc Natl Acad Sci USA 99:401–406

Watt F, Martorana A, Brookes DE, Ho T, Kingsley E, O'Keefe DS, Russell PJ, Heston WD, Molloy PL (2001) A tissue-specific enhancer of the prostate-specific membrane antigen gene folh1. Genomics 73:243–254

Weihua Z, Makela S, Andersson LC, Salmi S, Saji S, Webster JI, Jenson EV, Nilsson S, Warner M, Gustafsson J-A (2001) A role for estrogen receptor beta in the regulation of growth of the ventral prostate. Proc Natl Acad Sci USA 98:6330–6335

Weihua Z, Lathe R, Warner M, Gustafsson J-A (2002) An endocrine pathway in the prostate ERβ AR 5α-androstane-3β 17β-diol and CYP7B1 regulates prostate growth. Proc Natl Acad Sci USA 99:13589–13594

Whitacre DC, Chauhan S, Davis T, Gordon D, Cress AE, Miesfeld RL (2002) Androgen induction of in vitro prostate cell differentiation. Cell Growth & Differ 13:1–11

Wikstrom P, Westin P, Stattin P, Damber JE, Bergh A (1999) Early castration-induced upregulation of transforming growth factor beta1 and its receptors is associated with tumor cell apoptosis and a major decline in serum prostate-specific antigen in prostate cancer patients. Prostate 38:268–277

Wilson JD (1984) The endocrine control of sexual differentiation. Harvey Lect 79:145–172

Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai M-J, O'Malley BW (1998) Partial hormone resistance in mice with disruption of the steroid receptor coativator-1 (SRC-1) gene. Science 279:1922–1925

Yang G, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, Thompson TC, Harper JW (2002) Elevated Skp2 protein expression in human prostate cancer: Association with loss of the cyclin-dependent kinase inhibitor p27 and PTEn and with reduced recurrence-free survival. Clin Cancer Res 8:3419–3426

Yang G, Timme TL, Park SH, Thompson TC (1997) Transforming growth factor beta 1 transduced mouse prostate reconstitutions: I Induction of neuronal phenotypes. Prostate 33:151–156

Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ (2002) Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. Cancer Res 6:1008–1013

Zelivianski S, Igawa T, Lim S, Taylor R, Lin M (2002) Identification and characterization of regulatory elements of the human prostatic acid phosphatase promoter Oncogene 21:3696–3705

13

Clinical uses of testosterone in hypogonadism and other conditions

E. Nieschlag and H.M. Behre

Contents

13.1 Use of testosterone in male hypogonadism

The primary clinical use of testosterone is substitution therapy of male hypogonadism. Hypogonadism may be caused by hypothalamic, pituitary, testicular or

Table 13.1 Overview of disorders with male hypogonadism classified according to localisation of cause

Hypothalamic-pituitary origin (hypogonadotropic syndromes = secondary hypogonadism)
Idiopathic hypogonadotropic hypogonadism (IHH)
including Kallmann syndrome
Congenital adrenal hypoplasia
Prader-Labhart-Willi syndrome
Laurence-Moon-Biedl syndrome
Constitutional delay of puberty
Pituitary insufficiency/adenomas
Pasqualini syndrome
Isolated lack of FSH
Biologic inactive LH or FSH
Hyperprolactinemia
Hemochromatosis
Testicular origin (hypergonadotropic syndromes = primary hypogonadism)
Congenital anorchia
Acquired anorchia
Maldescended testes
Klinefelter syndrome
XYY syndrome
XX male
Noonan syndrome
Gonadal dysgenesis
Leydig cell tumors
Maldescended testes
Varicocele
Sertoli-cell-only syndrome
General disease e.g. renal failure, liver cirrhosis, diabetes, myotonia dystrophica
Male pseudohermaphroditism due to enzyme defects in testosterone biosynthesis or
LH-receptor defects (Leydig cell aplasia)
General diseases
Exogenous factors
Mixed primary and secondary hypogonadism
Late-onset hypogonadism
Target organ resistance to sex steroids
Complete androgen insensitivity (Testicular feminization)
Reifenstein syndrome
Perineoscrotal hypospadias with pseudovagina
Aromatase deficiency
Estrogen resistance

target organ lesions. An overview of the various disease entities and syndromes is provided in Table 13.1 and for a detailed description the reader is referred to the textbook by Nieschlag and Behre (2000).

The clinical symptoms of all syndromes and disease entities are predominantly due to a lack of testosterone or its action. The most frequent disorders requiring testosterone substitution are Klinefelter syndrome, Kallman syndrome, idiopathic hypogonadotropic hypogonadism (IHH), anorchia and pituitary insufficiency. Some disorders such as varicocele, orchitis, maldescended testes and Sertoli-cell-only syndrome will not, or only eventually require testosterone substitution. Although discrete endocrine alterations may be noted by laboratory tests in these patients, the endocrine capacity of the Leydig cells remains high enough to maintain serum testosterone in the lower physiological range.

In order to achieve fertility in patients with hypothalamic (IHH) or pituitary insufficiency, treatment with gonadotropins (hCG/hMG) or pulsatile GnRH is required temporarily (e.g. Büchter *et al.* 1998; Depenbusch *et al.* 2002; Depenbusch and Nieschlag 2004). Once a pregnancy has been induced these patients will go back on testosterone substitution. Cases with hypogonadism of testicular origin in whom infertility cannot be treated require testosterone substitution continuously. In all these patients testosterone substitution is a life-long therapy.

There is general agreement that patients with "classical" disorders of primary or secondary hypogondadism should receive testosterone substitution therapy. However, there is a relatively large group of patients in whom hypogonadism develops as a corollary of other acute or chronic diseases. Although these patients lack testosterone and show symptoms of hypogonadism, testosterone is usually not administered to them. Just why substitution is withheld is not quite clear. Probably in many physicians' minds testosterone is predominantly associated with sexual functions. However, the better the general effects of testosterone on well-being, mood, bones, muscles and red blood are understood, the more frequently testosterone substitution will be considered. Chapter 15 is dedicated to the possible use of testosterone in these non-gonadal diseases. Similarly, late-onset hypogonadism occurring with increasing incidence in ageing men, and representing a combined form of primary and secondary hypogonadism, is associated with symptoms of testosterone deficiency. But there is no general agreement on treatment strategies of this condition and Chapter 16 deals with late-onset hypogonadism and the controversies and unresolved problems surrounding this area. Furthermore, Chapter 3 covers the possible use of testosterone in androgen resistance syndromes.

For the time being the principle may be followed that any type of hypogonadism documented by decreased serum testosterone concentrations deserves testosterone substitution, unless there is a clear contraindication, of which there are only few.

Table 13.2 Symptoms of hypogonadism relative to age of manifestation

	Onset of lack of testosterone	
	before	after
Affected organ/function	completed puberty	
Larynx	No voice mutation	No change
Hair	Horizontal pubic hairline, straight frontal hairline, diminished beard growth	Diminishing secondary body hair, decreased beard growth
Skin	Absent sebum production, lack of acne, pallor, skin wrinkling	Decreased sebum production, lack of acne, pallor, skin wrinkling, hot flashes
Bones	Eunuchoid tall stature, Armspan > height, osteoporosis	Arm span = height, osteoporosis
Bone marrow	Low degree anaemia	Low degree anaemia
Muscles	Underdeveloped	Atrophy
Prostate	Underdeveloped	Atrophy
Penis	Infantile	No change of size
Testes	Small volume, often maldescended testes	Decrease of volume and consistency
Spermatogenesis	Not initiated	Arrest
Ejaculate	Not produced	Low volume
Libido	Not developed	Loss
Potency	Not developed	Erectile dysfunction

13.1.1 Classification and symptoms of hypogonadism

The time of onset of testosterone deficiency is of greater importance for the clinical symptoms than localization of the cause. Lack of testosterone or testosterone action during weeks 8 to 14 of fetal life, the period of sexual differentiation, leads to the development of intersexual genitalia (see Chapter 3). Lack of testosterone at the end of fetal life results in maldescended testes and small penis size. In later life the onset of testosterone deficiency before or after completion of puberty determines clinical appearance (Table 13.2).

If testosterone is lacking from the time of normal onset of puberty onwards, eunuchoidal body proportions will develop, i.e. arm span exceeds the standing height and lower length of body (from soles to symphysis), exceeds upper length (from symphysis to top of the cranium) and bone mass will not develop to its normal level. The distribution of fat will remain prepubertal and feminine, i.e. emphasis of

hips, buttocks and lower belly. Voice mutation will not occur. The frontal hairline will remain straight without lateral recession, beard growth is absent or scanty, the pubic hairline remains straight. Hemoglobin and erythrocytes will be in the lower normal to subnormal range. Early development of fine perioral and periorbital wrinkles are characteristic. Muscles remain underdeveloped. The skin is dry due to lack of sebum production and free of acne. The penis remains small, the prostate is underdeveloped. Spermatogenesis will not be initiated and the testes remain small. If an ejaculate can be produced it will have a very small volume. Libido and potency will not develop. A lack of testosterone occurring in adulthood cannot change body proportions, but will result in decreased bone mass and osteoporosis. Early-on lower backache and, at an advanced stage, vertebral fractures may occur. Once mutation has occurred the voice will not change again. Lateral hair recession and baldness when present will persist, the secondary sexual hair will become scanty and, in advanced cases, a female hair pattern may again develop. Mild anemia may develop. Muscle mass and power decrease. The skin will become atrophied and wrinkled. Gynecomastia may develop. The prostate will decrease in volume while the penis will not or only minimally change its size. Spermatogenesis will decrease and as a consequence, also the size of the testes, which will become softer. Libido and sexual arousability will decrease or disappear while potency will be less affected.

13.1.2 Initiation of substitution therapy and choice of preparation

Testosterone substitution is started when the diagnosis is established and serum testosterone levels below the normal range are found, taking into account the various influences on serum testosterone levels including diurnal variations. In order to establish a diagnosis by documenting low serum testosterone levels, usually determination of testosterone in a serum sample taken between 08.00 and 10.00 in the morning is sufficient (Vermeulen and Verdonck 1992). Pooled sera will not improve diagnostic accuracy (see Chapter 21).

The symptoms of androgen deficiency can be prevented or reversed by testosterone treatment. It is important that a preparation with natural testosterone is selected for treatment so that all functions of testosterone and its active metabolites DHT and estradiol can be exerted (Fig. 13.1). Of all testosterone preparations and routes of application described in Chapter 14, intramuscular injection or oral ingestion of testosterone esters were formerly the most widely accepted and practiced modalities for the treatment of all forms of hypogonadism. Over the last decade, transdermal testosterone preparations have become a valuable alternative, first transdermal patches and, more recently, transdermal gels. The transdermal preparations have the advantage that they can mimic the normal physiologic diurnal rhythm and thus represent the most physiologic form of substitution.

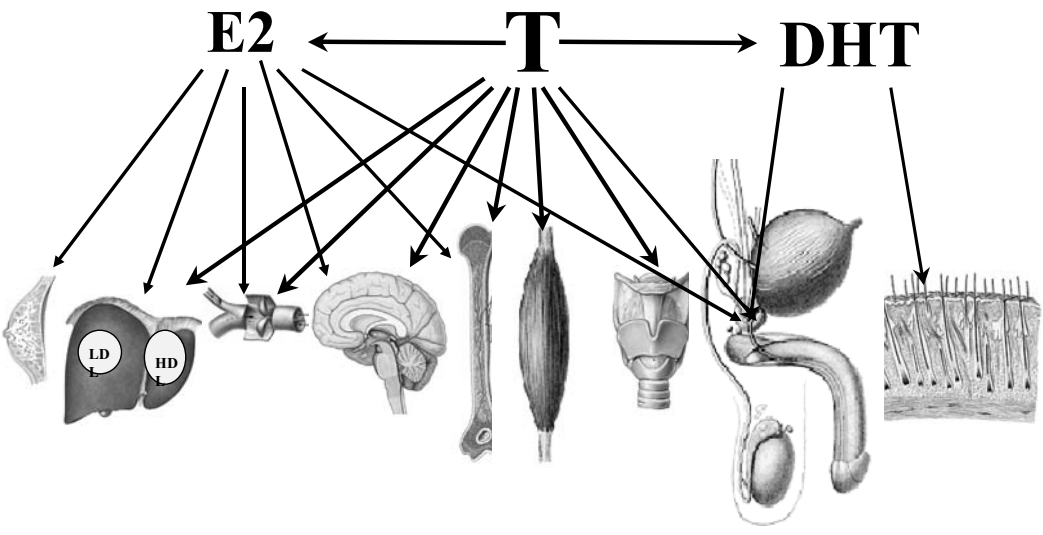


Fig. 13.1 Target organs of testosterone and its active metabolites DHT and estradiol indicating that testosterone needs to be converted to these metabolites to develop the full spectrum of its activity.

For full intramuscular substitution pharmacokinetic and clinical studies show that 200 to 250 mg testosterone enanthate or testosterone cypionate must be injected every two weeks (Cunningham *et al.* 1990; Davidson *et al.* 1979; Gooren 1987; Nieschlag *et al.* 1976; Schulte-Beerbühl and Nieschlag 1980; Snyder and Lawrence 1980; Sokol *et al.* 1982). More recently, 1000 mg testosterone undecanoate dissolved in castor oil and injected at 12-week intervals have been shown to be effective in substitution therapy (von Eckardstein and Nieschlag 2002). The long injection intervals and smooth serum levels in the normal range are appreciated by the patients and predict good acceptability of this preparation, once it has become licensed.

If oral substitution is preferred, 40 mg testosterone undecanoate capsules must be given two to four times daily. These doses have been shown to be effective in the majority of hypogonadal men in either open (Franchi *et al.* 1978; Franchimont *et al.* 1978; Gooren 1987; Maisey *et al.* 1981; Morales *et al.* 1997) or double-blind controlled studies (Luisi and Franchi 1980; Skakkebaek *et al.* 1981) when libido and potency as well as physical and mental activity were taken as parameters. Although relatively high testosterone doses are consumed with this regimen, liver function is not negatively affected, as could be shown in 35 men taking 80 to 200 mg testosterone undecanoate over ten years (Gooren 1994). The patients need to be instructed to ingest the capsules together with a meal in order to guarantee adequate absorption from the gut (Bagchus *et al.* 2003).

Transdermal testosterone preparations may be used as a first choice and are specifically suited for patients suffering from fluctuating symptoms caused by testosterone enanthate injections. Another advantage is the self-applicability of these preparations. First a transscrotal patch was developed, consisting of a film containing 10 or 15 mg natural testosterone. These patches are applied daily and lead to physiologic serum testosterone levels (Ahmed *et al.* 1988; Atkinson *et al.* 1998; Bals-Pratsch *et al.* 1988; Carey *et al.* 1988; Cunningham *et al.* 1989; Findlay *et al.* 1989). In our own experience of over ten years adequate long-term substitution can be achieved without serious side-effects under regular use. Serum testosterone levels are maintained in the lower normal range which is sufficient to induce e.g. normal bone density (Behre *et al.* 1999). Transdermal delivery systems on non-scrotal skin also result in physiological serum levels, depending on the number of patches used (Brocks *et al.* 1996). Regardless of application to different body areas e.g. back, abdomen, thigh or upper arm, rather similar pharmacokinetic patterns of serum testosterone are achieved (Meikle 1998). Due to the enhancers used in the patches to facilitate absorption, skin irritation occurs in a high percentage of patients, often leading to termination of this mode of testosterone application (Arver *et al.* 1997; Jordan 1997).

The latest development in androgen replacement therapy is an open testosterone delivery system using a hydroalcoholic gel which was first licensed in the United States and is now also available in Europe for the treatment of hypogonadal men. When applied to the skin, the gel dries rapidly and the steroid is absorbed into the stratum corneum, which serves as a reservoir. Pharmacokinetic studies of this gel (50 or 100 mg) applied to hypogonadal men indicate that testosterone levels increase into the normal range within 30 min, with steady-state levels achieved by 24 h. Studies over six months showed good clinical effects and tolerability, long-term trials are underway.

The choice of testosterone preparation and route of administration is finally up to the patient who over time may gather experience with several preparations and develop his own preference. Younger patients will be more inclined to choose long-acting preparations while the older patient ($>$50 years) should be advised to use a short-acting preparation (Nieschlag 1998). If therapy has to be stopped due to developing contraindications (e.g. prostate disease), serum testosterone levels will immediately decline to endogenous levels.

If a patient has pronounced androgen deficiency, has never received testosterone and has passed the age of puberty he is immediately treated with a full maintenance dose of testosterone. In cases of secondary hypogonadism when fertility is requested, testosterone therapy can be interrupted and GnRH or hCG/hMG therapy can be implemented until sperm counts increase and a pregnancy has been induced. Testosterone therapy does not prevent the chance of initiating or reinitiating

spermatogenesis with releasing or gonadotropic hormones. Once spermatogenesis has been induced it can be maintained for some time with hCG alone, keeping intratesticular testosterone concentrations high (Depenbusch *et al.* 2002); this is not the case with testosterone alone at clinically used doses (see also Chapter 5).

Patients with residual testosterone production may not require a full maintenance dose, e.g. Klinefelter patients in an early phase of testosterone deficiency. In these cases injection intervals of testosterone esters may be extended beyond the two-week period; these cases may also be suited for low-dose testosterone undecanoate therapy (i.e. 40 mg once or twice daily) or intermittent transdermal treatment. This dose does not entirely suppress the residual endogenous testosterone production and supplements the lacking hormone.

13.1.3 Surveillance of testosterone substitution therapy

The physiological effects of testosterone (Mooradian *et al.* 1987) can be used for monitoring the efficacy of testosterone substitution therapy. Since therapy aims at replacing the testosterone endogeneously lacking and since physiological serum concentrations are well known, serum testosterone levels also provide a good parameter for therapy surveillance. Guidelines for monitoring testosterone therapy in general have been issued by WHO (1992) and, with special focus on the ageing male, by others (Bhasin and Buckwalter 2001; Bhasin *et al.* 2003; Morales and Lunenfeld 2002; National Institute on Ageing 2001) and should be referred to for more details.

13.1.3.1 Behaviour and mood

The patient's general well-being is a good parameter to monitor the effectiveness of replacement therapy. Under sufficient testosterone replacement the patient feels physically and mentally active, vigorous, alert and in good spirits; too low testosterone levels will be accompanied by lethargy, inactivity and depressed mood (Burris *et al.* 1992; Wang *et al.* 1996; Zitzmann and Nieschlag 2001).

13.1.3.2 Sexuality

The presence and frequency of sexual thoughts and fantasies correlate with appropriate testosterone substitution, while loss of libido and sexual desire are a sign of subnormal testosterone values. Spontaneous erections such as those during sleep will not occur if testosterone replacement is inadequate; however, erections due to visual erotic stimuli may be present even with low testosterone levels. The frequency of ejaculations and sexual intercourse correlate with serum testosterone levels in the normal to subnormal range. Therefore, detailed psychological exploration or a diary on sexual activity are useful adjuncts in assessing testosterone substitution. For objective evaluation of psychosexual effects weekly questionnaires on sexual thoughts and fantasies, sexual interest and desire, satisfaction with sexuality,

frequency of erections and number of morning erections and ejaculations may be used (Lee *et al.* 2003). These clinical experiences are substantiated by studies on androgen replacement in hypogonadal men (Bals-Pratsch *et al.* 1988; Behre *et al.* 1992; Burris *et al.* 1992; Carani *et al.* 1992; Clopper *et al.* 1993; Cunningham *et al.* 1990; Jain *et al.* 2000; Morales *et al.* 1997), and by findings in normal men treated with GnRH analogues (Bagatell *et al.* 1994; Behre *et al.* 1994a; Buena *et al.* 1993) and in contraceptive trials (see Chapter 23).

Priapism has been reported to occur in individual cases at the beginning of testosterone substitution (Endres *et al.* 1987; Ruch and Jenny 1989; Zelissen and Stricker 1988). This is an extremely rare effect; in our own experience of 35 years of substitution therapy only one case is recollected. Decreasing the testosterone dose is the rational consequence, but intervention by aspirating blood from the corpora cavernosa may be acutely necessary.

13.1.3.3 Phenotype

Muscles and physical strength grow under testosterone treatment and the patient develops a more vigorous appearance (e.g. Wittert *et al.* 2003). Due to its anabolic effects body weight increases by about 5%. Therefore, accurate recording of body weight under comparable conditions is part of the routine control of the patient. The increase in lean body mass at the expense of body fat can be measured. Originally this was only possible by sophisticated equipment in the framework of clinical research (Young *et al.* 1993), but can now be done conveniently by equipment measuring bioimpedance (Rolf *et al.* 2002). Moreover, the distribution of subcutaneous fat that shows feminine characteristics in hypogonadism (hips, lower abdomen, nates) may change with increasing muscle mass. In particular, testosterone appears to reduce abdominal fat (Rebuffé-Scrive *et al.* 1991).

The appearance and maintenance of a male sexual hair pattern is a good parameter for monitoring testosterone replacement (see Chapter 6). In particular, beard growth and frequency of shaving can easily be recorded. Hair growth in the upper pubic triangle is an important indicator of sufficient androgen substitution. While women, boys and untreated hypogonadal patients have a straight frontal hairline, androgenization is accompanied by temporal recession of the hairline and – if a predisposition exists – by the development of baldness. The *pattern* of male sexual hair is of greater importance than the *intensity* of hair growth since no correlation could be found between the intensity of body hair growth and serum testosterone levels in the normal range (Knussmann *et al.* 1992). A well-substituted patient may have to shave daily. However, if there is no genetic disposition for dense beard growth, additional testosterone will not increase facial hair.

Sebum production correlates with circulating testosterone levels and hypogonadal men may suffer from dry skin. In an early phase of treatment patients may even complain about the necessity of shampooing more frequently; they have to

be informed that this is a part of normal maleness. The occurrence of acne may be a sign of supraphysiological testosterone levels and the dose should be reduced accordingly.

Gynecomastia may be caused by increased estradiol levels during testosterone therapy, especially under testosterone enanthate injections. After initiation of androgen therapy and consecutive decrease of estradiol serum levels, gynecomastia usually disappears. If gynecomastia preexists due to an increased estradiol/testosterone ratio in hypogonadal men, it may decrease during adequate testosterone therapy. However, in severe cases mastectomy by an experienced plastic surgeon may be required.

Patients who have not undergone pubertal development will experience voice mutation soon after initiation of testosterone therapy. During normal pubertal development the voice begins to break when serum testosterone levels reach about 10 nmol/l and SHBG drops (Pedersen *et al.* 1986). Mutation of the voice is very assuring for the patient and helps him to adjust to his environment by closing the gap between his chronological and biological age. It is specifically important for the patient to be recognized as an adult male on the phone. Once the voice has mutated it is no longer a useful parameter for monitoring replacement therapy since the size of the larynx, the vocal chords and thus the voice achieved will be maintained without requiring further androgens.

In prepubertal patients penis growth will be induced by testosterone treatment and normal erectile function will develop. Since penile androgen receptors diminish during puberty, growth will cease even under continued testosterone treatment (Shabsigh 1997; see also Chapter 11).

Patients who did not undergo puberty before the onset of hypogonadism may also develop eunuchoidal body proportions because of retarded closure of the epiphyseal lines of the extremities. Testosterone treatment will briefly stimulate growth, but will then lead to closure of the epiphyses and will arrest growth. In these patients, an X-ray of the left hand and distal end of the lower arm should be made before treatment to determine bone age. The epiphyseal closure may be followed by further X-rays during the course of treatment. In addition, body height and arm span – as measured from the tip of the right to the tip of the left middle finger – should be measured until no further growth occurs. Continued growth, in particular of the arm span, indicates inadequate androgen substitution.

13.1.3.4 Blood pressure

Overdosing androgens, as can be observed during misuse of testosterone and anabolic steroids, may increase blood pressure by increasing blood electrolytes and water retention, leading to edema. During effective testosterone substitution therapy in hypogonadal men such side-effects are not observed (e.g. Whitworth

et al. 1992). However, all androgens cause some degree of sodium retention and a small expansion of extracellular fluid volume that may contribute to weight increase in healthy individuals. Regular blood pressure measurement should be performed during testosterone therapy, especially during inception of treatment when the testosterone dosages have to be adjusted, and in men with additional problems of the heart and kidneys (Gooren 1994).

13.1.3.5 Serum testosterone

When serum testosterone levels are used to judge the quality of testosterone substitution it is necessary to be aware of the pharmacokinetic profiles of the different testosterone preparations (Chapter 14). Moreover, in longitudinal surveillance of testosterone therapy it is important to use assay systems that strictly undergo internal and external quality control (Chapter 21). Generally, testosterone serum levels should be measured just before the injection of the next dose of long-acting preparations. The time point of the last injection or administration of oral or transdermal testosterone must be recorded to interpret the serum levels measured.

Levels below the lower normal limit at the end of a three-week interval after testosterone enanthate injection or a 12-week interval after testosterone undecanoate injection should prompt shorter injection frequency of two-week intervals. Conversely, if the levels are in the high physiological range at the end of the injection interval, the dosing intervals may be extended. Low serum testosterone levels two to four hours after ingestion of oral testosterone undecanoate should prompt counseling of the patient so that the capsule is taken together with a meal and testosterone is better absorbed. However, it is difficult to base monitoring of treatment with oral testosterone undecanoate on serum testosterone levels and other parameters are of more importance if this mode of therapy is chosen.

When transdermal preparations are applied, serum testosterone levels may be measured just before the next dose is administered. Initial measurements, however, are only meaningful after two or three weeks following initiation of therapy since it takes time until the skin builds up a reservoir and steady state serum levels are reached. Transdermal patches may show poor adhesiveness, in particular in warm weather and when the patient sweats e.g. during athletic activity. This is not the case with gels which show good tolerability and only rarely skin irritation.

After initiation of testosterone substitution, measuring serum testosterone under the conditions mentioned above is recommendable after 3, 6 and 12 months and thereafter annually.

In blood, testosterone is bound to sex hormone binding globulin (SHBG) and other proteins. Only about 2% of testosterone is not bound and is available for biological action of testosterone (free testosterone). Since total testosterone correlates

well with free testosterone, separate determination of free testosterone is not necessary for routine monitoring (see also Chapter 21).

Testosterone can also be determined in saliva. The concentrations correlate to free testosterone concentrations in serum. Saliva collection can be easily performed without the help of medical staff and thus provides a useful procedure for monitoring substitution therapy (Navarro *et al.* 1994; Schürmeyer *et al.* 1983; Tschöp *et al.* 1998). However, since the available assays are not very robust, measurement of saliva testosterone has not become a widespread methodology and remains reserved for research projects.

13.1.3.6 Serum dihydrotestosterone (DHT)

Determination of dihydrotestosterone (DHT) does not play a role in routine monitoring of testosterone replacement therapy, but may be of importance in experimental use of testosterone preparations and monitoring biological effects of androgens. Due to the high 5α-reductase activity in skin, transdermal testosterone application is associated with increased serum DHT-levels; this applies especially to scrotal application. The DHT adds to the overall androgenicity of the preparation and a patient receiving transdermal treatment may be well substituted clinically although his serum testosterone does not reflect this. In these cases occasional measurement of serum DHT may be indicated (see Chapter 21).

13.1.3.7 Serum estradiol

In sensitive patients very high serum testosterone levels, as they may occur under testosterone enanthate, may be converted to estrogens and cause gynecomastia. This is an indication to reduce the dose or switch to another testosterone preparation. In this case monitoring serum estradiol levels may explain the clinical findings.

13.1.3.8 Gonadotropins

The determination of LH and FSH plays a key role in establishing the diagnosis of hypogonadotropic (i.e. secondary) or hypergonadotropic (i.e. primary) hypogonadism. However, during surveillance of testosterone therapy they are of less importance. Negative feedback regulation between hypothalamus, pituitary and testes causes negative correlation between serum testosterone and LH, as well as to some extent to FSH levels in normal men.

In cases with primary hypogonadism (e.g. intact hypothalamic and pituitary function) FSH and in particular LH increase with decreasing testosterone levels and may normalize under testosterone treatment. This is especially the case in patients with acquired anorchia (e.g. due to accidents or iatrogenic castration). However, in the most frequent form of primary hypogonadism, i.e. in patients with Klinefelter syndrome, LH and FSH often do not show significant suppression

during testosterone substitution. Moreover, oral or transdermal testosterone may have only little effect on gonadotropins. Therefore LH is not the best indicator of sufficient testosterone replacement therapy.

13.1.3.9 Erythropoiesis

Since erythropoiesis is androgen-dependant, hypogonadal patients usually present with mild anemia (with values in the female normal range) which normalizes under testosterone treatment. Therefore, hemoglobin, red blood cell count and hematocrit are good parameters for surveillance of replacement therapy. If sufficient stimulation is lacking despite adequate testosterone therapy, lack of iron should be ruled out and treated if necessary. At the beginning of therapy we check red blood values every three months, later on annually. If too much testosterone is administered, supraphysiological levels of hemoglobin, erythrocytes and hematocrit as a sign of polycythemia can develop, indicating that the testosterone dose should be scaled down (Hajjar *et al.* 1997; Matsumoto *et al.* 1985; Sih *et al.* 1997). In some cases phlebotomy may be required acutely.

Testosterone has been claimed to potentiate sleep apnea (see Chapter 15); however, only case reports about the incidence of sleep apnea during testosterone treatment have been published (Matsumoto *et al.* 1985) and paradoxically hypogonadism has also been cited as a cause of this condition (Luboshitzky *et al.* 2002). The two men who demonstrated worsening of obstructive apnea on testosterone replacement therapy had pathologically elevated erythrocyte counts and hematocrit (>59%), sufficient to require therapeutic phlebotomy. Increased hematocrit, increased mass of pharyngeal muscle bulk, as well as neuroendocrine effects of testosterone during therapy were discussed as possible reasons. The development of signs and symptoms of obstructive sleep apnea during testosterone therapy warrants a formal sleep study and treatment with continuous positive airway pressure (CPAP) if necessary. If the patient is unresponsive or cannot tolerate CPAP, the testosterone must be reduced or discontinued.

13.1.3.10 Liver function

The testosterone preparations proposed for testosterone replacement do not have negative side-effects on liver function (e.g. Gooren 1994). Nevertheless, many physicians believe that testosterone may disturb liver function. This impression derives from 17α-methyltestosterone and other 17α-alkylated anabolic steroids which are indeed liver toxic and which should no longer be used in the clinic (see Chapter 14). However, there may be ethnic differences since the weekly application of 200 mg testosterone enanthate, i.e. double the dose used for substitution, for several months led to a slight increase of liver transaminases in Chinese men while this

effect was not seen in non-Chinese men (Wu *et al.* 1996). Under more physiologic testosterone doses this phenomenon was not observed (Wang *et al.* 1991).

Monitoring liver function is of special interest in hypogonadal patients with concomitant diseases that affect liver function, or in patients whose hypogonadism is induced by general diseases. In such cases additional medication is necessary that may influence liver function and thus influence testosterone metabolism, e.g. by increasing SHBG production. We determine liver enzymes once per year routinely.

13.1.3.11 Lipid metabolism

Whether cardiovascular risk factors are affected by testosterone therapy remains a matter of debate (see Chapter 10). In adult male hypogonadism beneficial effects such as an increase in HDL-cholesterol as well as adverse effects such as a decrease in HDL-cholesterol or an increase in LDL-cholesterol have been demonstrated (Ozata *et al.* 1996; Sorva *et al.* 1988). In a one-year study, in which especially older, hypogonadal men were recruited, improvement in LDL cholesterol without effects on HDL cholesterol was reported (Zgliczynski *et al.* 1996). Clotting factors are also affected by testosterone treatment and their changes may compensate for any atherogenic effects of lipids (Zitzmann *et al.* 2002a).

Under testosterone replacement therapy, changes of lipid metabolism appear to occur within physiological ranges. The CAG repeat length of the androgen receptor has a modifying role in the effects on lipid parameters (Zitzmann *et al.* 2003) and pharmacogenetic considerations may in the future influence dose and route of testosterone administration. Currently, it appears sufficient to monitor lipids under testosterone therapy in those patients with grossly abnormal lipid profiles.

Besides lipid profiles other metabolic parameters such as overweight and especially accumulation of abdominal fat predispose men for cardiovascular diseases and diabetes. This condition is more frequent in men with low testosterone and SHBG levels (Tchernof *et al.* 1996). Leptin, the hormonal product of adipocytes, is a candidate link between these different metabolic systems. Substitution therapy of male hypogonadism normalizes initially elevated leptin concentrations and reduces obesity and therefore could be considered a useful marker of therapeutic effectiveness (Behre *et al.* 1997a; Jockenhövel *et al.* 1997; Sih *et al.* 1997).

13.1.3.12 Prostate and seminal vesicles

The prostate and seminal vesicles are androgen-sensitive organs and are small in hypogonadal patients. Their volumes increase under testosterone therapy. Testosterone induces their normal functions, as indicated by the appearance of seminal fluid. Well-substituted patients should have ejaculate volumes in the normal range (i.e. $\geq$ 2 ml).

There is much concern about the effects of testosterone with regard to the development of benign prostatic hyperplasia (BPH) and carcinoma of the prostate and this issue is specifically dealt with in Chapters 2 and 21. A widely accepted theory on the pathogenesis of BPH suggests that prostatic enlargement is mediated through the action of 5α-DHT and that these alterations are related to intraprostatic events rather than to increases in serum concentrations of testosterone or 5α-DHT (Meikle *et al.* 1997; Morgentaler *et al.* 1996; Nomura *et al.* 1988). Furthermore, estrogens may be involved in hormonal regulation of prostatic tissue (Thomas and Keeman 1994). Testosterone therapy increases prostate volume in hypogonadal men, but only to the prostate size seen in age-matched controls (Behre *et al.* 1994b). This is also the case in patients on scrotal testosterone treatment leading to somewhat elevated serum DHT levels. However, as in other androgen target organs, the androgen receptor modifies testosterone action in the prostate as well. Thus, under the same testosterone therapy patients with shorter CAG repeats may develop larger prostates than men with longer CAG repeats (Zitzmann *et al.* 2003). Those with shorter CAG repeats may also be more likely develop prostate cancer (see Chapter 2 and 12). These findings have, however, not yet been translated into clinical practice.

PSA levels increase slightly during therapy but remain within the normal range of a younger population (Behre *et al.* 1994b; Meikle *et al.* 1997; von Eckardstein and Nieschlag 2002). PSA levels must be monitored regularly under testosterone therapy. Though limited in accuracy, sensitivity and specificity, rectal palpation of the prostate for size, surface and consistency belongs to the regular check-up of patients under testosterone treatment. Palpation may be assisted by transrectal ultrasonography of the prostate.

Because of the incidence of benign prostatic hyperplasia and prostate carcinoma increasing with age and the risk of stimulating the growth of a preexisting carcinoma by testosterone, patients should be examined carefully before onset of testosterone therapy and thereafter at annual intervals if under 45 years of age. In addition, in patients over 45 PSA levels and prostate palpation should be performed 3, 6 and 12 months after initiation of testosterone therapy since it may activate a preexisting carcinoma. If a carcinoma is diagnosed, testosterone treatment is contraindicated and must be terminated immediately.

13.1.3.13 Bone mass

Hypogonadism is associated with decreased bone density by increased bone resorption and decreased mineralization, resulting in premature osteoporosis and increased risk of fractures (see Chapter 7). Testosterone replacement in hypogonadal patients results in an increase in bone density (Behre *et al.* 1997b; Leifke *et al.* 1998; Devogelaer *et al.* 1992; Zitzmann *et al.* 2002b) (Fig. 13.2). Since estrogens

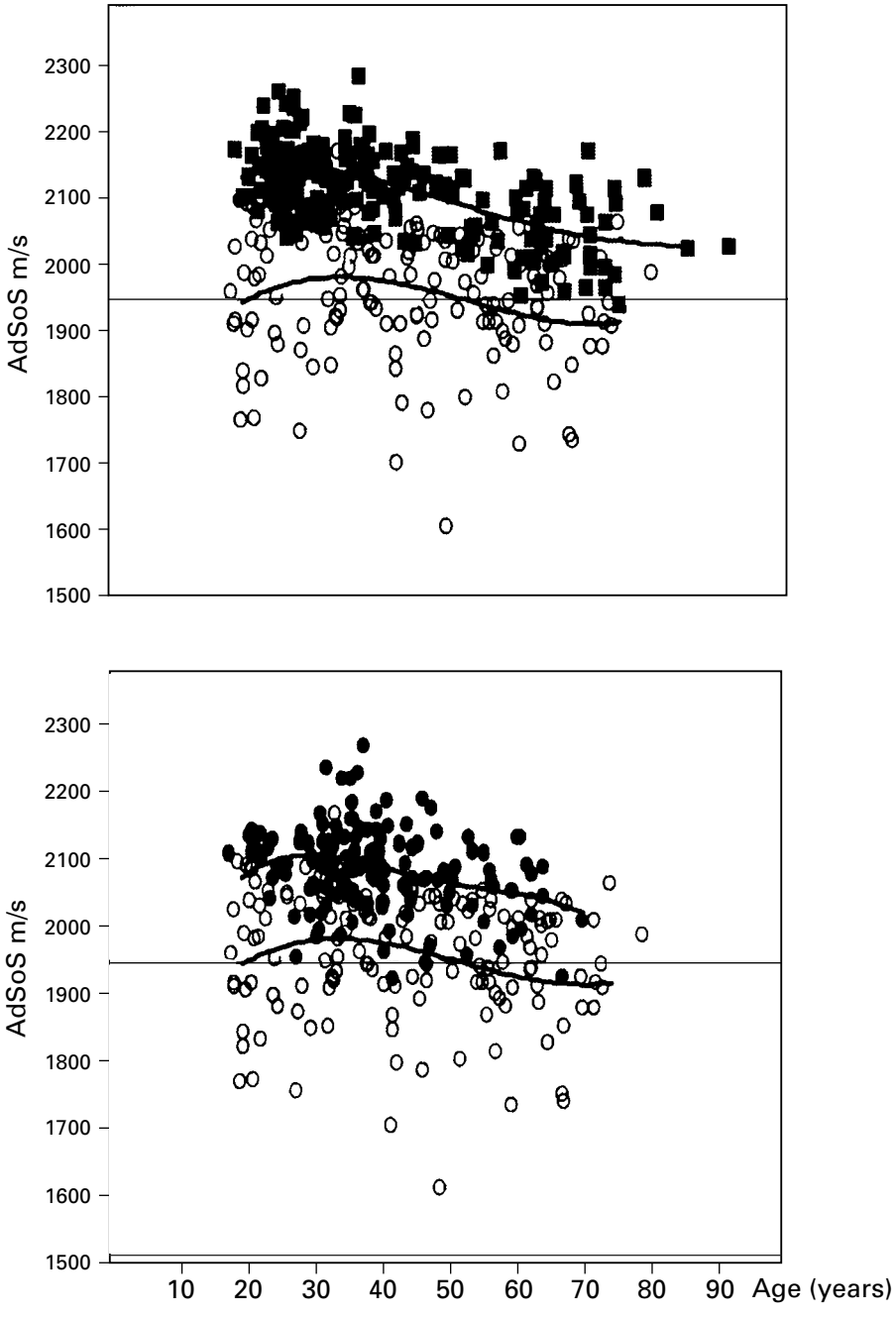


Fig. 13.2 Bone density as measured by phalangeal ultrasonographic osteodensitometry in 224 eugonadal men (squares), 156 hypogonadal patients (open circles) and 141 testosterone-substituted patients (closed circles) (modified from Zitzmann *et al*. 2002b).

play an important role in bone metabolism and structure it is important that the testosterone preparation used for substitution can be converted to estrogens, as is the case with natural testosterone.

Only advanced changes in bone density can be recognized by usual X-ray. For monitoring early signs of inadequate bone density different methods are available, e.g. dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA) or quantitative computer tomography of the lumbar spine (QCT) or the peripheral quantitative computer tomography of radial or tibial bone (pQCT). These methods are characterized by high accuracy and reproducibility, but are relatively time consuming and expensive. For routine surveillance of hypogonadal patients sonographic osteodensitometry appears to be sufficient to monitor the effects of testosterone therapy (Zitzmann *et al.* 2002b). In hypogonadal patients results from osteosonography of the phalanges agree well with those from QCT of the vertebrae so that we use this method routinely and subject patients to osteosonography on an annual basis for routine surveillance.

13.2 Treatment of delayed puberty in boys

Androgen replacement therapy in male adolescents with constitutional delay of growth and adolescence has been shown to be beneficial psychologically as well as physiologically, and should be initiated promptly on diagnosis (Albanese and Stanhope 1995; de Lange *et al.* 1979; Kaplan *et al.* 1973; Rosenfeld *et al.* 1982). Boys with delayed puberty are at risk for not obtaining adequate peak bone mass and for having deficiencies in developing social skills, an impaired body image, and low self-esteem. Younger boys with short stature, delayed bone age (at least 10.5 years), and delayed pubertal development in the absence of other endocrinological abnormalities can be treated with 50–100 mg of testosterone enanthate or cypionate im, every four weeks for three months, whereas boys >13 years old may be treated with 250 mg (im, every four weeks for three months). After a three-month "wait and see" period, another course of treatment may be offered if pubertal development does not continue. An increase in testes size is the most important indicator of spontaneous pubertal development (testes volume >3 ml). Overtreatment with testosterone may result in premature closure of the epiphyses of long bones, resulting in reduced adult height. Therefore, treatment of patients who have not yet reached full adult height has to be undertaken carefully.

Low-dose oral testosterone undecanoate has been tested for the treatment of constitutional delay of puberty (Albanese *et al.* 1994; Brown *et al.* 1995; Butler *et al.* 1992). For example, treatment of 11–14 year old prepubertal boys with 20 mg testosterone undecanoate per day for six months resulted in an increase in growth velocity without advancing bone age and pubertal development (Brown *et al.* 1995). Such

"mild" treatment appears to be suited for an early phase when virilization is not yet requested. Transdermal testosterone should also be a useful method to induce puberty. However, experience in a larger series of patients has not yet been reported. At the beginning of therapy it is often difficult to distinguish between boys with constitutional delay of growth and puberty, who require only temporary androgen replacement, and boys with idiopathic hypogonadotropic hypogonadism, who require lifelong androgen therapy to stimulate puberty and to maintain adult sexual function. However, boys with permanent hypogonadotropic hypogonadism will not have testicular growth induced by androgen therapy. Because pubertal growth is a product of the interaction of growth hormone (GH) and insulin-like growth factor I (IGF-I) and the hypothalamic-pituitary-gonadal axis, boys with concomitant GH deficiency will require the simultaneous administration of GH and androgens for the treatment of delayed puberty. In boys with secondary causes of delayed puberty, development can also be induced by pulsatile GnRH or hCG/hMG respectively. This therapy has the advantage that testicular development is induced simultaneously. However, we prefer to induce initial virilization by testosterone and to stimulate spermatogenesis at a later stage with the more demanding GnRH or gonadotropin therapy.

13.3 Overtall stature

The effect of testosterone on epiphysial closure may be used to treat boys who are dissatisfied with their prospective final overtall body height (for review Drop *et al.* 1998). Treatment has to start before the age of 14. Doses of 500 mg testosterone enanthate have to be administered every two weeks for at least a year to produce effects (Bettendorf *et al.* 1997). This treatment should be reserved for special cases since tall stature is not a disease but rather a cosmetic and psychological problem. However, social and psychological conflicts caused by this condition should not be underestimated. It should also be remembered that testosterone is not registered for this treatment, which has therefore to be considered "experimental". Combining ethinylestradiol with testosterone injections has no additional height-reducing effect (Decker *et al.* 2002).

An additional reservation comes from the possible effects of such high-dose testosterone treatment at this early age on fertility, the prostate, the cardiovascular system, on bones and other organs. Long-term follow-up of men treated on average ten years earlier with high-dose testosterone for tall stature revealed no negative effects on sperm parameters and reproductive hormones in comparison to controls (de Waal *et al.* 1995; Lemcke *et al.* 1996). Prostate morphology as evaluated by ultrasonsography did not show any abnormalities and serum lipids were not different from the control group. Slightly lower sperm motility was rather attributable to a

higher incidence of varicocele and maldescended testes in the treated men than to the treatment as such. Thus it appears that, as far as evaluated, high-dose treatment has no long-term negative side-effects in these adolescents.

13.4 Micropenis and microphallus

Enlargement of a micropenis or microphallus can be achieved in children by treatment with 25–50 mg of testosterone enanthate or cypionate (im, every 3 to 4 weeks for 3 months) or with 1.25–5% testosterone cream, 5% DHT cream, or 10% testosterone propionate cream (twice daily for 3 months). High-dose androgen therapy may be necessary to achieve some androgenization in male pseudohermaphroditism caused by 5α-reductase deficiency and certain androgen receptor defects (see Chapter 3).

13.5 Ineffective use of testosterone in male infertility

Since testosterone has been used so effectively in the treatment of endocrine insufficiency of the testes, its use has also been attempted in the treatment of idiopathic male infertility. Testosterone rebound was one of the earliest modalities in this regard. The published success rate in terms of pregnancies varied considerably from centre to centre, but remained low overall (Charny and Gordon 1978; Getzoff 1955; Lamensdorf *et al.* 1975; Rowley and Heller 1972). All studies were uncontrolled trials without placebo and double-blinding and therefore inconclusive. Testosterone rebound therapy cannot be recommended for treatment of infertility and is no longer practiced.

More recently, testosterone undecanoate has been tested for the treatment of idiopathic male infertility. However, a significant increase in pregnancy rates could not be demonstrated (Comhaire *et al.* 1995; Kloer *et al.* 1980; Pusch 1989). When testosterone undecanoate was given combined with tamoxifen and/or hMG, an improvement of semen parameters was observed (Adamopoulos *et al.* 1995; 1997). However, in these studies no pregnancy rates were reported. The therapeutic goal of every infertility treatment should be an increase in pregnancy rates, therefore, studies in which only improved semen parameters are reported, without examining the pregnancy rates, must be considered as inconclusive in terms of infertility treatment. Similarly, after many years of clinical use no significant effect of mesterolone on pregnancy rates could be demonstrated in an extensive WHO-sponsored multicentre trial (WHO 1989).

Thus, to date testosterone and other androgens have no place in evidence-based treatment of idiopathic male infertility (Kamischke and Nieschlag 1999).

13.6 Contraindications to testosterone treatment

Effects and side-effects of testosterone therapy have been described in detail above. Here the major reasons for not initiating or for interrupting testosterone therapy are briefly summarized.

The major contraindication to testosterone therapy is a *prostate carcinoma*. A patient with an existing prostate carcinoma should not receive testosterone. A carcinoma has to be excluded before starting therapy and the patient on testosterone should be checked regularly for prostate cancer (digital exploration, PSA, transrectal sonography and biopsy, if necessary) (see 13.1.3.2).

Breast cancer cells often are hormone-sensitive, especially estrogen-sensitive, and therefore, for reasons of safety, breast cancer is considered a contraindication to testosterone treatment. However, breast cancer is a relatively rare cancer in men and no cases of testosterone substitution and occurrence of breast cancer have been published, as an extended literature search revealed. Thus, this warning cannot be substantiated.

In some countries *sexual offenders* may be treated by castration or antiandrogenic therapy. It would be a serious mistake to administer testosterone to such patients. Relapses and renewed crimes could be the consequence and the responsibility of the prescribing physician.

Testosterone *suppresses spermatogenesis*, a phenomenon exploited for hormonal male contraception (see Chapter 23). In hypogonadal patients with reduced spermatogenetic function testosterone administration will also decrease sperm production. Such patients who wish to father children e.g. by techniques of artificial fertilization, should not receive full testosterone substitution therapy, at least not for the time their sperm are necessary for fertilization of eggs. This is of increasing importance as not only residual sperm in patients with secondary hypogonadism but also with Klinefelter syndrome may be able to fertilize eggs via intracytoplasmatic sperm injection (ICSI) and induce pregnancies (e.g. Friedler *et al.* 2001).

13.7 Overall effect of testosterone

Testosterone has many biological functions and, as demonstrated in this chapter, testosterone is a safe medication. There are only very few reasons why testosterone should be withheld from a hypogonadal patient (see 13.6). Nevertheless, to date many hypogonadal men do still not receive the benefit of testosterone therapy because they are not properly diagnosed and the therapeutic consequences are not drawn (e.g. Bojesen *et al.* 2003). Some physicians even believe that the shorter life expectancy of men compared to women could be attributed to effects

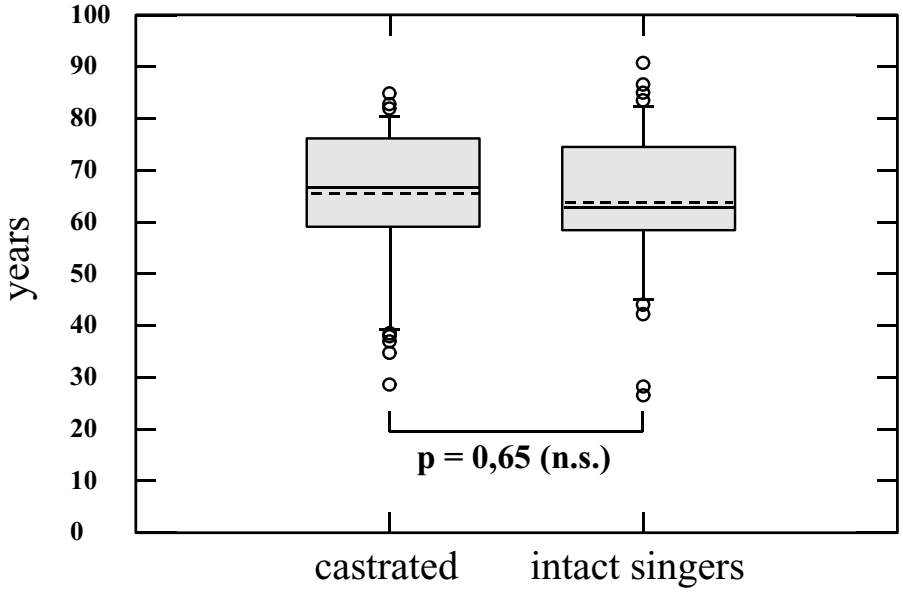


Fig. 13.3 Longevity of intact and castrated singers (50 in each group) born between 1580 and 1859 (matched pairs of intact and castrated singers with similar birth dates were formed) (Nieschlag *et al.* 1993).

of testosterone, possibly mediated through changes in lipid metabolism. Hence it may be asked whether testosterone may have a life-shortening effect on patients with hypogonadism under testosterone treatment. Appropriate controlled studies to answer this question directly are not available and are unlikely to be performed since it would be unethical to withhold testosterone lifelong from a hypogonadal control group. However, there are two retrospective historical studies available addressing the problem.

A retrospective analysis of the life expectancy of inmates of an institution for the mentally handicapped in the USA came to the conclusion that early castration would lead to a longer life expectancy (Hamilton and Mestler 1969). However, this could be explained by the preference of castration as treatment for the physically more active inmates, whereas lack of mobility is the major predictor of shortened life expectancy among institutionalized men. In contrast, the retrospective comparison of the life expectancy of singers born between 1580 and 1858 and castrated before puberty in order to preserve their high voices, to intact singers born at the same time did not reveal a significant difference between the lifespan of intact and castrated singers (Nieschlag *et al.* 1993) (Fig. 13.3). In contrast, among singers who died in the 20th century, basses had a tendency to live longer than tenors (67.4 $\pm$ 12.4 vs. 66.0 $\pm$ 14.4 years) (Basses have higher testosterone/estradiol ratios than tenors

(Meuser and Nieschlag 1977)). Sopranos, who are more estrogenized, lived significantly longer than altos, who are more androgenized (72.1 ± 14.3 vs. 67.5 ± 13.5 years) (Nieschlag *et al.* 2003). These findings can be interpreted that overall, a preponderance of isosexual hormones in the spectrum of sex steroids tends to extend life rather than shorten life.

Since neither the inmates nor the historical singers can be considered representative for the present population, these controversial studies can only provide hints but no conclusive answer. There is, however, no proof that testosterone is a life-shortening agent. Preventing a hypogonadal patient from receiving the necessary substitution would force him to continue a life of low quality. If testosterone in physiological doses should cause "side" effects, these would indeed be the normal biological effects. The risks inherent to testosterone, be it of endogenous or exogenous origin, would then appear to be the tribute men have to pay for being men.

13.8 Key messages

- The primary indications for testosterone therapy are the various forms of male hypogonadism. For substitution, testosterone preparations should be used that can be converted to 5α-dihydrotestosterone (DHT) as well as to estradiol in order to develop the full spectrum of testosterone action.
- Injectable, oral and transdermal testosterone preparations are available for clinical use. The best preparation is the one that replaces testosterone serum levels at as close to physiologic concentrations as possible.
- In six decades of clinical use testosterone has proven to be a very safe medication. No toxic effects are known. The only important contraindication is the presence of a prostate carcinoma which should be excluded before substitution is initiated.
- Testosterone therapy should be monitored by patients' well-being, alertness and sexual activity, by occasional measurement of serum testosterone levels, hemoglobin and hematocrit, by bone density measurements and prostate parameters (rectal examination, PSA and transrectal sonography).
- Testosterone can be used to initiate puberty in boys with constitutional delay of pubertal development. Careful dosing does not lead to premature closure of the epiphysis and reduced height.
- High-dose testosterone treatment in early puberty may prevent expected overtall stature in boys. Negative long-term effects of this treatment have not become evident to date.
- No evidence has been provided that testosterone treatment of male idiopathic infertility leads to higher pregnancy rates and it should therefore not be used for this indication.
- The risks inherent to testosterone, be it of endogenous or exogenous origin, appear to be the tribute men have to pay for being men.

13.9 REFERENCES

Adamopoulos DA, Nicopoulou S, Kapolla N, Vassipoulos P, Karamertzanis M, Kontogeorgos L (1995) Endocrine effects of testosterone undecanoate as a supplementary treatment to menopausal gonadotropins or tamoxifen citrate in idiopathic oligozoospermia. Fertil Steril 64:818–24

Adamopoulos DA, Nicopoulou St, Kapolla N, Karamertzanis M, Andreou E (1997) The combination of testosterone undecanoate with tamoxifen citrate enhances the effects of each agent given independently on seminal parameters in men with idiopathic oligozoospermia. Fertil Steril 67:756–62

Ahmed SR, Boucher AE, Manni A, Santen RJ, Bartholomew M, Demers LM (1988) Transdermal testosterone therapy in the treatment of male hypogonadism. J Clin Endocrinol Metab 66:546:551

Albanese A, Stanhope R (1995) Predictive factors in the determination of final height in boys with constitutional delay of growth and puberty. J Pediatr 126:545–550

Albanese A, Kewley GD, Long A, Pearl KN, Robins DG, Stanhope R (1994) Oral treatment for constitutional delay of growth and puberty in boys: a randomized trial of an anabolic steroid or testosterone undecanoate. Arch Dis Child 71:315–317

Arver S, Dobs AS, Meikle AW, Caramelli KE, Rajaram L, Sanders SW, Mazer NA (1997) Long-term efficacy and safety of a permeation-enhanced testosterone transdermal system in hypogonadal men. Clin Endocrinol 47:727–737

Atkinson LE, Chang Y-L, Snyder PJ (1998) Long-term experience with testosterone replacement through scrotal skin. In: Testosterone: Action, deficiency, substitution, 2nd edition Nieschlag E, Behre HM (eds) Springer Verlag, Heidelberg, pp 365–388

Bagatell CJ, Heimann JR, Rivier JE, Bremner WJ (1994) Effects of endogeneous testosterone and estradiol on sexual behaviour in normal young men. J Clin Endocrinol Metab 78:711–716

Bagchus WM, Hust R, Maris F, Schnabel PG, Houwing NS (2003) Important effect of food on the bioavailability of oral testosterone undecanoate. Pharmacotherapy 23:319–325

Bals-Pratsch M, Langer K, Place VA, Nieschlag E (1988) Substitution therapy of hypogonadal men with transdermal testosterone over one year. Acta Endocrinol 118:7–13

Behre HM, Nashan D, Hubert W, Nieschlag E (1992) Depot gonadotropin-releasing hormone agonist blunts the androgen-induced suppression of spermatogenesis in a clinical trial of male contraception. J Clin Endocrinol Metab 74:84–90.

Behre HM, Böckers A, Schlingheider A, Nieschlag E (1994a) Sustained suppression of serum LH, FSH testosterone and increase of high-density lipoprotein cholesterol by daily injections of the GnRH antagonist cetrorelix over 8 days in normal men. Clin Endocrinol 40:241–248

Behre HM, Bohmeyer J, Nieschlag E (1994b) Prostate volume in testosterone-treated and untreated hypogonadal men in comparison to age-matched normal controls. Clin Endocrinol 40:341–349

Behre HM, von Eckardstein S, Kliesch S, Nieschlag E (1999) Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. Clin Endocrinol 50: 629–635

Behre HM, Simoni M, Nieschlag E (1997a) Strong association between leptin and testosterone. Clin Endocrinol 47:237–240

Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E (1997b) Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. J Clin Endocrinol Metab 82:2386–2390

Bettendorf M, Heinrich UE, Schönberg DK, Grulich-Henn J (1997) Short-term, high dose testosterone treatment fails to reduce adult height in boys with constitutional tall stature. Eur J Pediatr 156:911–915

Bhasin S, Buckwalter JG (2001) Testosterone supplementation in older men: a rational idea whose time has not yet become. J Androl 22:718–731

Bhasin S, Singh AB, Phong Mac R, Carter B, Lee MI, Cunningham GR (2003) Managing the risks of prostate disease during testosterone replacement therapy in older men: recommendations for a standardized monitoring plan. J Androl 24:299–311

Bojesen A, Juul S, Gravholt CH (2003) Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 88:622–626

Brocks DR, Meikle AW, Boike SC, Mazer NA, Zariffa N, Audet PR, Jorkasky DK (1996) Pharmacokinetics of testosterone in hypogonadal men after transdermal delivery: influence of dose. J Clin Pharmacol 36:732–739

Brown D, Butler CGE, Kelnar CJH, Wu FCW (1995) A double blind, placebo controlled study of the effects of low dose testosterone undecanoate on the growth of small for age, prepubertal boys. Arch Dis Child 73:131–135

Büchter D, Behre HM, Kliesch S, Nieschlag E (1998) Pulsatile GnRH or human chorionic gonadotropin human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol 139:298–303

Buena F, Swerdloff RS, Steiner BS, Lutchmansingh P, Peterson MA, Pandian MR (1993) Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. Fertil Steril 59:1118–1123

Burris AS, Banks SM, Carter CS, Davidson JM, Sherins RJ (1992) A long-term prospective study of the physiologic and behavioural effects of hormone replacement in untreated hypogondadal men. J Androl 13:297–304

Butler GE, Sellar RE, Hendry RF, Qalker M, Kelnar CJH, Wu FCW (1992) Oral testosterone undecanoate in the management of delayed puberty in boys: pharmacokinetics and effects on sexual maturation and growth. J Clin Endocrinol Metabol 75:37–44

Carani C, Bancroft J, Granata A, Del Rio G, Marrama P (1992) Testosterone and erectile function: nocturnal penile tumescence and rigidity, and erectile response to visual erotic stimuli in hypogonadal men. Psychoneuroendocrinology 17:647–654

Carey PO, Howards SS, Vance ML (1988) Transdermal testosterone treatment of hypogonadal men. J Urol 140:76–79

Charny CW, Gordon JA (1978) Testosterone rebound therapy: a neglected modality. Fertil Steril 29:64–68

Clopper RR, Voorhess ML, MacGillivray MH, Lee PA, Mills B (1993) Psychosexual behavior in hypopituitary men: a controlled comparison of gonadotropin and testosterone replacement. Psychoneuroendocrinology 18:149–161

Comhaire F, Schoonjans F, Abelmassih R, Gordts S, Campo R, Dhont M, Milingos S, Gerris J (1995) Does treatment with testosterone undecanoate improve the in-vitro fertilizing capacity of spermatozoa in patients with idiopathic testicular failure? (Results of a double blind study). Hum Reprod 10:2600–2602

Cunningham GR, Cordero E, Thornby JI (1989) Testosterone replacement with transdermal therapeutic systems. JAMA 261:2525–2530

Cunningham GR, Hirshkowitz M, Korenman SG, Karacan I (1990) Testosterone replacement therapy and sleep-related erections in hypogonadal men. J Clin Endocrinol Metab 70:792–797

Davidson JM, Camargo CA, Smith ER (1979) Effects of androgen on sexual behavior in hypogonadal men. J Clin Endocrinol Metab 48:955

Decker R, Pratsch C-J, Sippell WG (2002) Combined treatment with testosterone (T) and ethinylestradiol (EE2) in constitutionally tall boys: is treatment with T plus EE2 more effective in reducing final height in tall boys than T alone? J Clin Endocrinol Metab 87:1634–1639

de Lange WE, Snoep MC, Doorenbos H (1979) The effect of short-term testosterone treatment in boys with delayed puberty. Acta endocrinol 91:177–183

Depenbusch M, von Eckardstein S, Simoni M, Nieschlag E (2002) Maintenance of spermatogenesis in hypogonadotropic hypogonadal men with hCG alone. Europ J Endocrinol 147:617–624

Depenbusch M, Nieschlag E (2004) (ed) Stimulation of spermatogenesis in hypogonadotropic men. In: Winters S (ed) Male hypogonadism: basic, clinical and therapeutic principles, Contemporary Endocrinology, Humana Press, Totowa NJ

Devogelaer JP, de Cooman S, Nagant de Deuxchaisnes C (1992) Low bone mass in hypogonadal males. Effect of testosterone substitution therapy, a densitometric study. Maturitas 15:17–23

De Waal WJ, Vreeburg JTM, Bekkering F, de Jongt FH, de Muinck Keizer-Schrama SMPF, Drop SLS, Weber RFA (1995) High dose testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood? Clin Endocrinol 43:87–95

Drop SLS, WJ de Waal, de Muinck Keizer-Schrama SMPF (1998) Sex steroid treatment of constitutionally tall stature. Endocr Rev 19:540–558

Endres W, Shin YS, Rieth M, Block T, Schmiedt E, Knorr D (1987) Priapism in Fabry's disease during testosterone treatment. Klin Wschr 65:925

Findlay JC, Place VA, Snyder PJ (1989) Treatment of primary hypogonadism in men by the transdermal administration of testosterone. J Clin Endocrinol Metab 68:369–373

Franchi F, Luisi M, Kicovic PM (1978) Long-term study of oral testosterone undecanoate in hypogonadal males. Int J Androl 1:270–278

Franchimont P, Kicovic PM, Mattei A, Roulier R (1978) Effects of oral testosterone undecanoate in hypogonadal male patients. Clin Endocrinol 9:313–320

Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R (2001) Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. Hum Reprod 16:2616–2620

Getzoff PL (1955) Clinical evaluation of testicular biopsy and the rebound phenomenon. Fertil Steril 6:465–474

Gooren LJG (1987) Androgen levels and sex functions in testosterone-treated hypogonadal men. Arch Sex Behav 16:463–473

Gooren LJG (1994) A ten year safety study of the oral androgen testosterone undecanoate. J Androl 15:212–215

Hajjar RR, Kaiser FE, Morley JE (1997) Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. J Clin Endocrinol Metab 82:3793–3796

Hamilton JB, Mestler GE (1969) Mortality and survival: comparison of eunuchs with intact men and women in a mentally retarded population. J Gerontology 24:395–411

Jain P, Rademarker AW, McVary KT (2000) Testosterone supplementation for erectile dysfunction: results of a meta-analysis. J Urol 164:371–375

Jockenhövel F, Blum WF, Vogel E, Englaro P, Müller-Wieland D, Reinwein D, Rascher W, Krone W (1997) Testosterone substitution normalizes elevated serum leptin levels in hypgonadal men. J Clin Endocrinol Metab 82:2510–2513

Jordan WP (1997) Allergy and topical irritation associated with transdermal testosterone administration: a comparison of scrotal and non-scrotal transdermal systems. Am J Cont Dermat 8:108–113

Kamischke A, Nieschlag E (1999) Analysis of medical treatment of male infertility. Hum Reprod 14: Suppl.11, 1–23

Kaplan JG, Monshant T, Bernstein R, Parks JS, Bingiovanni AM (1973) Constitutional delay of growth and development: Effect of treatment with androgens. J Pediatr 82:38–44

Kloer H, Hoogen H, Nieschlag E (1980) Trial of high-dose testosterone undecanoate in treatment of male infertility. Int J Androl 3:121

Knussmann R, Christiansen K, Kannmacher J (1992) Relations between sex hormone levels and character of hair and skin in healthy young men. Am J Phys Anthropol 88: 59–67

Lamensdorf H, Compere D, Begley G (1975) Testosterone rebound therapy in the treatment of male infertility. Fertil Steril 26:469–472

Lee KK, Berman N, Alexander GM, Hull L, Swerdloff RS, Wang C (2003) A simple self-report diary for assessing psychosexual function in hypogonadal men. J Androl 25:688–698

Leifke E, Körner HC, Link TM, Behre HM, Peters PE, Nieschlag E (1998) Effects of testosterone replacement therapy on cortical and trabecular bone mineral density, vertebral body area and paraspinal muscle area in hypogonadal men. Eur J Endocrinol 138:51–58

Lemcke B, Zentgraf J, Behre HM, Kliesch S, Bramswig JH, Nieschlag E (1996) Long-term effects on testicular function of high-dose testosterone treatment for excessively tall stature. J Clin Endocrinol Metab 81:296–301

Luboshitzky R, Aviv A, Hefetz A, Herer P, Shen-Orr Z, Lavie L, Lavie P. (2002) Decreased pituitary-gonadal secretion in men with obstructive sleep apnea. J Clin Endocrinol Metab 87:3394–3398.

Luisi M, Franchi F (1980) Double-blind group comparative study of testosterone undecanoate and mesterolone in hypogonadal male patients. J Endocrinol Invest 3:305–308

Maisey NM, Bingham J, Marks V, English J, Chakraborty J (1981) Clinical efficacy of testosterone undecanoate in male hypogonadism. Clin Endocrinol 14:625–629

Matsumoto AM, Sandblom RE, Schoene RB, Lee KA, Giblin EC, Pierson DJ, Bremner WJ (1985) Testosterone replacement in hypogonadal men: effects on obstructive sleep apnea, respiratory drives, and sleep. Clin Endocrinol 22:713–721

Meikle AW (1998) A permeation-enhanced non-scrotal testosterone transdermal system for the treatment of male hypogonadism. In: Nieschlag E, Behre HM (eds). Testosterone: Action, deficiency, substitution, 2nd edition, Springer, Heidelberg, 389–422

Meikle AW, Arver S, Dobs AS, Adolfsson J, Sanders SW, Middleton RG, Stephenson RA, Hoover DR, Rajaram L, Mazer NA (1997) Prostate size in hypogonadal men treated with a nonscrotal permeation-enhanced testosterone transdermal system. Urology 49:191–196

Meuser W, Nieschlag E (1977) Sex hormones and vocal register in adult men. Dtsch Med Wschr 102:261–264

Mooradian AD, Morley JE, Korenman SG (1987) Biological actions of androgens. Endocr Rev 8:1–28

Morales A, Johnston B, Heaton JPW, Lundie M (1997) Testosterone supplementation for hypogonadal impotence: assessment of biochemical measures and therapeutic outcomes. J Urol 157:849–854

Morales A, Lunenfeld B (2002) Investigation, treatment and monitoring of late-onset hypogonadism in males. Official recommendations of ISSAM. Aging Male 5:74–86

Morgentaler A, Carl MD, Bruning MD, De Wolf WC (1996) Occult prostate cancer in men with low serum testosterone levels. JAMA 276:1904–1906

National Institute on Ageing Advisory Panel (2001) Report of National Institute on Ageing Advisory Panel on Testosterone Replacement in Men. J Clin Endocrinol Metab 86:4611–4614

Navarro MA, Villabona CM, Blanco A, Gomez JM, Bonnin RM, Soler J (1994) Salivary excretory pattern of testosterone in substitutive therapy with testosterone enanthate. Fertil Steril 61: 125–128

Nieschlag E (1998) If testosterone, which testosterone? Which androgen regimen should be used for supplementation in older men? Formulation, dosing and monitoring issues. (1998) In: Bhasin S, Bagatell CJ, Bremmer WJ, Plymate SR, Tenover JL, Korenman SG, Nieschlag E (eds) Therapeutic perspective – Issues in testosterone replacement in older men. J Clin Endocrinol Metab 83:3443–3445

Nieschlag E, Behre HM (eds) (2000) Andrology: Male reproductive health and dysfunction. Springer, 2nd edition, Heidelberg, New York

Nieschlag E, Cüppers HJ, Wiegelmann W, Wickings EJ (1976) Bioavailability and LH suppressing effect of different testosterone preparations in normal and hypogonadal men. Horm Res 7:138

Nieschlag E, Kramer U, Nieschlag S (2003) Androgens shorten the longevity of women: sopranos last longer. Exp Clin Endocr Diab 111:230–231

Nieschlag E, Nieschlag S, Behre HM (1993) Life expectancy and testosterone. Nature 366:215

Nomura A, Heilbrunn LK, Stemmermann GN, Judd HL (1988) Prediagnostic serum hormones and the risk of prostate cancer. Cancer Res 48:3515–3517

Ozata M, Yildirimkaya M, Bulur M, Yilmaz K, Bolu E, Corakci A *et al.* (1996) Effects of gonadotropin and testosterone treatments on lipoprotein (a), high density lipoprotein particles and other lipoprotein levels in male hypogonadism. J Clin Endocrinol Metab 81:3372–3378

Pedersen MF, Moller S, Krabbe S, Bennett P (1986) Fundamental voice frequency measured by electroglottography during continuous speech. A new exact secondary sex characteristic in boys in puberty. Int J Ped Otohinol 11:21–27

Pusch HH (1989) Oral treatment of oligozoospermia with testosterone-undecanoate: results of a double-blind-placebo-controlled trial. Andrologia 21:76–82

Rebuffé-Scrive M, Marin P, Björntorp P (1991) Short communication: effect of testosterone on abdominal adipose tissue in men. Int J Obes 15:791–795

Rolf C, von Eckardstein S, Koken U, Nieschlag E (2002) Testosterone substitution of hypogonadal men prevents the age-dependent increases in body mass index, body fat and leptin seen in healthy ageing men: results of a cross-sectional study. Eur J Endocrinol 146:505–511

Rosenfeld RG, Northcraft GB, Hintz RL (1982) A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents. Pediatrics 69:681–687

Rowley MJ, Heller CG (1972) The testosterone rebound phenomenon in the treatment of male infertility. Fertil Steril 23:498–504

Ruch W, Jenny P (1989) Priapism following testosterone administration for delayed male puberty. Am J Med 86:256

Schulte-Beerbühl M, Nieschlag E (1980) Comparison of testosterone dihydrotestosterone, luteinizing hormone, and follicle-stimulating hormone in serum after injection of testosterone enanthate or testosterone cypionate. Fertil Steril 33:201–203

Schürmeyer T, Wickings EJ, Freischem CW, Nieschlag E (1983) Saliva and serum testosterone following oral testosterone undecanoate administration in normal and hypogonadal men. Acta endocrinol 102:456–462

Shabsigh R (1997) The effects of testosterone on the cavernous tissue and erectile function. World J Urol 15:21–26

Sih R, Morley JE, Kaiser FE, Perry HM, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Skakkebaek NE, Bancroft J, Davidson DW, Warner P (1981) Androgen replacement with oral testosterone undecanoate in hypogonadal men: a double blind controlled study. Clin Endocrinol 14:49–61

Snyder PJ, Lawrence DA (1980) Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 51:1335

Sokol RZ, Palacios A, Campfield LA, Saul C, Swerdloff RS (1982) Comparison of the kinetics of injectable testosterone in eugonadal and hypogonadal men. Fertil Steril 37:425–430

Sorva R, Kuusi T, Taskinen MR, Perheentupa J, Nikkila EA (1988). Testosterone substitution increases the activity of lipoprotein lipase and hepatic lipase in hypogonadal males. Atherosclerosis 69:191–197

Tchernof A, Labrie F, Belanger A, Despres JP (1996) Obesity and metabolic complications: contribution of dehydroepiandrosterone and other steroid hormones. J Endocrinol 150 Suppl:155–164

Thomas JA, Keenan EJ (1994) Effects of estrogens on the prostate. J Androl 15:97–99

Tschöp M, Behre HM, Nieschlag E, Dressendörfer RA, Strasburger CJ (1998) A time-resolved fluorescence immunoassay for the measurement of testosterone in saliva: monitoring of testosterone replacement therapy with testosterone buciclate. Clin Chem Lab Med 36:223–230

Vermeulen A, Verdonck G (1992) Representativeness of a single point plasma testosterone level for the long term hormonal milieu in men. J Clin Endocrinol Metab 74:939–942

von Eckardstein S, Nieschlag E (2002) Treatment of male hypogonadism with testosterone undecanoate injections at extended intervals of 12 weeks: a phase II study. J Androl 23:419–425

Wang C, Alexander G, Berman N, Salehian B, Davidson T, McDonald V, Steiner B, Hull H, Callegari C, Swerdloff R (1996) Testosterone replacement therapy improves mood in hypogonadal men – a clinical research center study. J Clin Endocrinol Metab 81:3578–3583

Wang L, Shi DC, Lu SY, Fang RY (1991) The therapeutic effect of domestically produced testosterone undecanoate in Klinefelter syndrome. New Drugs Mark 8:28–32

Whitworth JA, Scoggins BA, Andrews J, Williamson PM, Brown MA (1992) Haemodynamic and metabolic effects of short term administration of synthetic sex steroids in humans. Clin Exp Hypertens 14:905–922

Wittert GA, Chapman IM, Haren MT, Mackintosh S, Coates P, Morley JE (2003) Oral testosterone supplementation increases muscle and decreases fat mass in healthy elderly males with low-normal gonadal status. J Gerontol A Biol Sci Med Sci 58:618–625

WHO (World Health Organization) Nieschlag E, Wang C, Handelsman DJ, Swerdloff RS, Wu F, Einer-Jensen N, Waites G (1992) Guidelines for the use of androgens. WHO, Geneva

WHO (World Health Organization) Task Force on the Diagnosis and Treatment of Infertility (1989) Mesterolone and idiopathic male infertility: a double-blind study. Int J Androl 12:254–264

Wu FCW, Farley TMM, Peregoudov A, Waites GMH, World Health Organisation Task Force on Methods for the Regulation of Male Fertility (1996) Effects of testosterone enanthate in normal men: experience from a multicenter contraceptive efficacy study. Fertil Steril 65:626–636

Young NR, Baker HWG, Liu G, Seeman E (1993) Body composition and muscle strength in healthy men receiving testosterone enanthate for contraception. J Clin Endocrinol Metab 77:1028–1032

Zelissen PMJ, Stricker BHC (1988) Severe priapism as a complication of testosterone substitution therapy. Am J Med 85:273

Zgliczynksi S, Ossowski M, Slowinska-Srzednicka J, Brzezinska A, Zgliczynski W, Soszynksi P (1996) Effects of testosterone replacement therapy on lipids and lipoproteins in hypogonadal and elderly men. Atherosclerosis 121:35–43

Zitzmann M, Nieschlag E (2001) Testosterone levels in healthy men and their relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 144:183–197

Zitzmann M, Junker R, Kamischke A, Nieschlag E (2002a) Contraceptive steroids influence the hemostatic activation state in healthy men. J Androl 23:503–511

Zitzmann M, Rolf C, Brune M, Vieth V, Nieschlag E (2002b) Monitoring bone density in hypogonadal men by quantitative phalangeal ultrasound. Bone 31:422–429

Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E (2003) Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: a longitudinal pharmacogenetic study. J Clin Endocrinol Metab 88:2049–2054

14

Pharmacology of testosterone preparations

H.M. Behre, C. Wang, D.J. Handelsman and E. Nieschlag

Contents

14.1 Historical development of testosterone therapy

The first experimental proof that the testes produce a substance responsible for virility was provided by Berthold (1849). He transplanted testes from roosters into

the abdomen of capons and recognized that the animals with the transplanted testes behaved like normal roosters: "They crowed quite considerably, often fought among themselves and with other young roosters and showed a normal inclination toward hens". Berthold concluded that the virilizing effects were exerted by testicular secretions reaching the target organs via the bloodstream. Berthold's investigation is generally considered the origin of experimental endocrinology (Simmer and Simmer 1961). Following his observation various attempts were made to use testicular preparations for therapeutic purposes. The best known experiments are those by Brown-Séquard (1889), who tried testis extracts on himself which can at best have had placebo effects (Cussons *et al.* 2002). In the 1920s Voronoff transplanted testes from animals to humans for the purpose of rejuvenation (Voronoff 1920), but the effectiveness of his methods was disproven by a committee of the Royal Society London. The first testicular extracts with demonstrable biological activity were prepared by Loewe and Voss (1930) using the seminal vesicle as a test organ. Finally, the groundstone for modern androgen therapy was laid when steroidal androgens were first isolated from urine by Butenandt (1931), testosterone was obtained in crystalline form from bull testes by David *et al.* (1935) and testosterone was chemically synthesized by Butenandt and Hanisch (1935) and Ruzicka and Wettstein (1935).

Immediately after its chemical isolation and synthesis, testosterone was introduced into clinical medicine (unthinkable had it happened today) and used for the treatment of hypogonadism. Since testosterone was ineffective orally it was either compressed into pellets and applied subcutaneously or was used in the form of 17α-methyltestosterone. In the 1950s longer-acting injectable testosterone esters (Junkmann 1957) became the preferred therapeutic modality. In the 1950s and 1960s chemists and pharmacologists concentrated on the chemical modification of androgens in order to emphasize their erythropoetic (Gardner and Besa 1983) or anabolic effects (Kopera 1985). These preparations never played an important role in the treatment of hypogonadism and were abandoned for purposes of clinical medicine. In the late 1970s the orally effective testosterone undecanoate was added to the spectrum of testosterone preparations used clinically (Coert *et al.* 1975; Nieschlag *et al.* 1975). In the mid 1990s, transdermal testosterone patches applied either to scrotal skin (Bals-Pratsch *et al.* 1986) or non-scrotal skin (Mazer *et al.* 1992) were introduced into clinical practice. In 2000, a transdermal testosterone gel became available for treatment of male hypogonadism, first in the US and subsequently in other countries as well (Wang *et al.* 2000).

14.2 General considerations

Although testosterone has been in clinical use for almost 70 years, it has only slowly attracted interest from clinical researchers. This is partly due to the fact that hypogondal men requiring testosterone treatment constitute only a minority of all

patients and hypogonadism is not a life-threatening disease. Since development of new preparations is mainly a task of the pharmaceutical industry and hypogonadal patients did not promise to contribute a substantial economic profit, development of testosterone preparations was slow. Only recently has the question of testosterone treatment of senescent men (see Chapter 16) and, to a certain extent also the search for a hormonal male contraceptive (see Chapter 23) spurred interest in the pharmacology and application of testosterone.

Today oral, buccal, injectable, implantable and transdermal testosterone preparations are available for clinical use. There are only a few studies available comparing the various preparations with the goal of identifying the optimal preparation for substitution purposes (Conway *et al.* 1988). While the older injectable preparations, which are still the predominant form for substitution, produce supraphysiological serum testosterone levels, newer preparations achieve levels closer to the physiological range. We are only beginning to understand which serum levels are required to achieve the various biological effects of testosterone and to avoid adverse side-effects. In particular, very little is known about long-term effects of testosterone therapy inherent to different preparations. Similarly, the role of the androgen receptor polymorphism in modifying testosterone action individually is becoming understood only slowly, but may lead to a pharmacogenetic concept for the therapeutic application of testosterone (e.g. Zitzmann *et al.* 2003). Under these circumstances it appears that the consensus reached by a Workshop Conference on Androgen Therapy organised jointly by WHO, NIH and FDA in 1990 still provides the best therapeutic guidelines: "The consensus view was that the major goal of therapy is to replace testosterone levels at as close to physiologic concentrations as is possible" (WHO 1992). Until other evidence is provided, all testosterone preparations will best be judged by this principle.

An important question is which androgen preparation should be used for clinical purposes. Numerous androgenic steroids have been synthesized and used clinically in the past. The synthetic androgens were produced with the aim to enhance selectively certain aspects of testosterone activity e.g. the anabolic effect on muscles or the hematopoietic effect. Some of these molecules proved to have toxic side-effects, in particular upon long-term use (as required for substitution of hypogonadism) or the desired efficacy and safety were inadequate in controlled clinical trials (as advocated by evidence-based medicine). In addition, some of these steroids cannot be converted to 5α-DHT or estrogen, as is testosterone, and therefore cannot develop the full spectrum of activities of testosterone. The important biological significance of these conversions is described in Chapters 1 to 3 of this volume. For these reasons, synthetic preparations have almost disappeared from the market and testosterone as produced naturally is the prevailing androgen used in clinical medicine. In its various preparations testosterone has been available for over six decades and, as one of the oldest "drugs" in clinical use, has demonstrated its high safety.

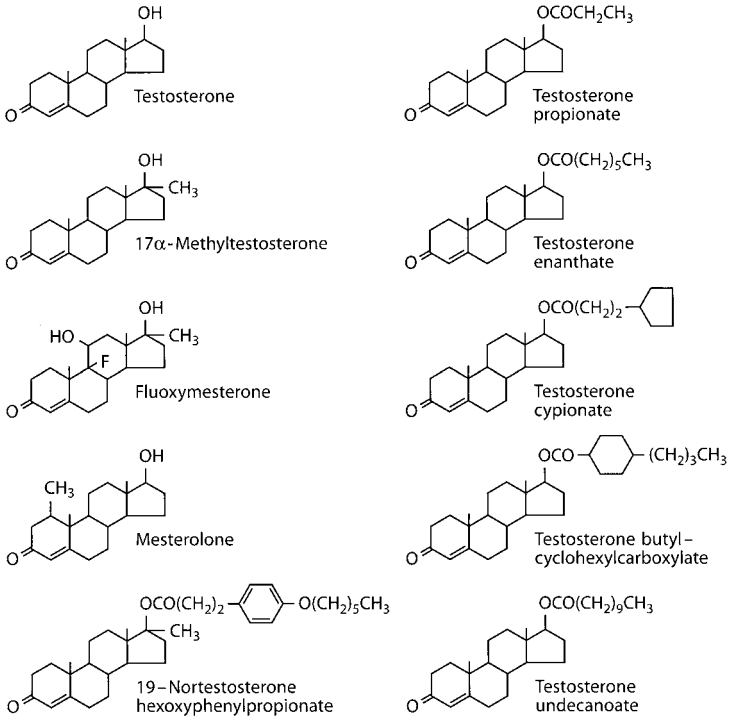


Fig. 14.1 Molecular structure of testosterone and clinically used testosterone esters and derivatives.

However, new insights into the molecular mechanisms of androgen action may lead to the development of steroids suited for specific purposes (see Chapter 20). 7α-methyl-19-nortestosterone serves as an example, as it is experiencing a renaissance due to its high androgenicity combined with low prostatotropic effects shown in hypogonadal patients (Anderson *et al.* 2003). Whether such steroids may become useful and safe for clinical use remains to be seen.

This chapter provides an overview of the various conventional and new testosterone preparations used in clinical medicine.

14.3 Pharmacology of testosterone preparations

As all other androgens, testosterone derives from the basic structure of androstane. This molecule consists of three cyclohexane and one cyclopentane ring (perhydrocyclopentanephenanthrene ring) and a methyl group each in position 10 and 13. Androstane itself is biologically inactive and gains activity through oxygroups in position 3 and 17. Testosterone, the quantitatively most important androgen synthesized in the organism, is characterized by an oxy group in position 3, a hydroxy group in position 17 and a double bond in position 4 (Fig. 14.1).

Table 14.1 Mode of application and dosage of various testosterone preparations

Preparation	Route of application	Full substitution dose
In clinical use		
Testosterone undecanoate	oral	2–4 capsules à 40 mg per day
Testosterone tablets	buccal	30 mg / twice daily
Testosterone enanthate	intramuscular injection	200–250 mg every 2–3 weeks
Testosterone cypionate	intramuscular injection	200 mg every 2 weeks
Testosterone undecanoate	intramuscular injection	1000 mg every 10–12 weeks
Testosterone implants	implantation under the abdominal skin	4 implants à 200 mg every 5–6 months
Transdermal testosterone patch	scrotal skin	1 patch per day
Transdermal testosterone patch	non-scrotal skin	1 or 2 systems per day
Transdermal testosterone gel	non-scrotal skin	5 to 10 g gel per day
Under development		
Testosterone cyclodextrin	sublingual	not yet determined
Testosterone buciclate	intramuscular injection	not yet determined
Testosterone microcapsules	subcutaneous injection	not yet determined
Obsolete		
17α-Methyltestosterone	oral	
Fluoxymesterone	sublingual/oral	

To make testosterone therapeutically effective three approaches have been used:

1) different routes of administration,
2) esterification in position 17, and
3) chemical modification of the molecule.

In addition, these approaches have been combined. Since of practical clinical relevance, the route of administration is used here for categorizing the various testosterone preparations (overview in Table 14.1).

14.3.1 Oral administration

14.3.1.1 Unmodified testosterone

Unmodified testosterone as physiologically secreted by the testes would appear to be the first choice when considering substitution therapy. When ingested orally in its unmodified form testosterone is absorbed well from the gut but is effectively metabolized and inactivated in the liver before it reaches the target organs ("first-pass-effect"). Only when a dose of 200 mg is ingested which exceeds 30fold the amount of testosterone produced daily by a normal man, is the metabolizing capacity of the liver overcome. With such doses an increase in peripheral testosterone blood levels becomes measurable and clinical effects can be observed (Daggett

et al. 1978; Johnsen *et al.* 1974; Nieschlag *et al.* 1975; 1977). The testosterone-metabolizing capacity of the liver, however, is age- and sex-dependent. An oral dose of 60 mg unmodified testosterone does not affect peripheral testosterone levels in normal adult men, but produces a significant rise in prepubertal boys and women (Nieschlag *et al.* 1977). This demonstrates that testosterone induces liver enzymes responsible for its own metabolism (Johnsen *et al.* 1976). When the liver is severely damaged its metabolizing capacity decreases. Thus, in patients with liver cirrhosis a dose of 60 mg testosterone (ineffective in normal men) produces high serum levels (Nieschlag *et al.* 1977).

In hypogonadal men with normal liver function, 400–600 mg testosterone must be administered daily if the patient is to be substituted by oral testosterone (Johnsen 1978; Johnsen *et al.* 1974), a dose exceeding the testosterone production of a normal man almost 100fold. Aside from being uneconomical, the possibility of adverse effects of such huge testosterone doses cannot be excluded, especially when given over long periods of time as required for substitution therapy. However, in a small group of patients treated for as long as seven years with oral testosterone no serious side-effects were observed (Johnson 1978). Nevertheless, oral administration of unmodified testosterone has not become a generally accepted method for therapeutic purposes.

As a relict of experiments performed last century (see 14.1), preparations containing animal testis or plant extracts or dried organ powder are still being manufactured and are available on the market. Although synthesized in the testis, the testosterone content of these preparations is negligible since the testis, in contrast to other endocrine glands (such as the thyroid), does not store its hormonal products (Cussons *et al.* 2002). Moreover, the testosterone in these orally consumed products cannot become effective for the reasons described above. Such preparations may at best exert placebo effects and do not belong to a rational therapeutic repertoire. Similarly, there is no evidence that ingestion of animal testes as food has endocrine effects.

14.3.1.2 17α-methyltestosterone

Several attempts have been made to modify the testosterone molecule by chemical means in order to render it orally effective, i.e. to delay metabolism in the liver. In this regard, the longest known testosterone derivative is 17α-methyltestosterone (Ruzicka *et al.* 1935) which is a fully effective oral androgen preparation. 17α-methyltestosterone is quickly absorbed and maximal blood levels are observed 90 to 120 minutes after ingestion. The half-life in blood amounts to approximately 150 minutes (Alkalay *et al.* 1973).

Ever since this steroid was introduced for clinical use, hepatotoxic side-effects such as an increase in serum liver enzymes (Carbone *et al.* 1959), cholestasis of the liver (de Lorimer *et al.* 1965; Werner *et al.* 1950), and peliosis of the liver (Westaby *et al.* 1977) have been reported repeatedly. It is of interest that humans are more

susceptible to the hepatotoxic effects of nethyltestosterone than rats (Heywood *et al.* 1977a) or dogs (Heywood *et al.* 1977 b). Later, an association between long-term methyltestosterone treatment and liver tumors was found (Bird *et al.* 1979; Boyd and Mark 1977; Coombes *et al.* 1978; Falk *et al.* 1979; Farrell *et al.* 1975; Goodman and Laden 1977; McCaughan *et al.* 1985; Paradinas *et al.* 1977). While these side-effects appear to be clearly related to methyltestosterone administration, the isolated observation of a seminoma in a 36-year old man on high-dose methyltestosterone seems incidental (Vogelzang *et al.* 1986).

The hepatotoxic side-effects are due to the alkyl group in the 17α position and have also been reported for other steroids with this configuration (Krüskemper and Noell 1967). Because of the side-effects methyltestosterone should no longer be used therapeutically for hypogonadism, in particular since effective alternatives are available (Nieschlag 1981). The German Endocrine Society declared methyltestosterone obsolete in 1981 and the German Federal Health Authority ruled that methyltestosterone should be withdrawn from the market (Methyltestosterone 1988). In other countries, however, methyltestosterone is still in use, a practice which should be terminated.

14.3.1.3 Fluoxymesterone

The androgenic activity of fluoxymesterone was enhanced over that of testosterone by the introduction of fluorine and the addition of a hydroxy group into the steroid skeleton of testosterone. This substance also contains a 17α-methyl group and accordingly there is a risk of hepatotoxicity with long-term use. Therefore, this androgen has disappeared from the market.

14.3.1.4 Mesterolone

Mesterolone can be considered a derivative of the 5α-reduced testosterone metabolite 5α-dihydrotestosterone (DHT) which is protected from rapid metabolism in the liver by a methyl group in position 1 (Gerhards *et al.* 1966) and thus becomes orally active. It is free of liver toxicity. Unlike testosterone, mesterolone cannot be metabolized to estrogens (Breuer and Gütgemann 1966) and at a molecular level acts like DHT. Because of its limited effectiveness in suppressing pituitary gonadotropin secretion (Aakvaag and Stromme 1974; Gordon *et al.* 1975) it can only be considered an incomplete androgen. Altogether, mesterolone is not suited for the substitution of hypogonadism. Nevertheless, in 2001 it still represented 12% of all androgen sales in Germany.

14.3.1.5 Testosterone undecanoate

When testosterone is esterified in the 17ß-position with a long fatty acid side chain such as undecanoic acid and given orally, its route of absorption from the gastrointestinal tract is slightly shifted from the vena portae to the lymph and

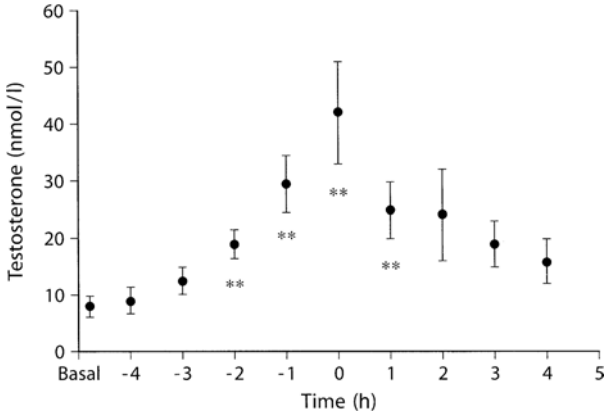


Fig. 14.2 Single-dose pharmacokinetics of testosterone undecanoate after oral administration of 120 mg of the ester to 8 hypogonadal patients. Because of high interindividual variability of testosterone serum concentrations after administration of testosterone undecanoate, individual curves were all centralized about the time of maximal serum concentrations (time 0). *Asterisks* indicate significantly higher testosterone serum concentrations compared to pretreatment values (basal) (mean ± SEM).

reaches the circulation via the thoracic duct (Coert *et al.* 1975; Horst *et al.* 1976; Shackleford *et al.* 2003). Absorption is improved if the ester is taken in arachis oil (Nieschlag *et al.* 1975) and with a meal (Frey *et al.* 1979; Bagchus *et al.* 2003). After oral ingestion of a 40 mg capsule, of which 63% i.e. 25 mg is testosterone, maximum serum levels are reached two to six hours later (Nieschlag *et al.* 1975). Thus, with 2 to 4 capsules (80 to 160 mg) per day substitution of hypogonadism can be achieved.

Testosterone undecanoate pharmacokinetics after single-dose administration were tested in eight hypogonadal patients and twelve normal men (Schürmeyer *et al.* 1983). Directly before and at hourly intervals after oral application of three times 40 mg of testosterone undecanoate in arachis oil taken together with a standardized breakfast, matched saliva samples, as a parameter for free testosterone at the tissue level, and blood samples were collected hourly for up to 8 h. After administration of testosterone undecanoate, serum and saliva testosterone always showed a parallel rise and fall, as demonstrated by a constant saliva/serum testosterone ratio. On average maximum levels could be observed five hours after testosterone undecanoate administration. However, the serum testosterone profile showed high interindividual variability of the time when maximum concentrations were reached, as well as of the maximum levels themselves that ranged from 17 to 96 nmol/l. When the individual serum concentration versus time curves were centralized about the time of maximal serum concentrations, serum concentrations significantly different from basal values were seen only two hours before and one hour after the time of maximal serum concentrations in hypogonadal patients (Fig. 14.2) (Schürmeyer

et al. 1983). Based on this observation it can be deduced that even with administration of testosterone undecanoate 3 times daily, only short-lived testosterone peaks resulting in high fluctuations can be obtained.

This judgment is in agreement with the data of a two-month multiple-dose study with testosterone undecanoate for replacement therapy in hypogonadal men (Skakkebaek *et al.* 1981). Applying a double blind cross-over design, serum testosterone levels were studied in 12 hypogonadal patients to whom 80 mg of testosterone undecanoate had been administered twice per day 12 hours apart. Whereas four hours after administration of testosterone undecanoate a significant increase of testosterone serum levels was observed compared to the placebo group, twelve hours after administration no significant difference in testosterone serum levels between treatment and placebo control group was seen. Even four hours after administration, in four of twelve patients testosterone levels were still below the lower level of the normal range after both one month and two months of treatment. A significant marked variability between subjects as well as within the same subjects has also been observed in other clinical studies (Cantrill *et al.* 1984; Conway *et al.* 1988).

The original preparation of oral testosterone undecanoate had to be refrigerated (2–8°C) in the pharmacy for reasons of stability, whereas patients must store it at room temperature to ensure optimal absorption. The shelf-life at room temperature is only three months. Therefore, a new, more stable pharmaceutical formulation of testosterone undecanoate was developed in which the oleic acid solvent was replaced by castor oil and propylene glycol laurate. This new formulation can be stored at room temperature (15–30°C) for three years (Bagchus *et al.* 2003). According to an unpublished randomized multicenter study in 49 hypogonadal men, oral administration of 2 × 80 mg or 3 × 80 mg of the reformulated testosterone undecanoate might result in more physiological and stable serum testosterone levels.

14.3.2 Sublingual application

17α-methyltestosterone was found to be more effective when applied sublingually than when ingested orally (Escamilla 1949). This type of substitution should, however, not be practised because of the liver toxicity of methyltestosterone summarized above. The solubility of the hydrophobic testosterone molecule can be enhanced by incorporation into hydroxypropyl-ß-cyclodextrins (Pitha *et al.* 1986) which are macro-ring structures consisting of cyclic oligosaccharides. When testosterone incorporated into such cyclodextrins is administered sublingually steep increases in serum testosterone occur lasting for one or two hours (Stuenkel *et al.* 1991). Hypogonadal men treated with three daily doses for 60 days showed improvement of their condition (Salehian *et al.* 1995; Wang *et al.* 1996). This is an interesting approach to testosterone substitution, but unless more constant serum levels can

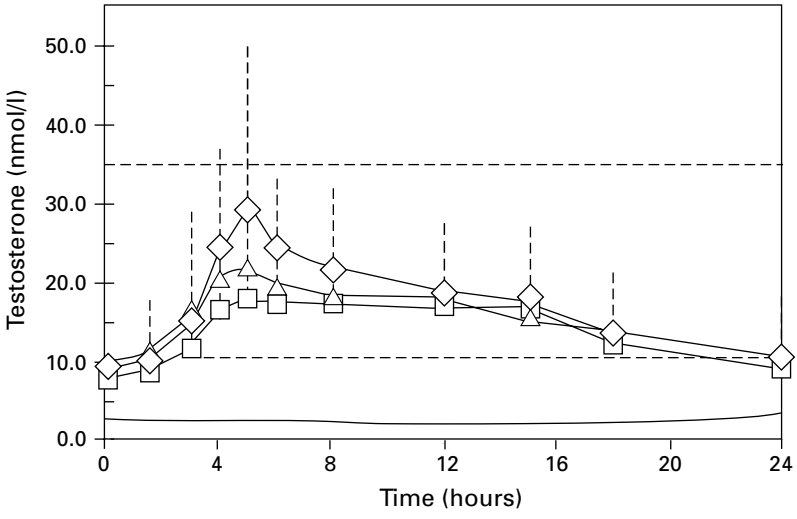


Fig. 14.3 Mean (±SD), baseline-adjusted serum concentrations of testosterone after application of placebo (solid line), 10 mg (squares), 20 mg (triangles) and 30 mg (diamonds) of buccal testosterone at steady state (day 10 of dosing). Broken lines indicate normal range of testosterone (adapted from Baisley *et al.* 2002, reproduced by permission of the Society of Endocrinology).

be achieved this therapy would require repeated daily applications and would have the same disadvantages as conventional oral testosterone undecanoate therapy.

14.3.3 Buccal application

Administration of testosterone via the buccal mucosa bypasses the liver and avoids first-pass clearance by delivering the drug directly into systemic circulation. Compared to sublingual administration, buccal mucosa is less permeable and potentially better suited for sustained delivery systems. An initial randomized, double-blind, placebo-controlled study in hypogonadal patients receiving 10 mg testosterone or placebo buccal tablets showed unfavourable pharmacokinetics with serum levels of testosterone far above the upper normal range and returning to baseline as soon as four to six hours after administration (Dobs *et al.* 1998).

Significantly improved pharmacokinetics were obtained with newly formulated buccal tablets. In a randomized, double-blind, crossover design 24 healthy men received a GnRH agonist for suppression of endogenous testosterone (Baisley *et al.* 2002). Buccal tablets containing 10, 20 or 30 mg testosterone were taken daily at 8.00 h for 10 days. Steady state was reached by day 5. Peak total and free testosterone were reached eight to nine hours after tablet application (Fig. 14.3). Hormone concentrations increased with the testosterone dose of the tablets, but this increase was less than dose-proportional. Whereas the average concentration of testosterone did

not exceed the normal range, some individual blood samples still showed supraphysiological testosterone concentrations. About half of the volunteers reported local discomfort at the buccal application site, in most subjects during the first treatment period. The advantage of this buccal testosterone preparation seems to be the mimicking of the physiological circadian testosterone rhythm, however, long-term studies in hypogonadal patients including evaluation of acceptability are awaited.

A new testosterone bioadhesive buccal system was designed to adhere rapidly to the buccal mucosa and gellify for delivering testosterone steadily into the circulation. The pharmacokinetics were evaluated in 82 hypogonadal men. The tablet (30 mg testosterone) was applied twice daily to the upper gums for three months. 73% of the patients reached an average testosterone concentration over 24 hours within the physiological range. Local problems associated with tablet use were transient and minimal. This bioadhesive buccal system is approved for use in hypogonadal men in the U.S.A. and approval in Europe is expected.

14.3.4 Nasal application

The first-pass effect of the liver can also be avoided by applying testosterone to the nasal mucosa (Danner and Frick 1980). However, unreliable absorption patterns and short-lived serum peaks prevent this form of application from becoming a desirable option for long-term substitution therapy and it has never passed the experimental state.

14.3.5 Rectal application

In order to avoid the first-pass effect of the liver, testosterone can be applied rectally in suppositories (Hamburger 1958). Administration of a suppository containing 40 mg testosterone results in an immediate and steep rise of serum testosterone lasting for about four hours. Effective serum levels can be achieved by repeated applications (Nieschlag *et al.* 1976). This therapy, however, never gained much popularity probably because the patients find it unacceptable to use suppositories three times daily on a long-term routine basis.

14.3.6 Intramuscular application

The most widely-used testosterone substitution therapy is the intramuscular injection of testosterone esters. Unmodified testosterone has a half-life of only ten minutes and would have to be injected very frequently. Esterification of the testosterone molecule at position 17, e.g., with propionic or enanthic acid, prolongs the activity of testosterone in proportion to the length of the side chain when administered intramuscularly (Junkmann 1952; 1957). The deep intramuscular injection of testosterone esters in oily vehicle is generally safe and well tolerated, but can cause minor side-effects such as local pain (Mackey *et al.* 1995).

Studies applying gas chromatography-mass spectrometry that allow discrimination between endogenous testosterone and exogenously administered deuterium-labelled testosterone propionate-19,19,19-d_3 and its metabolite testosterone-19,19,19-d_3 were able to show that after intramuscular administration, the testosterone ester is slowly absorbed into the general circulation and then rapidly converted to the active unesterified metabolite (Fujioka *et al.* 1986). The observation that the duration at the injection site is the major factor determining the residence time of the drug in the body agrees with pharmacokinetic studies in rats showing that the androgen ester 19-nortestosterone decanoate, when injected into the musculus gastrocnemius of the rat in vivo, is absorbed unchanged from the injection depot in the muscle into the general circulation according to first-order kinetics with a long half-life of 130 h (van der Vies 1965). Comparisons of the absorption kinetics of different testosterone esters clearly show that the half-lives of the absorption of the esters increase when the esterified fatty acids have a longer chain (van der Vies 1985). In addition, pharmacokinetics are influenced by the oily vehicle, the injection site and the injection volume (Minto *et al.* 1997).

After absorption from the intramuscular depot, the testosterone ester is rapidly hydrolysed in plasma, as could be shown by in vitro rat studies (van der Vies 1970) and in vivo human studies (Fujioka *et al.* 1986). The rate of hydrolysis again depends on the structure of the acid chain, but this process is much faster than release from the injection depot (van der Vies 1985). The metabolism of the testosterone ester to the unesterified testosterone occurs rapidly so that testosterone enanthate or testosterone have nearly identical intravenous pharmacokinetics (Sokol and Swerdloff 1986). Similarly, the duration of action of the orally effective ester testosterone undecanoate seems to be dependent on the duration of absorption of the uncleaved lipophilic testosterone undecanoate via the ductus thoracicus from the gut (Maisey *et al.* 1981; Schürmeyer *et al.* 1983).

In men treated with testosterone, the testosterone concentration measurable in the serum is the sum of endogenous testosterone and exogenous testosterone hydrolysed from the injected ester. Hypogonadal patients are characterized by impaired or absent endogenous testosterone secretion; exogenous testosterone administration can further suppress endogenous testosterone secretion only to a limited degree, if at all. Accordingly, in hypogonadal patients the serum concentration versus time profile is mainly a reflection of the pharmacokinetics of exogenously administered testosterone ester alone. In this chapter the evaluation of pharmacokinetic parameters for different testosterone esters is based on the increases of testosterone serum concentrations over basal levels in hypogonadal patients.

Table 14.2 Comparative pharmacokinetics of different testosterone esters after intramuscular injection to hypogonadal patients

Testosterone ester	Terminal elimination half-life (d)
Testosterone propionate	0.8
Testosterone enanthate	4.5
Testosterone buciclate	29.5
Testosterone undecanoate	33.9

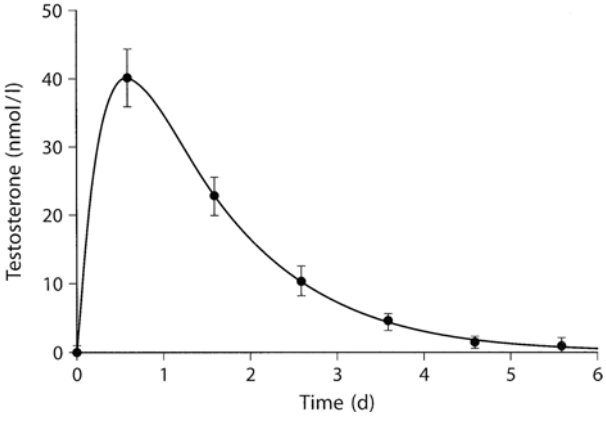


Fig. 14.4 Single-dose pharmacokinetics of testosterone propionate in seven hypogonadal patients. *Closed circles*, mean ± SEM of testosterone serum concentrations actually measured; *curve*, best-fitted pharmacokinetic profile.

14.3.6.1 Testosterone propionate

Single-dose pharmacokinetics of 50 mg testosterone propionate after intramuscular injection to seven hypogonadal patients and the best-fitted pharmacokinetic profile are shown in Fig. 14.4 (Nieschlag *et al.* 1976). Maximal testosterone levels in the supraphysiological range were seen shortly after injection (40.2 nmol/l, t_{max} = 14 h). Testosterone levels below the normal range were observed following day 2 (57 h) after injection. The calculated values for AUC were 1843 nmol * h/l, for MRT 1.5 d and 0.8 d for terminal half-life (Table 14.2).

Based on single-dose pharmacokinetic parameters, a multiple-dose pharmacokinetic simulation was performed. Expected testosterone serum concentrations after multiple dosing of 50 mg testosterone propionate twice per week (e.g. injections Mondays and Thursdays, 8.00 h) are shown in Fig. 14.5. Shortly after injection high supraphysiological testosterone serum concentrations of up to 45 nmol/l are observed. At the end of the injection interval (three and four days, respectively)

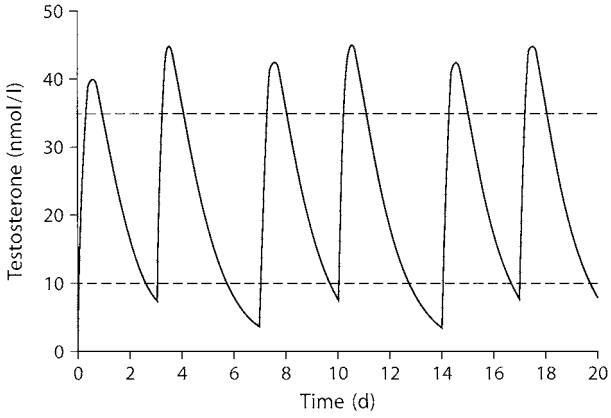


Fig. 14.5 Multiple-dose pharmacokinetics of testosterone propionate after injection of 50 mg testosterone propionate twice per week (e.g. Mondays and Thursdays). *Solid curve*, pharmacokinetic simulation; *broken lines*, range of normal testosterone values.

testosterone serum concentrations below the lower range of normal testosterone values are projected (7 nmol/l and 3 nmol/l, respectively).

Judged by the data from pharmacokinetic analysis and simulation, administration of testosterone propionate is not suitable for substitution therapy of male hypogonadism because of its short-term kinetics resulting in wide fluctuations of testosterone serum concentrations and maximal injection intervals of three days for the 50 mg dose.

14.3.6.2 Testosterone enanthate

Single-dose pharmacokinetics of testosterone enanthate after intramuscular administration of 250 mg testosterone enanthate to seven hypogonadal patients and the best-fitted pharmacokinetic profile are shown in Fig. 14.6 (Nieschlag *et al.* 1976). Maximal testosterone levels in the supraphysiological range were seen shortly after injection (39.4 nmol/l, t_{max} = 10 h). Testosterone levels below the normal range were observed following day 12 after injection. The calculated values were 9911 nmol * h/l for AUC, 8.5 d for MRT and 4.5 d for terminal half-life (Table 14.2).

Based on the pharmacokinetic parameters of single-dose pharmacokinetics multiple-dose pharmacokinetic simulations for equal doses of 250 mg testosterone enanthate and injection intervals of one to four weeks were performed. With weekly injection intervals supraphysiological maximal testosterone serum concentrations up to 78 nmol/l are observed at steady state shortly after injection and supraphysiological minimal testosterone serum concentrations up to 40 nmol/l just before the next injection (Fig. 14.7). Injecting 250 mg of testosterone enanthate every two

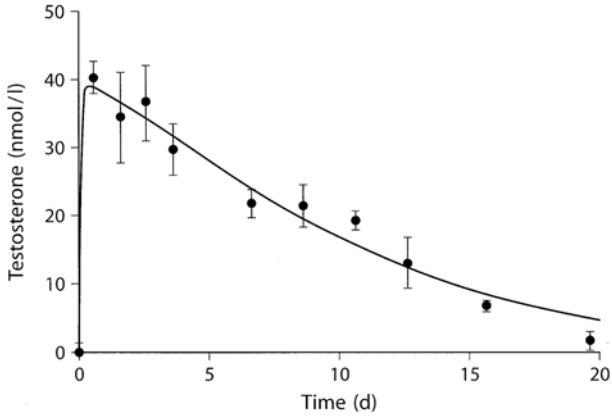


Fig. 14.6 Single-dose pharmacokinetics of testosterone enanthate in seven hypogonadal patients. *Closed circles*, mean ± SEM of testosterone serum concentrations actually measured; *curve*, best-fitted pharmacokinetic profile.

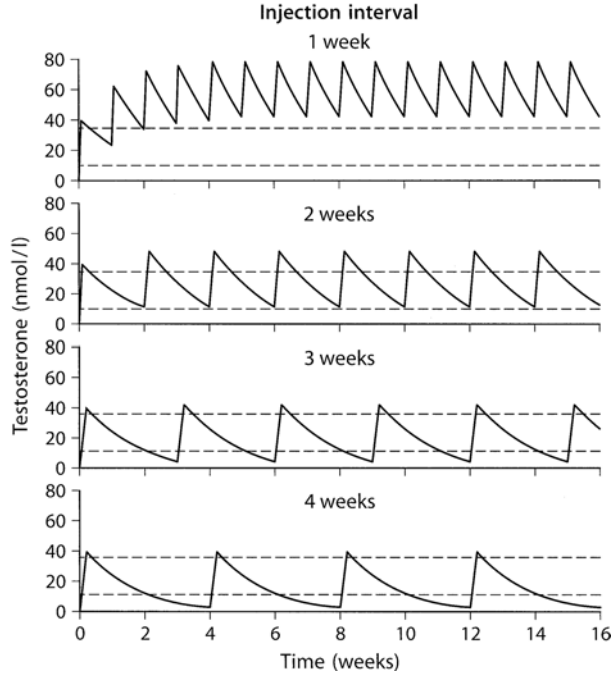


Fig. 14.7 Multiple-dose pharmacokinetics of testosterone enanthate after injection of 250 mg testosterone enanthate every week (*upper panel*), every second week (*upper middle panel*), every three weeks (*lower middle panel*) and every four weeks (*lower panel*). *Solid curves*, pharmacokinetic simulations; *broken lines*, range of normal testosterone values.

weeks results in maximal supraphysiological testosterone serum concentrations of up to 51 nmol/l shortly after injection and testosterone serum levels at the lower range for normal testosterone serum concentration shortly before the next injection. If the injection interval is extended to three weeks, testosterone serum concentrations below the normal range are observed 14 days after injection. With injection intervals of four weeks, testosterone serum concentrations are in the subnormal range at week three and four and effective testosterone substitution is not guaranteed (Fig. 14.7).

The calculated testosterone serum concentrations at steady state obtained by computer simulation correspond well to the results of published studies describing multiple-dose testosterone enanthate pharmacokinetics. In a clinical trial for male contraception 20 healthy men were injected with 200 mg/wk of testosterone enanthate for 12 weeks (Cunningham *et al.* 1978). Minimal serum concentrations of testosterone at steady state, i.e. the testosterone serum concentration just before the next injection, were measured at 31.2 nmol/l to 39.5 nmol/l after weekly injections of 200 mg testosterone enanthate. Very similar data were obtained in further contraceptive studies when normal men received 200 mg/wk testosterone enanthate injections for 18 months (Anderson and Wu 1996; Wu *et al.* 1996). The data of these studies fit well with the computer-calculated minimal testosterone serum concentrations of 40 nmol/l and maximal testosterone levels 78 nmol/l after multiple injections of testosterone enanthate at a dosage of 250 mg/wk.

Snyder and Lawrence (1980) administered 100 mg/wk (n = 12), 200 mg/2 wks (n = 10), 300 mg/3 wks (n = 9) and 400 mg/4 wks (n = 6) testosterone enanthate to hypogonadal patients during a study period of three months. Blood was drawn during the last injection period, when steady state had been reached, every day (100 mg/wk) up to every fourth day (400 mg/4 wks). Similar to the computer simulation described above for 250 mg testosterone enanthate and injections intervals of one to four weeks, initial supraphysiological testosterone serum levels were seen shortly after injection. In the 100 mg/wk treatment group, where daily blood sampling was performed, mean peak serum concentrations were seen 24 h after injection. Comparable to the results of the computer simulation, after injection of 200 mg/2 wks testosterone enanthate, following initial supraphysiological testosterone serum levels, values fell to progressively lower values before the next injection, eventually reaching the lower normal limit (Snyder and Lawrence 1980). Similar results were described after injection of 300 mg/3 wks or 400 mg/4 wks testosterone enanthate. The authors conclude that the testosterone enanthate doses of 200 mg have to be injected every two weeks or doses of 300 mg every 3 weeks to guarantee effective substitution therapy.

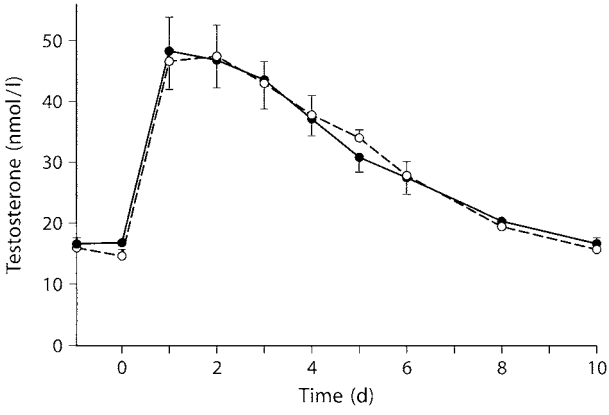


Fig. 14.8 Comparative pharmacokinetics of 194 mg of testosterone enanthate and 200 mg of testosterone cypionate after intramuscular injection to 6 normal volunteers. *Closed circles*, mean ± SEM of testosterone enanthate kinetics; *open circles*, mean ± SEM of testosterone cypionate kinetics.

14.3.6.3 Testosterone cypionate and testosterone cyclohexanecarboxylate

Testosterone cypionate (cyclopentylpropionate) pharmacokinetics were compared with those of testosterone enanthate in a cross-over study involving six healthy men aged 20–29 years. Three subjects received 194 mg of testosterone enanthate, followed seven weeks later by 200 mg of testosterone cypionate and vice versa (amount of unesterified testosterone 140 mg in both preparations). The serum testosterone profiles were identical after injection of both preparations in equivalent doses, both in terms of maximal concentrations and in terms of duration of elevation above basal levels (Fig. 14.8) (Schulte-Beerbühl and Nieschlag 1980).

In a subsequent clinical study the pharmacokinetics of testosterone cyclohexanecarboxylate were compared to the pharmacokinetics of testosterone enanthate in a single-blind cross-over study in seven healthy young men (Schürmeyer and Nieschlag 1984). After injection of either testosterone enanthate or testosterone cyclohexanecarboxylate, testosterone concentrations in serum increased sharply and reached maximum levels, 4–5 times above basal, 8–24 h after injection. During following days a parallel decay of testosterone levels occurred after injection of either ester preparations, with testosterone serum concentrations slightly, but significantly lower after testosterone cyclohexanecarboxylate injection compared to testosterone enanthate injection two, three and seven days after administration. Basal serum levels were reached seven days after testosterone cyclohexanecarboxylate administration and nine days after injection of testosterone enanthate.

Because testosterone cypionate, testosterone cyclohexanecarboxylate and testosterone enanthate had comparable suppressing effects on LH and consequently on endogenous testosterone secretion, it can be concluded from these studies in normal volunteers that all three esters with similar molecular structure possess comparable pharmacokinetics of exogenous testosterone serum concentrations. Testosterone cypionate or testosterone cyclohexanecarboxylate do not provide a more advantageous pharmacokinetic profile than testosterone enanthate. This observation is in agreement with a clinical study of replacement therapy with single-dose administration of 200 mg of testosterone cypionate in 11 hypogonadal patients (Nankin 1987).

14.3.6.4 Testosterone ester combinations

Testosterone ester mixtures have been widely used for substitution therapy of male hypogonadism (e.g. *Testoviron*^{R}*Depot 50*: 20 mg testosterone propionate and 55 mg testosterone enanthate; *Testoviron*^{R}*Depot 100*: 25 mg testosterone propionate and 100 mg testosterone enanthate; *Sustanon*^{R} 250: 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaproate and 100 mg testosterone decanoate). These combinations are used following the postulate that the so-called short-acting testosterone ester (e.g testosterone propionate) is the effective testosterone for substitution during the first days of treatment and the so-called long-acting testosterone (e.g. testosterone enanthate) warrants effective substitution for the end of injection interval. However, this assumption is not supported by the pharmacokinetic parameters of the individual testosterone esters. Both testosterone propionate and testosterone enanthate cause highest testosterone serum concentrations shortly after injection (Fig. 14.4 and Fig. 14.6). Accordingly, addition of testosterone propionate to testosterone enanthate only increases the initial undesired testosterone peak and worsens the pharmacokinetic profile that ideally should follow zero-order kinetics (Fig. 14.9). The computer simulation agrees well with the limited published single-dose testosterone values that have been measured in hypogonadal patients treated with the combination of testosterone propionate and testosterone enanthate. Maximal increases of approximately 40 nmol/l testosterone over basal values are described one day after intramuscular administration of a testosterone ester combination of 115.7 mg testosterone enanthate and 20 mg testosterone propionate to three hypogonadal patients (Fukutani *et al.* 1974).

A comparison of computer-simulated testosterone serum concentrations after multiple-dose injections of Testoviron^{R} Depot 100 (110 mg testosterone enanthate and 25 mg testosterone propionate = 100 mg unesterified testosterone) every 10 d and 139 mg testosterone enanthate (= 100 mg unersterified testosterone) every 10 d is shown in Fig. 14.10. As can be expected by the single-dose kinetics of the

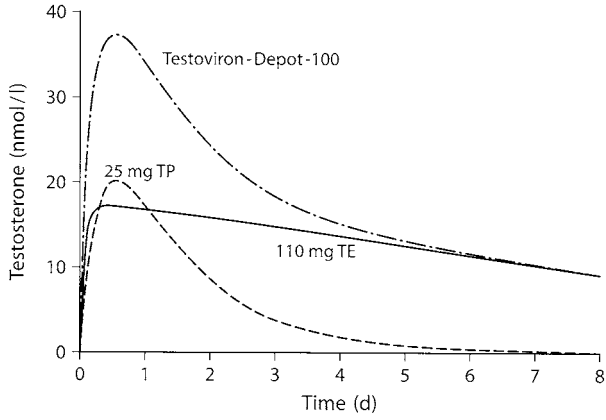


Fig. 14.9 Pharmacokinetic profile of Testoviron^{R} Depot 100 (110 mg testosterone enanthate and 25 mg testosterone propionate) in comparison to the pharmacokinetics of the individual testosterone esters of the mixture. *Curves*, pharmacokinetic simulations.

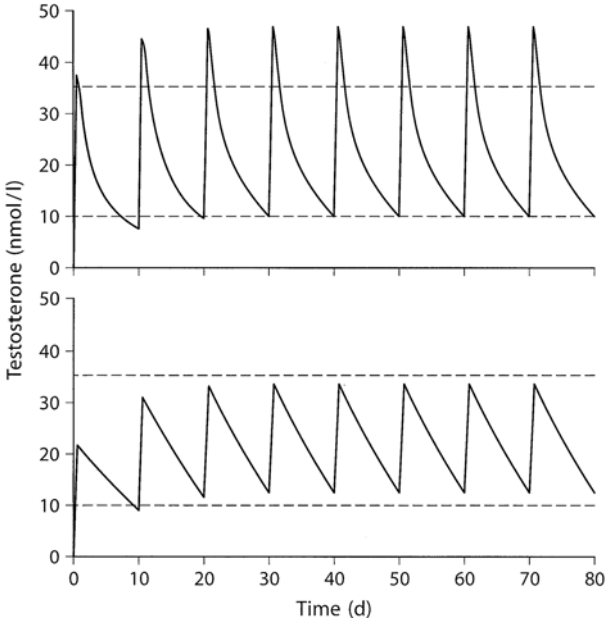


Fig. 14.10 Multiple-dose pharmacokinetics of the testosterone ester mixture Testoviron^{R} Depot 100 (110 mg testosterone enanthate and 25 mg testosterone propionate = 100 mg unesterified testosterone, upper panel) every 10 d in comparison to 139 mg testosterone enanthate (= 100 mg unersterified testosterone, lower panel) every 10 d. *Solid curves*, pharmacokinetic simulations; *broken lines*, range of normal testosterone values.

individual esters, injection of the testosterone ester mixture (upper panel) produces a much wider fluctuation of testosterone serum concentrations relative to injection of testosterone enanthate alone (lower panel). This simulation shows that injections of testosterone enanthate alone produce a more favourable pharmacokinetic profile in comparison to injections of testosterone propionate and testosterone enanthate ester mixtures in comparable doses. For treatment of male hypogonadism there is no advantage in combining the available short- and long-acting testosterone esters.

14.3.6.5 Testosterone buciclate

The disadvantage of all esters described so far is that they produce initially supraphysiological testosterone levels which may exceed normal levels severalfold and then slowly decline, so that before the next injection pathologically low levels may be reached. Some patients recognize these ups and downs of testosterone levels in parallel variations of general well-being, sexual activity and emotional stability. Despite these disadvantages testosterone enanthate and cypionate are still the standard therapy for male hypogonadism.

Because of these shortcomings of the available esters the World Health Organization (WHO) initiated a steroid synthesis programme (Crabbé *et al.* 1980) out of which a series of new testosterone esters was developed. When tested in laboratory rodents a specific ester was identified that showed greatly prolonged activity, namely *testosterone-trans-4-n-butylcyclohexyl-carboxylate*, generic name *testosterone buciclate*. This preparation is injected intramuscularly in an aqueous solution, in contrast to the other testosterone esters which are dissolved in oily solution.

A first study on the pharmacokinetics of the new WHO/NIH androgen ester testosterone buciclate was performed in two groups of orchiectomized cynomolgus monkeys (Weinbauer *et al.* 1986). Intramuscular injections of testosterone enanthate resulted in supraphysiological serum levels of testosterone for eight days, followed by a rapid decline with levels lower than the physiological limit after three weeks. In contrast, testosterone buciclate produced a moderate increase of serum testosterone levels into the physiological range, and serum levels remained in this range for a period of four months. These favourable results on the pharmacokinetics of testosterone buciclate were confirmed in castrated rhesus monkeys (Rajalakshmi and Ramakrishnan 1989).

To assess the pharmacokinetics of testosterone buciclate in men the first clinical study was performed in eight men with primary hypogonadism (Behre and Nieschlag 1992). The men were randomly assigned to two study groups and were given either 200 (group I) or 600 mg (group II) testosterone buciclate intramuscularly. Whereas in group I serum androgen levels did not rise to normal values, in group II androgens increased significantly and were maintained in the normal range up to 12 weeks with maximal serum levels (c_{max}) of 13.1 $\pm$ 0.9 nmol/l

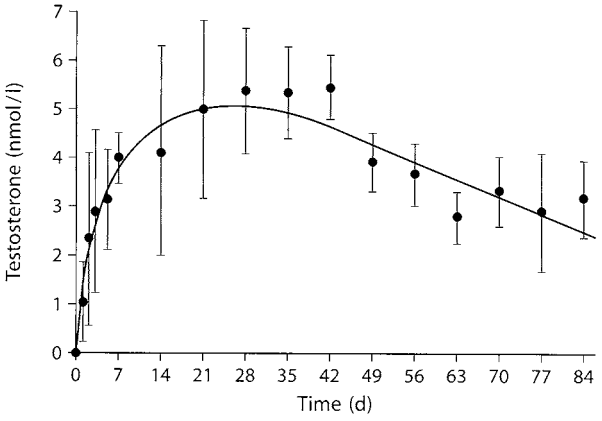


Fig. 14.11 Single-dose pharmacokinetics of testosterone buciclate after intramuscular injection of 600 mg of the ester to four hypogonadal patients. *Closed circles*, mean ± SEM of testosterone serum concentrations actually measured; *curve*, best-fitted pharmacokinetic profile.

(mean ± SEM) in study week 6 (t_{max}). No initial burst release of testosterone was observed in either study group. Pharmacokinetic analysis revealed a terminal elimination half-life of 29.5 ± 3.9 days (Fig. 14.11) (Table 14.2).

Because of the promising results of the first clinical study with testosterone buciclate, a follow-up study was initiated. After complete wash-out from previous therapy all hypogonadal men received a single intramuscular injection of 1000 mg testosterone buciclate. As in the previous study with lower doses, no initial burst release of testosterone was observed. Maximal testosterone serum levels were observed nine weeks (t_{max}) after injection with a mean value of 13.1 ± 1.8 nmol/l (c_{max}). Following peak concentrations, testosterone serum levels gradually declined and remained within the normal range up to week 16. This study demonstrated that an increase of the injected dose of testosterone buciclate from 600 to 1000 mg prolongs the duration of action significantly, but does not lead to significantly higher maximal serum levels of testosterone.

The long duration of action of testosterone buciclate was also demonstrated in a contraceptive study with this new testosterone ester. After a single intramuscular injection of 1200 mg testosterone buciclate at a concentration of 400 mg/ml to eight normal men, serum levels of testosterone remained within the normal range, whereas gonadotropins and spermatogenesis was significantly suppressed for at least 18 weeks (Behre *et al.* 1995). These studies demonstrate that the long-acting testosterone buciclate is well suited for substitution therapy of male hypogonadism as well as for male contraception. However, this compound has not been developed into a marketable product and is currently not available.

14.3.6.6 Testosterone undecanoate

While testosterone undecanoate has been available for oral substitution for more than two decades, it was first demonstrated in China that intramuscular administration of testosterone undecanoate in tea seed oil (125 mg/ml) has a prolonged duration of action (Wang *et al.* 1991). Therefore the pharmacokinetics of testosterone undecanoate in comparison to testosterone enanthate were tested in two groups of orchiectomized cynomolgus monkeys (Partsch *et al.* 1995). After injection of 10 mg/kg body weight of the respective esters serum levels of testosterone remained above the lower limit of normal for 108 days, compared to 31 days after testosterone enanthate injection. Pharmacokinetic analysis revealed a terminal half-life of 25.7 $\pm$ 4.0 days for testosterone undecanoate, compared to 10.3 $\pm$ 1.1 days for testosterone enanthate. The maximal testosterone concentration of 72.6 $\pm$ 11.7 nmol/l after testosterone undecanoate injection was significantly lower than 177.0 $\pm$ 21.3 nmol/l after testosterone enanthate injection.

In a recent monkey study it was demonstrated that biological effects of testosterone esters are determined by the pharmacokinetics and degree of aromatization rather than the total dose administered (Weinbauer *et al.* 2003). Twenty adult male cynomolgus monkeys were randomly assigned to treatment for 28 weeks with either testosterone enanthate every four weeks, testosterone buciclate every seven weeks, or testosterone undecanoate every ten weeks. Each injection delivered 20 mg pure testosterone per kilogram body weight. Despite a smaller total dose of testosterone, increase in body weight or lowering effects on serum lipids were significantly stronger with the long-acting testosterone undecanoate or buciclate compared to testosterone enanthate.

In a clinical study in Asian hypogonadal men, eight patients received one intramuscular injection of 500 mg and 7 of the initial 8 hypogonadal patients one injection of 1000 mg testosterone undecanoate (in eight milliliters tea seed oil) in a cross-over design (Zhang *et al.* 1998). Follow-up blood samples were obtained weekly up to week 9 after injection. In both study groups, mean serum levels of testosterone were above the upper limit of normal during the first two weeks after injection. Thereafter, mean serum concentration remained in the normal range up to week 7 after injection in the 500 mg-dose group and at least up to week 9 in the 1000 mg-dose group. The terminal elimination half-lives were 18.3 $\pm$ 2.3 and 23.7 $\pm$ 2.7 days for the 500 mg-dose und 1000 mg-dose groups, respectively. Administration of 500 mg of this testosterone preparation every four weeks, after an initial loading dose of 1000 mg, for up to 12 months to 308 healthy men for male contraception maintained serum levels of testosterone in the normal range when measured directly before the next injection (Gu *et al.* 2003).

In the first study in Caucasian men, intramuscular injections of 250 mg or 1000 mg testosterone undecanoate in tea seed oil were given to 14 hypogonadal

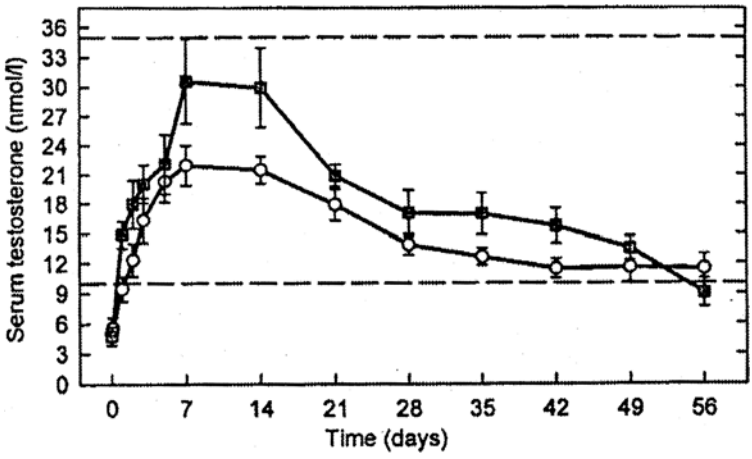


Fig. 14.12 Serum concentrations (mean ± SEM) of testosterone after single-dose intramuscular injections of 1000 mg testosterone undecanoate in tea seed oil in 7 hypogonadal men (squares) or castor oil in 14 hypogonadal men (circles). Broken lines indicate normal range of testosterone (adapted from Behre *et al*. 1999a, reproduced by permission of the European Journal of Endocrinology).

patients (Behre *et al.* 1999a). Follow-up examinations were performed 1, 2, 3, 5 and 7 days after injection and then weekly up to study week 8. Whereas no prolonged increase of testosterone was observed in the 250 mg-group, serum levels of testosterone in the higher dose group increased from 4.8 ± 0.9 nmol/l (mean ± SEM) to maximum levels of 30.5 ± 4.3 nmol/l at day 7 (t_{max}). Testosterone levels remained within the normal range up to week 7 (13.5 ± 1.2 nmol/l). Non-linear regression analysis revealed a terminal elimination half-life for intramuscular testosterone undecanoate of 20.9 ± 6.0 days (Fig. 14.12).

Similar to the preclinical study in monkeys, the clinical study in hypogonadal men demonstrated favourable pharmacokinetics of intramuscular testosterone undecanoate. Because of the relatively low concentration of 125 mg testosterone undecanoate per milliliter tea seed oil, however, administration of the 1000 mg dose requires an injection volume of 8 ml which renders intramuscular administration impracticable. Therefore, the preparation was reformulated and testosterone undecanoate dissolved in castor oil at a higher concentration of 250 mg/ml. 14 hypogonadal patients received one intramuscular injection of 1000 mg of the reformulated testosterone undecanoate preparation (Behre *et al.* 1999a). Maximal serum levels with the reformulated preparation were lower than with the Chinese preparation and remained within the mid-normal range (Fig. 14.12). Pharmacokinetic analysis revealed a long terminal elimination half-life of 33.9 ± 4.9 days (Table 14.2).

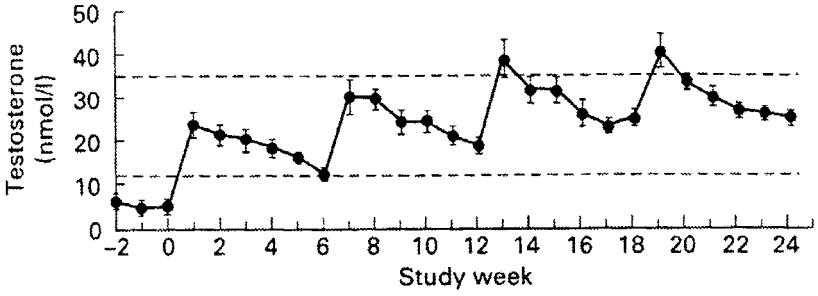


Fig. 14.13 Serum concentrations (mean ± SEM) of testosterone after multiple intramuscular injections of 1000 mg testosterone undecanoate in castor oil in 13 hypogonadal men. Broken lines indicate normal range of testosterone (adapted with permission from Nieschlag *et al.* 1999, copyright 1999, Blackwell Publishing).

Due to these favourable pharmacokinetics a first, prospective, open-label study with repeated intramuscular injection was initiated (Nieschlag *et al.* 1999). 13 hypogonadal men received four intramuscular injections of 1000 mg testosterone undecanoate in castor oil at six-week intervals. Following the first injection, mean serum levels of testosterone were never found below the lower limit of normal (Fig. 14.13). However, peak and trough serum concentrations of testosterone increased during the six-month treatment, with testosterone levels above the upper normal limit after the third and fourth injection. Therefore, in seven of the 13 hypogonadal men injections were given at gradually increasing intervals between the fifth and tenth injection, and from then on every 12 weeks (von Eckardstein and Nieschlag 2002). During steady state, serum levels of testosterone remained in the normal range with maximal concentrations of 32.0 ± 11.7 nmol/l (mean ± SD) one week after injection and nadir levels before the next injection of 12.6 ± 3.7 nmol/l (Fig. 14.14). As this preparation has been approved for clinical use in Europe, intramuscular testosterone undecanoate in castor oil will become a significantly improved testosterone preparation for treatment of male hypogonadism as well as for male contraception (see Chapter 23).

14.3.6.7 Testosterone decanoate

Testosterone decanoate differs from testosterone undecanoate by one carbon atom in the ester side chain. It has been widely administered for many years as part of a mixture with shorter-action testosterone esters, however, it has not been available as a single preparation. To date there are no detailed studies published on the pharmacokinetics of administration of testosterone decanoate to hypogonadal men. Recently, intramuscular injections of 400 mg of testosterone decanoate were given four times every four weeks to normal men in a contraceptive study (Anderson *et al.* 2002). Endogenous testosterone was suppressed by concomitant administration

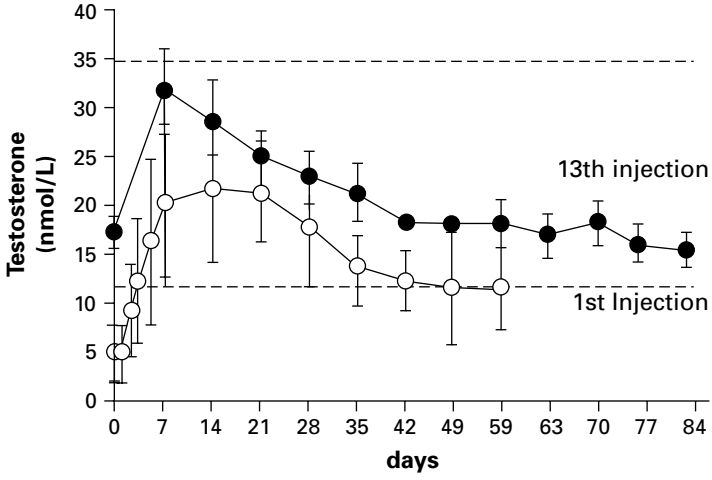


Fig. 14.14 Serum concentrations (mean ± SD) of testosterone after single injection of 1000 mg testosterone undecanoate in castor oil in 14 hypogonadal men (open circles) and during multiple injections with the same dose every 12 weeks. Broken lines indicate normal range of testosterone (adapted with permission from von Eckardstein and Nieschlag. 2002).

of etonogestrel implants. Nadir testosterone levels before the next injection were in the lower normal range, whereas serum levels were at the upper normal limit one week after injection. From these limited data it can be concluded that testosterone decanoate seems to have an improved pharmacokinetic profile over testosterone enanthate, but does not allow similar prolonged injection intervals of about 12 weeks, as demonstrated for testosterone undecanoate in hypogonadal men.

14.3.6.8 Testosterone microspheres

Drugs can be incorporated into biodegradable microspheres. When injected intramuscularly, such drug-loaded microspheres provide controlled release of the substance for several weeks or even months. As an example, microencapsulated GnRH agonists have become a valuable modality in the treatment of prostatic carcinoma. Testosterone has been incorporated into poly(DL-lactide-co-glycolide) microspheres. When first tested in castrated monkeys single injections resulted in an elevation of serum levels above the lower limit of normal for several months (Asch *et al.* 1986). When similar microsphere injections containing 315 mg of testosterone were given to eight hypogonadal men, serum testosterone levels slowly increased to peak levels at about eight weeks and fell thereafter to reach pathological levels again by 11 weeks (Burris *et al.* 1988). In a later study the size-range and the testosterone loading of the microspheres were adjusted so that in hypogonadal men single intramuscular injections resulted in relatively constant serum levels within the normal range for about 70 days (Bhasin *et al.* 1992). These two clinical studies demonstrated

that the microspheres can be adapted to the required needs and the results were encouraging. However, this formulation of microspheres is technically difficult to manufacture consistently and requires two painful, large-volume intramuscular injections that limits its appeal for long-term therapy.

14.3.7 Subdermal application

14.3.7.1 Testosterone pellets

Subdermal testosterone pellet implantation was among the earliest effective modalities employed for clinical application of testosterone which became an established form of androgen replacement therapy by 1940 (Deansley and Parks 1938; Vest and Howard 1939). With the advent of other modalities, e.g. intramuscular testosterone ester injections, they went out of general use. However, investigations in the 1990s redefined the favourable pharmacokinetic profiles and clinical pharmacology of testosterone implants (Handelsman *et al.* 1990; Jockenhövel *et al.* 1996).

The original testosterone implants were manufactured by high-pressure tableting of crystalline steroid with a cholesterol excipient. These proved brittle, hard to standardize or sterilize and exhibited surface unevenness and fragmentation during *in-vivo* absorption to produce an uneven late release rate. These limitations were overcome in the 1950s by switching to high-temperature moulding whereby molten testosterone was cast into cylindrical moulds to produce more robust implants. These have more uniform composition, resulting in a more steady and prolonged release and reduced tissue reaction. Sterilization is achieved by a combination of high-temperature exposure during manufacture together with surface sterilization or, more recently, gamma-irradiation. The testosterone implants are currently available in two sizes with a common diameter of 4.5 mm: 6 mm length for the 100 mg and 12 mm length for the 200 mg implant. Pellets are usually implanted under the skin of the lower abdominal wall under sterile conditions using a trochar and cannula.

The estimated half-life of absorption of testosterone from subdermal implants is 2.5 months. On average, approximately 1.3 mg of testosterone are released per day from the 200 mg pellet. Testosterone implants demonstrate a minor and transient accelerated initial "burst" release, which lasts for 1–2 days (Jockenhövel *et al.* 1996). The most comprehensive pharmacokinetic evaluation of testosterone implants was done in a random-sequence, cross-over clinical study of 43 androgen-deficient men with primary or secondary hypogonadism (Handelsman *et al.* 1990). Patients were treated sequentially with 3 regimens – six 100 mg, three 200 mg or six 200 mg implants – at intervals of at least six months. Implantation of testosterone pellets resulted in a highly reproducible and dose-dependent time-course for circulation of total and free testosterone. Testosterone concentrations reached baseline by six months after either of the 600 mg dose regimens but remained significantly elevated

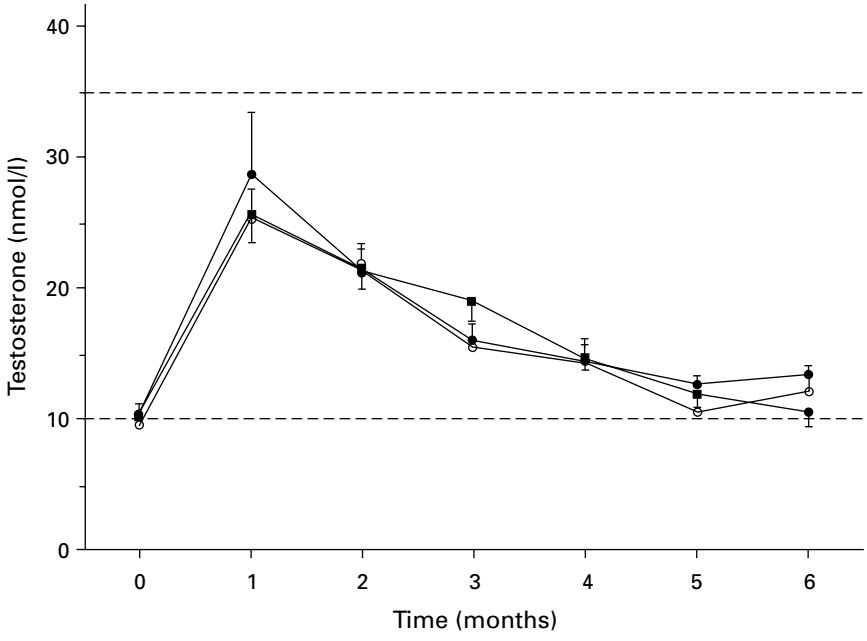


Fig. 14.15 Blood total testosterone in 43 hypogonadal men receiving four 200 mg pellets (800 mg) implanted either under the skin of the lateral abdominal wall (in 4 tracks [filled circles], n = 9; or in 2 tracks [open circles], n = 16) or in the hip region (filled squares, n = 18) (adapted with permission from Kelleher *et al.* 2001, copyright 2001, Blackwell Publishing).

after six months following the 1200 mg dose. The standard dose for hypogonadal men is 800 mg every six months, which can be titrated individually (Fig. 14.15).

Pellet implantation has few side-effects and is generally well tolerated. Adverse events after implantations were extrusions (8.5–12% per procedure), bruising (2.3–8.8%) and infections (0.6–4%) (Handelsman *et al.* 1997; Kelleher *et al.* 1999). Due to the long-lasting effect and the inconvenience of removal, preferably pellets should be used by men in whom the beneficial effects and tolerance for androgen replacement therapy have already been established by treatment with shorter-acting testosterone preparations.

14.3.7.2 Testosterone microcapsules

Testosterone can be encapsuled in a biodegradable matrix composed of lactide/glycolide copolymer which is suitable for subcutaneous injection. The pharmacokinetics and pharmacodynamics of this microcapsule formulation were tested in fourteen hypogonadal men in an open-label, prospective study (Amory *et al.* 2002). Patients received either 267 mg (n = 7, injection of 2.5 ml of the formulation) or 534 mg of testosterone (n = 7, two injections of 2.5 ml). Peak serum

concentrations were already seen at the first follow-up examination on day 1. In the higher-dose group, mean serum concentrations were at the upper limit of normal at this time-point. Thereafter, testosterone levels declined rapidly in both groups with mean serum levels below 10 nmol/l after 5 and 7 weeks, respectively. In the higher-dose group, serum levels of free testosterone, bioavailable testosterone, estradiol and DHT exceeded the normal range for at least the first week after injection. Two subjects complained of transient tenderness and fullness at the injection sites. Multiple-dose studies are still outstanding, and therefore the appropriate injection interval for long-term therapy has not yet been determined. One disadvantage of the testosterone microcapsule formulation seems to be the early burst release of testosterone, which limits the clinically acceptable dose and shortens the maximal injection interval.

14.3.8 Transdermal application

The skin easily absorbs steroids and other drugs and transdermal drug delivery has become a widely used therapeutic modality. The scrotum shows the highest rate of steroid absorption, about 40-fold higher than the forearm (Feldmann and Maibach 1967). This difference in absorption rates has been exploited for the development of a transdermal therapeutic system (TTS) to deliver testosterone. 40 and 60 cm^2 large polymeric membranes loaded with 10 or 15 mg testosterone when attached to the scrotal skin deliver sufficient amounts of the steroid to provide hypogonadal men with serum levels in the physiological range (Bals-Pratsch *et al.* 1986; 1988; Findlay *et al.* 1987; Korenmann *et al.* 1987). The application of the patch to scrotal skin requires hair clipping or shaving to optimize adherence. The membranes need to be renewed every day. When applied in the morning and worn until the next morning the resulting serum testosterone levels resemble the normal diurnal variations of serum testosterone in normal men without supraphysiological peaks (Bals-Pratsch *et al.* 1988). Long-term therapy up to ten years with daily administration of the scrotal patch in 11 hypogonadal men produced steady-state serum levels of testosterone and estradiol in the normal range and serum levels of DHT at or slightly above the higher limit of normal without significant adverse side-effects (Fig. 14.16) (Behre *et al.* 1999b).

While testosterone is readily absorbed by genital skin, transdermal systems for use on non-genital skin require enhancers to facilitate sufficient testosterone passage through the skin. The permeation enhanced testosterone patch delivers 2.5 mg/day testosterone when applied to non-scrotal skin. If one or two such systems are worn for 24 hours physiologic serum testosterone levels can be mimicked, as with scrotal patches (Fig. 14.17) (Brocks *et al.* 1996; Meikle *et al.* 1996). Due to the alcoholic enhancer used and the occlusive nature of the systems, the application is associated with skin irritation in up to 60% of the subjects, with most users discontinuing

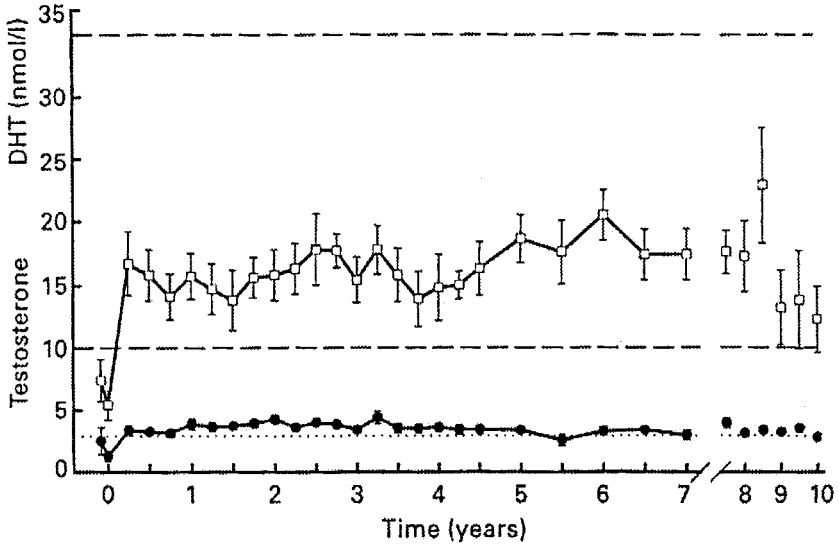


Fig. 14.16 Serum concentrations (mean ± SEM) of testosterone (squares) and DHT (circles) in 11 hypogonadal men before and during treatment with transscrotal testosterone patches. Broken lines indicate normal range of testosterone, dotted line upper normal limit of DHT (adapted with permission from Behre *et al*. 1999b, copyright 1999, Blackwell Publishing).

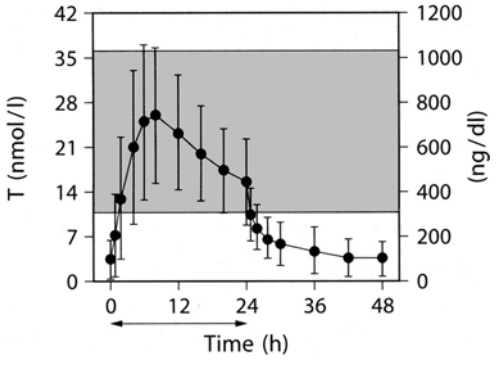


Fig. 14.17 Serum concentrations (mean ± SD) of testosterone during and after nighttime application of two non-scrotal testosterone systems to the backs of 34 hypogonadal men. Shaded area indicates normal range of testosterone (adapted with permission from Meikle *et al*. 1996, copyright 1996, The Endocrine Society).

application because of the skin irritation (Jordan 1997; Parker and Armitage 1999). Preapplication of corticosteroid cream to the skin has been reported to decrease the severity of skin irritation, although the effects on pharmacokinetics of testosterone are unclear. Another larger non-scrotal patch causes less skin irritation (about 12% itching and 3% erythema) but may create adherence problems (Jordan *et al*. 1998).

Nevertheless, both transdermal modalities through either scrotal or non-genital skin provide physiologic serum testosterone levels and have been shown to reverse the signs and symptoms of male hypogonadism with only minor systemic side-effects (Behre *et al.* 1999b; Dobs *et al.* 1999).

In 2000, a 1% colourless hydroalcoholic gel containing 25 or 50 mg testosterone in 2.5 or 5 g gel was approved for clinical use in hypogonadism. The gel dries in less than 5 min without leaving a visible residue on the skin. About 9 to 14% of the testosterone in the gel is bioavailable. Application of the testosterone gel increased serum testosterone levels into the normal range within one hour after application (Wang *et al.* 2000). Steady-state serum levels are achieved 48–72 hours after initiation of therapy, whereas pre-treatment serum testosterone levels are seen four days after stopping application. The application of the testosterone gel at four sites (application skin areas approximately four times that of one site) resulted in an area under the curve of testosterone which was 23% higher compared to application of the same amount of gel on one site. However, this difference did not achieve statistical significance in the nine hypogonadal men tested (Wang *et al.* 2000).

Long-term pharmacokinetics of the transdermal testosterone gel were evaluated in 227 hypogonadal men (Swerdloff *et al.* 2000). Patients were randomly assigned to application of 5 or 10 g of the testosterone gel or two patches of a non-scrotal testosterone system. After 90 days of testosterone gel treatment, the dose was titrated up (5 to 7.5 g) or down (10 to 7.5 g) if the preapplication serum testosterone levels were outside the normal adult male range. During long-term treatment mean serum levels of testosterone were maintained in the mid normal range with 5 g of gel and in the upper normal range with 10 g of gel (Fig. 14.18). Testosterone gel application resulted in dose-proportionate increases in serum DHT and E_2 as well as dose-proportionate decreases of gonadotropins.

The advantages of the testosterone gel over the testosterone patch are a lower incidence of skin irritation, the ease of application, the invisibility of the dried gel, and the ability to deliver testosterone dose-dependently to the low, mid or upper normal range. A potential adverse side-effect of testosterone gel application is the transfer of testosterone to women or children upon close contact with the skin. Transfer of transdermal testosterone from the skin can be avoided by applying gel to skin covered by clothing or showering after application. This preparation has gained a significant market share of androgen formulations in Europe and the United States, although it is marketed at a slightly higher price than the patches and at a much higher price than injectable testosterone.

Currently, a number of other testosterone gels and creams are being developed. Two recent randomized controlled studies demonstrated a dose-dependent increase

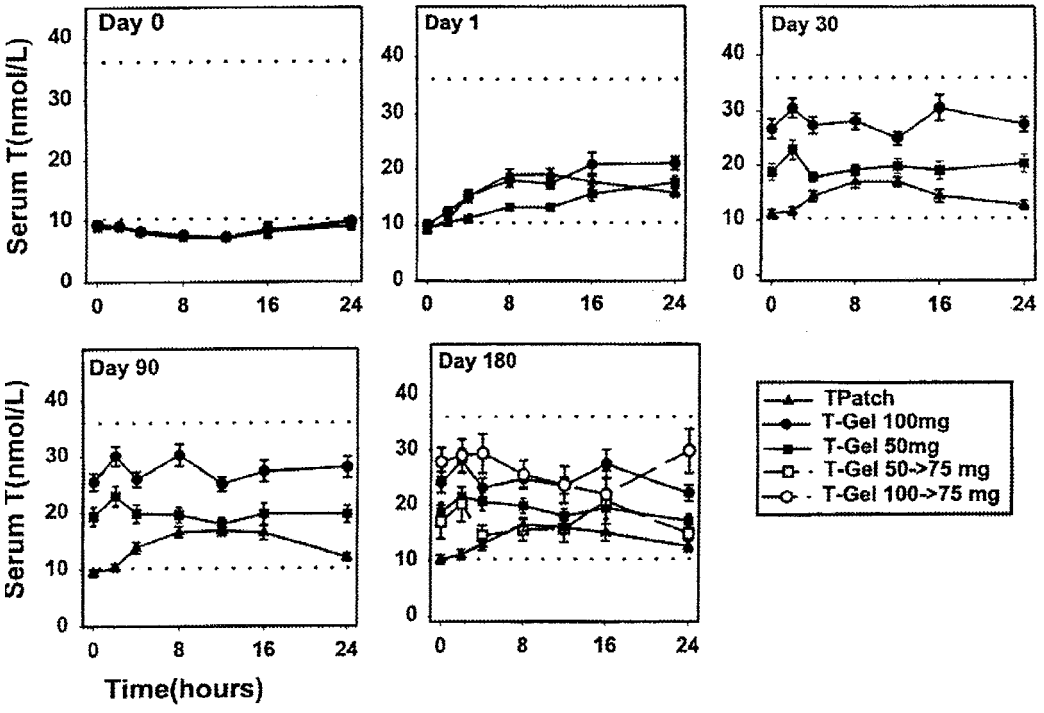


Fig. 14.18 Serum concentrations (mean ± SEM) of testosterone before (day 0) and after transdermal testosterone applications on days 1, 30, 90, and 180. Time 0 was 0800 h, when blood sampling usually began. On day 90, the dose in the subjects applying testosterone gel 50 or 100 mg was up- or down-titrated if their preapplicaton serum testosterone levels were below or above the normal adult male range, respectively. Dotted lines denote the adult normal range (adapted with permission from Swerdolff *et al.* 2000, copyright 2000, The Endocrine Society).

of testosterone serum levels to the normal range in hypogonadal men after 90 days of application of 5 g/d or 10 g/d of another hydroalcoholic topical gel containing 1% testosterone compared to non-scrotal testosterone patches (n = 208, McNicholas *et al.* 2003) or compared to non-scrotal testosterone patches and placebo gel (n = 406, Steidle *et al.* 2003). Application of 5 g/d of a 2.5% hydroalcoholic gel increased serum levels of testosterone to the normal range in 14 gonadotropin-suppressed normal men (Rolf *et al.* 2002a). Washing of the skin after 10 min. did not influence the pharmacokinetic profile. No interpersonal testosterone transfer could be detected after evaporation of the alcohol vehicle of this testosterone gel (Rolf *et al.* 2002b). This gel preparation can also be administered at a dose of 1 g/d to the scrotal skin. Ongoing randomized controlled studies in hypogonadal patients indicate the efficacy and practicability of administration of this gel to normal or scrotal skin.

14.4 Key messages

- Oral, buccal, injectable, subdermal implantable and transdermal testosterone preparations are available for clinical use. The best preparation is the one that replaces testosterone serum levels at as close to physiologic concentrations as possible.
- Oral administration of the currently available testosterone undecanoate preparation results in high interindividual and intraindividual variability of serum testosterone values.
- Daily or twice daily buccal administration of testosterone tablets increases serum testosterone to the normal range. Acceptability of this application form has yet to be determined.
- The available testosterone esters for intramuscular injection (testosterone propionate, testosterone enanthate, testosterone cypionate, testosterone cyclohexanecarboxylate) are still widely used but suboptimal for the treatment of male hypogonadism. Doses and injection intervals most frequently used in the clinic lead to initial supraphysiological testosterone levels and subnormal values before the next injection. To obtain testosterone serum concentrations continuously in the normal range, unacceptably frequent small doses would have to be injected.
- Intramuscular injection of 1000 mg testosterone undecanoate to hypogonadal men maintains serum levels of testosterone within the normal range for up to 12 weeks. Recently approved for clinical use, intramuscular testosterone undecanoate will become a valuable preparation for depot substitution therapy of male hypogonadism and for male contraception.
- A single implantation procedure of testosterone pellets provides serum levels of testosterone in the normal range for up to six months. Pellet extrusion occurs in about 10% of the implantation procedures. Due to the long-lasting effect and the inconvenience of removal, preferably pellets should be used by men in whom the beneficial effects and tolerance for androgen replacement therapy have already been established.
- Subcutaneous injection of testosterone microcapsules in hypogonadal men increases serum testosterone levels to the normal range for five to seven weeks. One disadvantage of the testosterone microcapsules formulation seems to be the early burst release of testosterone.
- Transdermal application of testosterone by scrotal or non-scrotal patches increases serum levels of testosterone to the normal range and even mimics the physiological circadian testosterone rhythm. Non-scrotal testosterone patches cause skin irritations in up to 60% of patients, or might have adherence problems.
- Daily administration of testosterone gel increases serum levels of testosterone in hypogonadal patients dose-dependently to the normal range. Acceptability of the gel is high and it has become a standard replacement therapy within the first years following its approval.

14.5 REFERENCES

Aakvaag A, Stromme SB (1974) The effect of mesterolone administration to normal men on the pituitary-testicular function. Acta Endocrinol 77:380–386

Alkalay D, Khemani L, Wagner WE, Bartlett MF (1973) Sublingual and oral administration of methyltestosterone. A comparison of drug bioavailability. J Clin Pharmac 13:142–151

Amory JK, Anawalt BD, Blaskovich PD, Gilchriest J, Nuwayser ES, Matsumoto AM (2002) Testosterone release from a subcutaneous, biodegradable microcapsule formulation (Viatrel) in hypogonadal men. J Androl 23:84–91

Anderson RA, Wallace AM, Sattar N, Kumar N, Sundaram K (2003) Evidence for tissue selectivity of the synthetic androgen 7alpha-methyl-19-nortestosterone in hypogonadal men. J Clin Endocrinol Metab 88:2784–2793

Anderson RA, Wu FC (1996) Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. II. Pharmacokinetics and pharmacodynamics of once weekly administration of testosterone enanthate. J Clin Endocrinol Metab 81:896–901

Anderson RA, Zhu H, Cheng L, Baird DT (2002) Investigation of a novel preparation of testosterone decanoate in men: pharmacokinetics and spermatogenic suppression with etonogestrel implants. Contraception 66:357–364

Asch RH, Heitman TO, Gilley RM, Tice TR (1986) Preliminary results on the effects of testosterone microcapsules. In: Zatuchni GI, Goldsmith A, Spieler JM, Sciarra JJ (eds) Male contraception: advances and future prospects, Harper & Row, Philadelphia, pp 347–360

Bagchus WM, Hust R, Maris F, Schnabel PG, Houwing NS (2003) Important effect of food on the bioavailability of oral testosterone undecanoate. Pharmacotherapy 23:319–325

Baisley KJ, Boyce MJ, Bukofzer S, Pradhan R, Warrington SJ (2002) Pharmacokinetics, safety and tolerability of three dosage regimens of buccal adhesive testosterone tablets in healthy men suppressed with leuprorelin. J Endocrinol 175:813–819

Bals-Pratsch M, Knuth UA, Yoon YD, Nieschlag E (1986) Transdermal testosterone substitution therapy for male hypogonadism. Lancet ii:943–946

Bals-Pratsch M, Langer K, Place VA, Nieschlag E (1988) Substitution therapy of hypogonadal men with transdermal testosterone over one year. Acta Endocrinol (Copenh) 118:7–13

Behre HM, Nieschlag E (1992) Testosterone buciclate (20 Aet-1) in hypogonadal men: pharmacokinetics and pharmacodynamics of the new long-acting androgen ester. J Clin Endocrinol Metab 75:1204–1210

Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E (1995) Potential of testosterone buciclate for male contraception: endocrine differences between responders and nonresponders. J Clin Endocrinol Metab 80:2394–2403

Behre HM, Abshagen K, Oettel M, Hubler D, Nieschlag E (1999a) Intramuscular injection of testosterone undecanoate for the treatment of male hypogonadism: phase I studies. Eur J Endocrinol 140:414–419

Behre HM, von Eckardstein S, Kliesch S, Nieschlag E (1999b) Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. Clin Endocrinol (Oxf) 50:629–635

Berthold AA (1849) Transplantation der Hoden. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin, Berlin, 42–46

Bhasin S, Swerdloff RS, Steiner B, Peterson MA, Meridores T, Galmirini M, Pandian MR, Goldberg R, Berman N (1992) A biodegradable testosterone microcapsule formulation provides uniform eugonadal levels of testosterone for 10–11 weeks in hypgonadal men. J Clin Endocrin Metab 74:75–83

Bird D, Vowles K, Anthony PP (1979) Spontaneous rupture of a liver cell adenoma after long term methyltestosterone: report of a case successfully treated by emergency right hepatic lobetomy. Br J Surg 66:212–213

Boyd PR, Mark GJ (1977) Multiple hepatic adenomas and a heptatocellular carcinoma in a man on oral methyl testosterone for eleven years. Cancer 40:1765–1770

Breuer H, Gütgemann D (1966) Wirkung von 1alpha-Methyl-5alpha-androstan-17ß-ol-3-on (Mesterolon) auf die Steroidausscheidung beim Menschen. Arzneimittelforschung 16:759–762

Brocks DR, Meikle AW, Boike SC, Mazer NA, Zariffa N, Audet PR, Jorkasky DK (1996) Pharmacokinetics of testosterone in hypogonadal men after transdermal delivery: influence of dose. J Clin Pharmacol 36:732–739

Brown-Séquard CE (1889) Des effets produits chez l'homme par des injections souscutanées d'un liquide retiré des testicules frais de cobaye et de chien. C R Séanc Soc Biol 1:420–430

Burris AS, Ewing LL, Sherins RJ (1988) Initial trial of slow-release testosterone microspheres in hypogonadal men. Fertil Steril 50:493–497

Butenandt A (1931) Über die chemische Untersuchung des Sexualhormons. Z Angew Chem 44:905–908

Butenandt A, Hanisch G (1935) Über Testosteron. Umwandlung des Dehydroandrosterons in Androstendiol und Testosteron; ein Weg zur Darstellung des Testosterons aus Cholesterin. Hoppe-Seyler's Z Physiol Chem 237:89–98

Cantrill AJ, Dewis P, Large DM, Newman M, Anderson DC (1984) Which testosterone replacement therapy? Clin Endocrinol 21:97–107

Carbone JV, Grodsky GM, Hjelte V (1959) Effect of hepatic dysfunction on circulating levels of sulphobromopthalein and its metabolites. J Clin Invest 38:1989–1996

Coert A, Geelen J, de Visser J, van der Vies J (1975) The pharmacology and metabolism of testosterone undecanoate (TU), a new orally active androgen. Acta Endocrinol 79:789–800

Conway AJ, Boylan LM, Howe C, Ross G, Handelsman DJ (1988) Randomized clinical trial of testosterone replacement therapy in hypogonadal men. Int J Androl 11:247–264

Coombes GB, Reiser J, Paradinas FJ, Burn I (1978) An androgen-associated hepatic adenoma in a trans-sexual. Br J Surg 65:869–870

Crabbé P, Diczfalusy E, Djerassi C (1980) Injectable contraceptive synthesis: an example of international cooperation. Science 209:992–994

Cunningham GR, Silverman VE, Kohler PO (1978) Clinical evaluation of testosterone enanthate for induction and maintenance of reversible azoospermia in man. In: Patanelli DJ (ed) Hormonal control of male fertility. Department of Health, Education and Welfare. National Institutes of Health, Bethesda, Md, Publ No (NIH) 78–1097, pp 71–87

Cussons AJ, Bhagat CI, Fletcher SJ, Walsh JP (2002) Brown-Sequard revisited: a lesson from history on the placebo effect of androgen treatment. Med J Aust 177:678–679

Daggett PR, Wheeler MJ, Nabarro JDN (1978) Oral testosterone: a reappraisal. Horm Res 9:121–129

Danner C, Frick G (1980) Androgen substitution with testosterone-containing nasal drops. Int J Androl 3:429–431

David K, Dingemanse E, Freud J, Laquer E (1935) Über krystallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron. Hoppe-Seyler's Z physiol Chem 233:281–282

Deansley R, Parkes AS (1938) Further experiments on the administration of hormones by the subcutaneous implantation of tablets. Lancet ii:606–608

de Lorimer AA, Gordan GS, Löwe RC, Carbone JV (1965) Methyltestosterone: related steroids and liver function. Arch Intern Med 116:289–294

Dobs AS, Hoover DR, Chen MC, Allen R (1998) Pharmacokinetic characteristics, efficacy, and safety of buccal testosterone in hypogonadal males: a pilot study. J Clin Endocrinol Metab 83:33–39

Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA (1999) Pharmacokinetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in comparison with bi-weekly injections of testosterone enanthate for the treatment of hypogonadal men. J Clin Endocrinol Metab 84:3469–3478

Escamilla RF (1949) Treatment of preadolescent eunuchoidism with (methyl)testosterone linguets. Amer Pract 3:425

Falk H, Thomas LB, Popper H, Ishak KG (1979) Hepatic angiosarcoma associated with androgenic-anabolic steroids. Lancet ii:1120–1123

Farrell GC, Joshua DE, Uren RF, Baird PJ, Perkins KW, Kronenberg H (1975) Androgen-induced hepatoma. Lancet i:430–432

Feldmann RJ, Maibach HI (1967) Regional variation in percutaneous penetration of 14C cortisol in man. J Invest Dermatol 48:181–183

Findlay JC, Place VA, Snyder PJ (1987) Transdermal delivery of testosterone. J Clin Endocrinol Metab 64:266–268

Frey H, Aakvaag A, Saanum D, Falch J (1979) Bioavailability of oral testosterone in males. Eur J Clin Pharmacol 16:345–349

Fujioka M, Shinohara Y, Baba S, Irie M, Inoue K (1986) Pharmacokinetic properties of testosterone propionate in normal men. J Clin Endocrinol Metab 63:1361–1364

Fukutani K, Isurugi K, Takayasu H, Wakabayashi K, Tamaoki B-I (1974) Effects of depot testosterone therapy on serum levels of luteinizing hormone and follicle-stimulating hormone in patients with Klinefelter's syndrome and hypogonadotropic eunuchoidism. J Clin Endocrinol Metab 39:856–864

Gardner FH, Besa EC (1983) Physiologic mechanisms and the hematopoietic effects of the androstanes and their derivatives. Curr Top Hematol 4:123–195

Gerhards E, Gibian H, Kolb KH (1966) Zum Stoffwechsel von 1alpha-Methyl-5alpha androstan-17ß-ol-3-on (Mesterolon) beim Menschen. Arneimittelforschung 16:458–463

Goodman MA, Laden AMJ (1977) Hepatocellular carcinoma in association with androgen therapy. Med J Aust i:220–221

Gordon RD, Thomas MJ, Poyntin JM, Stocks AE (1975) Effect of mesterolone on plasma LH, FSH and testosterone. Andrologia 7:287–296

Gu YQ, Wang XH, Xu D, Peng L, Cheng LF, Huang MK, Huang ZJ, Zhang GY (2003) A multicenter contraceptive efficacy study of injectable testosterone undecanoate in healthy Chinese men. J Clin Endocrinol Metab 88:562–568

Hamburger C (1958) Testosterone treatment and 17-ketosteroid excretion. Acta Endocrinol 28:529–536

Handelsman DJ, Conway AJ, Boylan LM (1990) Pharmacokinetics and pharmacodynamics of testosterone pellets in man. J Clin Endocrinol Metab 70:216–222

Handelsman DJ, Mackey MA, Howe C, Turner L, Conway AJ (1997) Analysis of testosterone implants for androgen replacement therapy. Clin Endocrinol 47:311–316

Heywood R, Hunter B, Green OP, Kennedy SJ (1977a) The toxicity of methyl testosterone in the rat. Toxicology Letters 1:27–31

Heywood R, Chesterman H, Ball SA, Wadsworth PF (1977b) Toxicity of methyl testosterone in the beagle dog. Toxicology 7:357–365

Horst HJ, Höltge WJ, Dennis M, Coert A, Geelen J, Voigt KD (1976) Lymphatic absorption and metabolism of orally administered testosterone undecanoate in man. Klin Wschr 54: 875–879

Jockenhövel F, Vogel E, Kreutzer E, Reinhard W, Lederbogen S, Reinwein D (1996) Pharmacokinetics and pharmacodynamics of subcutaneous testosterone implants in hypogonadal man. Clin Endocrinol 45:61–71

Johnsen SG (1978) Long-term androgen therapy with oral testosterone. In: Patanelli DJ (ed) Hormonal control of male fertility. DHEW publication No (NIH) 78–1097. Bethesda, pp 123–143

Johnsen SG, Bennet EP, Jensen VG (1974) Therapeutic effectiveness of oral testosterone. Lancet 2:1473–1475

Johnsen SG, Kampmann JP, Bennet EP, Jörgensen F (1976) Enzyme induction by oral testosterone. Clin Pharmacol Ther 20:233–237

Jordan WP (1997) Allergy and topical irritation associated with transdermal testosterone administration: a comparison of scrotal and nonscrotal transdermal systems. Am J Cont Dermat 8:108–113

Jordan WP, Atkinson LE, Lai C (1998) Comparison of the skin irritation potential of two testosterone transdermal systems: an investigational system and a marketed product. Clin Ther 20:80–87.

Junkmann K (1952) Über protrahiert wirksame Androgene. Arch Path Pharmacol 215:85–92

Junkmann K (1957) Long-acting steroids in reproduction. Rec Progr Horm Res 13:389–419

Kelleher S, Conway AJ, Handelsman DJ (1999) A randomized controlled clinical trial of antibiotic impregnation of testosterone pellet implants to reduce extrusion rate. Eur J Endocrinol 146:513–518

Kelleher S, Conway AJ, Handelsman DJ (2001) Influence of implantation site and track geometry on the extrusion rate and pharmacology of testosterone implants. Clin Endocrinol (Oxf) 55:531–536

Kopera H (1985) The history of anabolic steroids and a review of clinical experience with anabolic steroids. In: Eikelboom FA, van der Vies J (eds) Anabolics in the '80s. Acta endocrinol suppl 271:11–18

Korenman SG, Viosca S, Garza D, Guralnik M, Place V, Campbell P, Stanik Davis S (1987) Androgen therapy of hypogonadal men with transscrotal testosterone system. Am J Med 83:471–478

Krüskemper HI, Noell G (1967) Steroidstruktur und Lebertoxizität. Acta endocrinol 54:73–80

Loewe S, Voss HE (1930) Der Stand der Erfassung des männlichen Sexualhormons (Androkinins). Klin Wschr 9:481–487

Maisey NM, Bingham J, Marks V, English J, Chakraborty J (1981) Clinical efficacy of testosterone undecanoate in male hypogonadism. Clin Endocrinol 14:625–629

Mackey MA, Conway AJ, Handelsman DJ (1995) Tolerability of intramuscular injections of testosterone ester in oil vehicle. Hum Reprod 10:862–865

Mazer NA, Heiber WE, Moellmer JF, Meikle AW, Stringham JD, Sanders SW, Tolman KG, Odell WD (1992) Enhanced transdermal delivery of testosterone: a new physiological approach for androgen replacement in hypogonadal men. J Control Release 19:347–362

McCaughan GW, Bilous MJ, Gallagher ND (1985) Long-term survival with tumor regression in androgen-induced liver tumors. Cancer 56:2622–2626

McNicholas TA, Dean JD, Mulder H, Carnegie C, Jones NA (2003) A novel testosterone gel formulation normalizes androgen levels in hypogonadal men, with improvements in body composition and sexual function. BJU Int 91:69–74

Meikle AW, Arver S, Dobs AS, Sanders SW, Rajaram L, Mazer N (1996) Pharmacokinetics and metabolism of a permeation-enhanced testosterone transdermal system in hypogonadal men: influence of application site – a clinical research center study. J Clin Endocrinol Metab 81:1832–1840

Methyltestosteron-Monographie (Anonymous) (1988) Bundesanzeiger 40:5140–5141

Minto CF, Howe C, Wishart S, Conway AJ, Handelsman DJ (1997). Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: effects of ester, injection site and injection volume. J Pharmacol Exp Ther 281:93–102

Nankin HR (1987) Hormone kinetics after intramuscular testosterone cypionate. Fertil Steril 47:1004–1009

Nieschlag E (1981) Ist die Anwendung von Methyltestosteron obsolet? Dtsch med Wschr 106:1123–1125

Nieschlag E, Mauss J, Coert A, Kicovic P (1975) Plasma androgen levels in men after oral administration of testosterone or testosterone undecanoate. Acta endocrinol 79:366–374

Nieschlag E, Cüppers HJ, Wiegelmann W, Wickings EJ (1976) Bioavailability and LH-suppressing effect of different testosterone preparations in normal and hypogonadal men. Horm Res 7:138–145

Nieschlag E, Cüppers EJ, Wickings EJ (1977) Influence of sex, testicular development and liver function on the bioavailability of oral testosterone. Euro J Clin Invest 7:145–147

Nieschlag E, Büchter D, von Eckardstein S, Abshagen K, Simoni M, Behre HM (1999) Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol (Oxf) 51:757–763

Paradinas FJ, Bull TB, Westaby D, Murray-Lyon IM (1977) Hyperplasia and prolapse of hepatocytes into hepatic veins during longterm methyltestosterone therapy: possible relationships of these changes to the development of peliosis hepatis and liver tumours. Histopathology 1:225–246

Parker S, Armitage M (1999) Experience with transdermal testosterone replacement therapy for hypogonadal men. Clin Endocrinol (Oxf) 50:57–62

Partsch CJ, Weinbauer GF, Fang R, Nieschlag E (1995) Injectable testosterone undecanoate has more favourable pharmacokinetics and pharmacodynamics than testosterone enanthate. Eur J Endocrinol 132:514–519

Pitha J, Harman SM, Michel ME (1986) Hydrophilic cyclodextrin derivatives enable effective oral administration of steroidal hormones. J Pharm Sci 75:165–167

Rajalakshmi M, Ramakrinshnan PR (1989) Pharmacokinetics and pharmacodynamics of a new long-acting androgen ester: maintenance of physiological androgen levels for 4 months after a single injection. Contraception 40:399–412

Rolf C, Kemper S, Lemmnitz G, Eickenberg U, Nieschlag E (2002a) Pharmacokinetics of a new transdermal testosterone gel in gonadotrophin-suppressed normal men. Eur J Endocrinol 146:673–679

Rolf C, Knie U, Lemmnitz G, Nieschlag E (2002b) Interpersonal testosterone transfer after topical application of a newly developed testosterone gel preparation. Clin Endocrinol (Oxf) 56:637–641

Ruzicka L, Wettstein A (1935) Synthetische Darstellung des Testishormons, Testosteron (Androsten 3-on-17-ol). Helv chim Acta 18:1264–1275

Ruzicka L, Goldberg MW, Rosenberg HR (1935) Herstellung des 17α-Methyl- testosterons und anderer Androsten- und Androstanderivate. Zusammenhänge zwischen chemischer Konstitution und männlicher Hormonwirkung. Helv Chim Acta 18:1487–1498

Salehian B, Wang C, Alexander G, Davidson T, McDonald V, Berman N, Dudley RE, Ziel F, Swerdloff RS (1995) Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate – a clinical research center study. J Clin Endocrinol Metab 80:3567–3575

Schulte-Beerbühl M, Nieschlag E (1980) Comparison of testosterone, dihydrotestosterone, luteinizing hormone, and follicle-stimulating hormone in serum after injection of testosterone enanthate or testosterone cypionate. Fertil Steril 33:201–203

Schürmeyer T, Nieschlag E (1984) Comparative pharmacokinetics of testosterone enanthate and testosterone cyclohexanecarboxylate as assessed by serum and salivary testosterone levels in normal men. Int J Androl 7:181–187

Schürmeyer T, Wickings EJ, Freischem CW, Nieschlag E (1983) Saliva and serum testosterone following oral testosterone undecanoate administration in normal and hypogonadal men. Acta endocrinol 102:456–462

Shackleford DM, Faassen WA, Houwing N, Lass H, Edwards GA, Porter CJ, Charman WN (2003) Contribution of lymphatically transported testosterone undecanoate to the systemic exposure of testosterone after oral administration of two Andriol formulations in conscious lymph duct-cannulated dogs. J Pharmacol Exp Ther 306:925–933

Simmer H, Simmer I (1961) Arnold Adolph Berthold (1803–1861). Zur Erinnerung an den hundertsten Todestag des Begründers der experimentellen Endokrinologie. Dtsch med Wschr 86:2186–2192

Skakkebaek NE, Bancroft J, Davidson DW, Warner P (1981) Androgen replacement with oral testosterone undecanoate in hypogonadal men: a double blind controlled study. Clin Endocrinol 14:49–61

Snyder PJ, Lawrence DA (1980) Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 51:1335–1339

Sokol RZ, Swerdloff RS (1986) Practical considerations in the use of androgen therapy. In: Santen JR, Swerdloff RS (eds) Male reproductive dysfunction. Marcel Dekker, New York, pp 211–225

Steidle C, Schwartz S, Jacoby K, Sebree T, Smith T, Bachand R; North American AA2500 T Gel Study Group (2003) AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. J Clin Endocrinol Metab 88:2673–2681

Stuenkel CA, Dudley RE, Yen SC (1991) Sublingual administration of testosterone hydroxypropyl-β-cyclodextrin inclusion complex simulates episodic androgen release in hypogonadal men. J Clin Endocrinol Metab 72:1054–1059

Swerdloff RS, Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Longstreth J, Berman N (2000) Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. J Clin Endocrinol Metab 85:4500–4510

van der Vies J (1965) On the mechanism of action of nandrolone phenylpropionate and nandrolone decanoate in rats. Acta endocrinol 49:271–282

van der Vies J (1970) Model studies in vitro with long-acting hormonal preparations. Acta endocrinol 64:656–669

van der Vies J (1985) Implications of basic pharmacology in the therapy with esters of nandrolone. In: Eikelboom FA, van der Vies J (eds) Anabolics in the '80s. Acta endocrinol, suppl 271, 110:38–44

Vest SA, Howard JE (1939) Clinical experiments with androgens. IV: a method of implantation of crystalline testosterone. J Am Med Assoc 113:1869–1872

Vogelzang NJ, Arnold JL, Chodak GJ, Schoenberg H (1986) Androgen and germ cell testicular cancers. JAMA 255:906–906

von Eckardstein S, Nieschlag E (2002) Treatment of male hypogonadism with testosterone undecanoate injected at extended intervals of 12 weeks: a phase II study. J Androl 23:419–425

Voronoff S (1920) Testicular grafting from ape to man. Brentanos Ltd., London

Wang C, Eyre R, Clark D, Kleinberg C, Newman I, Iranmanesh A, Veldhuis R, Dudley RE, Berman N, Davidson T, Barstow TS, Sinow R, Alexander G, Swerdloff R (1996) Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption and increases bone formation markers in hypogonadal men – a clinical research center study. J Clin Endocrinol Metab 81:3654–3662

Wang C, Berman N, Longstreth JA, Chuapoco B, Hull L, Steiner B, Faulkner S, Dudley RE, Swerdloff RS (2000) Pharmacokinetics of transdermal testosterone gel in hypogonadal men: application of gel at one site versus four sites: a general clinical research center study. J Clin Endocrinol Metab 85:964–969

Wang L, Shi DC, Lu SY, Fang RY (1991) The therapeutic effect of domestically produced testosterone undecanoate in Klinefelter syndrome. New Drugs Mark 8: 28–32

Weinbauer GF, Marshall GR, Nieschlag E (1986) New injectable testosterone ester maintains serum testosterone of castrated monkeys in the normal range for four months. Acta Endocrinol 113:128–132

Weinbauer GF, Partsch CJ, Zitzmann M, Schlatt S, Nieschlag E (2003) Pharmacokinetics and degree of aromatization rather than total dose of different preparations determine the effects of testosterone: a nonhuman primate study in *Macaca fascicularis*. J Androl 24:765–774

Werner SC, Hamger FM, Kritzler RA (1950) Jaundice during methyltestosterone therapy. Am J Med 8:325–331

Westaby D, Ogle SJ, Paradinas FJ, Randell JB, Murray-Lyon IM (1977) Liver damage from long-term methyltestosterone. Lancet ii:261–263

World Health Organization, Nieschlag E, Wang C, Handelsman DJ, Swerdloff RS, Wu F, Einer-Jensen N, Waites G (1992) Guidelines for the use of androgens. WHO, Geneva

Wu FCW, Farley TMM, Peregoudov A, Waites GMH, WHO (1996) Effects of testosterone enanthate in normal men: experience from a multicenter contraceptive efficacy study. Fertil Steril 65:626–636

Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ (1998) A pharmacokinetic study of injectable testosterone undecanoate in hypogonadal men. J Androl 19:761–768

Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E (2003) Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: a longitudinal pharmacogenetic study. J Clin Endocrinol Metab 88:2049–2054

15

Androgen therapy in non-gonadal disease

P.Y. Liu and D.J. Handelsman

Contents

15.1 Introduction

Male reproductive function is influenced by non-gonadal disease so that mild androgen deficiency is a regular feature of chronic disease, which, if sufficiently severe or prolonged may contribute to the pathophysiology. Additionally, androgens are potent therapeutic drugs with effects on androgen-sensitive tissues such as muscle, bone, brain, liver or adipose tissue that may be exploited for therapeutic benefit.

As an adjunct to standard medical care, androgen therapy may be considered as either physiological androgen replacement or pharmacological androgen therapy. Androgen replacement therapy aims to replicate endogenous androgen exposure thereby limiting it to the use of testosterone in doses intended to produce physiological blood concentrations. To the degree it replicates endogenous androgen exposure, the expectation for safety may reasonably be compared with the benchmark of life-long health experience of eugonadal men. By contrast, pharmacological androgen therapy is no different from pharmacotherapy with any xenobiotic drug used to achieve a therapeutic goal. It utilises an androgen, without restriction to testosterone or reference to replacement doses, to optimal effect as judged by the standards of efficacy, safety and cost-effectiveness applicable to other drugs. Pharmacological androgen therapy has often involved synthetic oral androgens rather than testosterone, usually the hepatotoxic 17α-alkylated androgens now considered obsolete for androgen replacement therapy. While occasionally justified by the need to avoid parenteral injections due to bleeding disorders or for deliberate hepatic targeting via oral first pass effects, this involves an avoidable risk of hepatotoxicity.

Development of selective designer androgens in the future should open new possibilities for further investigation of adjuvant pharmacological androgen therapy. These designer androgens would be based on tissue-specific activation to aromatised and/or 5α-reduced metabolites or on co-regulator distribution patterns, rather than being "anabolic steroids", an outmoded term referring to non-virilising androgens targeted exclusively to muscle, a concept lacking biological proof of principle.

The goals of androgen therapy for non-gonadal disease must be considered in relation to the natural history of the underlying disease. Mild androgen deficiency is a frequent biochemical accompaniment of systemic disease. If unusually severe or prolonged, androgen deficiency may contribute to morbidity from the underlying disease. Physiological androgen replacement therapy in itself is unlikely to influence mortality, as complete androgen deficiency due to congenital androgen resistance or castration before or after puberty does not appear to reduce life expectancy (Liu *et al.* 2003a; Nieschlag *et al.* 1993). Hence most studies of pharmacological androgen therapy in systemic disease aim ideally to modify the natural history of the underlying disease but otherwise to palliate symptoms and improve quality of life. The natural history of the underlying disease must also be considered to evaluate the relative importance of potential long-term hazards (such as acceleration of prostate or cardiovascular disease) compared with possible immediate benefits that pharmacological androgen therapy might achieve for quality of life.

This chapter focuses on controlled clinical studies reported over the last few decades rather than the plethora of studies performed over the six decades since testosterone was first used clinically (Foss 1939; Hamilton 1937). A comprehensive account of early, mostly uncontrolled studies of androgen therapy up to the mid-1970s is contained in two classical textbooks (Kopera 1976; Kruskemper 1968). Recognising there are few if any well-established indications for pharmacological androgen therapy, placebo controls remain the *conditio sine qua non* for high-quality clinical studies. In addition, other optimal trial design features include adequate power and duration with valid, objective end-points. Unfortunately, few studies fulfil these stringent requirements, most comprising observational and/or mechanistic studies, which provide little reliable guidance for practical therapeutics. Observational studies of systemic disease effects on male reproductive health are reviewed elsewhere (Handelsman 2001).

15.2 Liver disease

15.2.1 Cirrhosis

In studies dating back to the 1960's, androgen therapy does not alter the natural history of *alcoholic cirrhosis*. The earliest controlled studies of androgen therapy to

ameliorate the natural history of alcoholic cirrhosis claimed a survival benefit in 26 men treated with testosterone propionate (100 mg alternate days for ~4 weeks) compared with 27 placebo-treated men (Wells 1960). This study had defective randomisation and could not be replicated in another study of 17 men treated with either 100 mg of *testosterone propionate* or *methenolone* acetate every second day for one month. They had no survival advantage after 6 months compared with 10 placebo-treated controls (Fenster 1966). Neither study was large nor long enough to be definitive. The best evidence is derived from the Copenhagen Study Group for Liver Disease which enrolled 221 men with alcoholic cirrhosis in a 3 year prospective double-blinded, randomised, placebo-controlled study testing oral micronised testosterone (600 mg daily). This study showed convincingly no benefit in mortality (Copenhagen Study Group for Liver Diseases 1986), hepatic histology (Gluud *et al.* 1987a), liver hemodynamics and biochemical function (Gluud *et al.* 1987c) or improvement in sexual dysfunction (Gluud *et al.* 1988b). The negative outcome with sufficient power to exclude a 35% decrease in mortality was at variance with many enthusiastic but poorly controlled previous reports (Kopera 1976). The observation of *portal vein thrombosis* in three men treated with testosterone may be related to the extreme portal testosterone levels created by oral administration of very high androgen dosage. Characteristic of testosterone pharmacokinetics in chronic liver disease (Gluud *et al.* 1981; Nieschlag *et al.* 1977), this regimen produced markedly supraphysiological peripheral blood testosterone concentrations (Gluud *et al.* 1988a; Gluud *et al.* 1987b) which suggests even more extreme portal testosterone concentrations.

15.2.2 Hepatitis

Short-term controlled studies of androgen therapy in men with *alcoholic hepatitis* do not provide convincing evidence of any benefit. A prospective randomised multicentre Veterans Administration study claimed a mortality benefit after 30 days of *oxandrolone* treatment (80 mg daily), compared with placebo in 263 men presenting with alcoholic hepatitis (Mendenhall *et al.* 1984). The poorly defined entry and end-point definitions have been criticised (Maddrey 1986) and the benefits, if any, appeared to be short-term. The same authors reported a further study of 271 poorly nourished men with alcoholic hepatitis randomised to treatment with oxandrolone plus high calorie food supplements compared with a group receiving placebo without dietary supplementation (Mendenhall *et al.* 1993). This study showed no overall survival benefit by an intention-to-treat analysis; however, subgroup analysis demonstrated a significant doubling of survival at one month, which persisted for six months in those with "moderate" but not severe malnutrition at entry. Due to the study design, the benefit of androgen therapy relative to enhanced nutrition could not be resolved. Another randomised controlled study of 19 men

and 20 women with alcoholic hepatitis treated with 80 mg oxandrolone, parenteral nutrition, both or neither for 21 days demonstrated modest improvement in hepatic biochemical function but did not report other clinical end-points (Bonkovsky *et al.* 1991).

15.2.3 Androgen-induced liver disorders

A consistent adverse feature of pharmacological androgen therapy, regardless of indication, is the risk of androgen-induced liver disorders (Ishak and Zimmerman 1987). These involve biochemical effects on hepatic function, hepatotoxicity (hepatitic or cholestatic) and liver tumor development (benign or malignant) and peliosis hepatis. These risks are a class-specific adverse effect of 17α-alkylated androgens, especially when used orally but no reliable estimates of the incidence or prevalence are available. The East German national sports doping programme involving oral *17α-alkylated androgens* resulted in deaths from liver failure and chronic liver disease (Franke and Berendonk 1997). Every marketed 17α-alkylated androgen is associated with hepatotoxicity, whereas other androgens (1-methyl androgens, nandrolone, testosterone, dihydrotestosterone) are not hepatotoxic. Cholestasis and functional impairment of liver function (BSP retention, antipyrine clearance) are consistently impaired by oral 17α-alkylated androgens. Androgen-induced hepatitis and tumours are less frequent but unpredictable. If claims that low-dose methyltestosterone used in post-menopausal women has minimal hepatotoxicity risk (Gelfand and Wiita 1997; Gitlin *et al.* 1999; Simon 2001) are correct, then the therapeutic index is low and such safer doses would be ineffective in men. Blood *SHBG* concentrations are significantly reduced by any oral androgen as well as supra-physiological circulating testosterone concentrations in peripheral or portal blood (Conway *et al.* 1988). This indicates that SHBG can serve as a useful, sensitive index of hepatic androgen over-dosage.

15.3 Hematological disorders

15.3.1 Erythropoiesis and marrow stimulation

Androgen therapy has long been used clinically to stimulate erythropoiesis since the original observational study of 68 women with breast cancer which demonstrated significant, sometimes dramatic, increases in hemoglobin levels after the administration of 100 mg *testosterone* or *dihydrotestosterone* propionate injections three times weekly (Kennedy and Gilbertsen 1957). In addition, androgen therapy has smaller and less consistent effects on other bone marrow cell lineages that produce neutrophils and platelets. Androgen therapy increases *hemoglobin* in healthy men (Palacios *et al.* 1983; Wu *et al.* 1996) as well as augmenting the hemoglobin responses to recombinant human *erythropoietin* (EPO) in renal anemia (Ballal *et al.* 1991) and

iron supplementation in iron deficiency anemia (Victor *et al.* 1967). Although the hemoglobin response to androgen therapy is usually modest in magnitude (typically ~10g/l), a small proportion (~1%) of normal (Palacios *et al.* 1983; Wu *et al.* 1996) or hypogonadal (Drinka *et al.* 1995; Krauss *et al.* 1991) men exhibit idiosyncratic polycythemic responses to androgen therapy. Such androgen-induced *polycythemia* may be asymptomatic or produce significant clinical effects due to hyperviscosity and/or ischemia. Androgen-induced polycythemia is usually reversible following cessation of treatment but occasionally clinical circumstances (e.g. unstable angina or transient ischemic attack) may warrant venesection. The biochemical basis of the idiosyncratic polycythemic response to testosterone is not fully understood, but it appears to be more frequent with testosterone ester injections, presumably reflecting the transient supraphysiological peak blood testosterone concentrations following each injection (see Chapter 14). More stable testosterone delivery with transdermal patches (Meikle *et al.* 1996) or implants (Handelsman *et al.* 1997) are rarely associated with polycythemia (Jockenhövel *et al.* 1997). Following recovery from testosterone-induced polycythemia, testosterone treatment may be resumed with careful monitoring by using a more steady-state preparation. Androgen-induced polycythemia is also occasionally related to underlying sleep apnea or respiratory failure. It is not known whether or not the androgen receptor *CAG triplet repeat* polymorphism in androgen sensitivity is involved (Zitzmann and Nieschlag 2003).

15.3.2 Anemia due to marrow failure

In severe *aplastic anemia*, a major study of 110 patients compared HLA-identical marrow transplantation with oral, intramuscular or no androgen therapy (Camitta *et al.* 1979). This showed a major survival advantage (70% vs. 35% six month survival) for 47 patients having HLA-identical *bone marrow transplantation* compared with 63 patients in whom no donor was available who were randomised to oral (oxymetholone 3–5 mg/kg/day), intramuscular (nandrolone decanoate 3–5 mg/kg/wk) or no androgen therapy (Camitta *et al.* 1979). The latter three groups did not differ in survival, a finding consistent with another small randomised study that showed no survival benefit due to androgen therapy (50–100 mg nandrolone phenylpropionate weekly) compared with placebo vehicle injections (Branda *et al.* 1977).

In standard non-transplantation treatment for aplastic anemia, a randomised cross-over study of 44 patients concluded that anti-thymocyte globulin (ATG) was superior to androgen therapy (nandrolone decanoate 5 mg/kg/wk). This conclusion was, however, flawed as half the patients had failed prior androgen therapy, thus constituting an entry bias against androgen therapy (Young *et al.* 1988). Coupled with ATG, androgen therapy appears to offer morbidity but not mortality benefit in aplastic anemia. A randomised, controlled multi-centre study of the

European Bone Marrow Transplantation in Severe Aplastic Anaemia Study Group of 134 patients with newly diagnosed severe aplastic anemia receiving standard therapy (including ATG and methylprednisolone) demonstrated an improvement in transfusion independence due to treatment with oxymetholone (2 mg/kg/day) compared with placebo (Bacigalupo *et al.* 1993). However there was no overall benefit in survival that was determined principally by the severity of disease based on leucocyte count. These findings confirmed the benefit of androgen therapy on transfusion independence but not survival from two smaller randomised placebo-controlled studies involving 61 patients using oral methenolone acetate (2–3 mg/kg/day) (Kaltwasser *et al.* 1988; Li Bock *et al.* 1976) but contradict another randomised placebo-controlled study which found no benefit from androgen therapy (fluoxymesterone 25 mg/m^2/day or oxymetholone 4 mg/kg/day) over placebo in 53 patients (Champlin *et al.* 1985). None of these studies reported survival benefits or formally evaluated quality of life and all frequently observed female virilisation.

An important pair of studies attempted to define the optimal dosage and type of androgen therapy for aplastic anemia (French Cooperative Group for the Study of Aplastic and Refractory Anaemias 1986). In the first study, 110 patients were randomised into four groups according to androgen (norethandrolone, fluoxymesterone) and dose (high 1 mg/kg/d, low 0.2 mg/kg/d). Survival was mainly influenced by disease severity but, in less severe cases, high-dose androgen therapy significantly improved survival over low-dose androgen therapy. Despite randomisation, there were imbalances between treatment groups with respect to disease severity and age that undermine the interpretability of the findings. In the second study, 125 patients were randomised to four different androgens – norethandrolone, stanozolol, fluoxymesterone (all at 1 mg/kg/day) or *testosterone undecanoate* (1.7 mg/kg/day). The fluoxymesterone treatment group had the best and stanozolol the worst survival with norethandrolone and testosterone undecanoate being equivalent and intermediate in efficacy. Once again, however, the treatment groups were unbalanced with respect to disease severity and age. Hence the reported benefit limited to the less severe and older (>30 yr) cases remains dubious. The superiority of any specific androgen remains to be unequivocally demonstrated with particular difficulty in comparing effective doses of different androgens.

The French Cooperative Study Group also reported a series of cohort studies examining the efficacy of androgen therapy in patients with aplastic anemia. Their initial cohort randomised 352 men and women to treatment with methandrostenolone (1 mg/kg/day), oxymetholone (2.5 mg/kg/day), methenolone acetate (2.5 mg/kg/day) or norethandrolone (1 mg/kg/day). The methandrostenolone group had the best, whereas oxymetholone and methenolone groups exhibited equally the worst two-year survival from randomisation (Cooperative

Group for the Study of Aplastic and Refractory Anaemias 1979). However, treatment groups were unbalanced for disease severity, the principal determinant of survival. Despite post-hoc stratified analyses, it remains ultimately difficult to conclude whether underlying disease prognosis or drug effects explained the differences in group survival. In a follow-up study from the same cohort who survived at least two years from initial randomisation, 137 patients were re-randomised to rapid (3 month) or slow (20 month) withdrawal of their original androgen therapy. The slow withdrawal group had a higher rate of maintained remission consistent with androgen therapy having maintained a clinical benefit, presumably via maintenance of hemoglobin levels, but no survival data were reported (Najean and Joint Group for the Study of Aplastic and Refractory Anaemias 1981).

Overall, androgen therapy does not improve survival in aplastic anemia but provides a morbidity benefit by maintaining hemoglobin and transfusion independence, although the improved quality of life has not been quantified. In severe aplastic anemia, bone marrow transplantation from an HLA-identical sibling (if feasible) is the preferred treatment and superior to androgen therapy. Androgen therapy may be useful in less severe aplastic anemia for which bone marrow transplantation is not available or justified. However, the relative merits of androgen therapy compared with HLA non-identical bone marrow transplantation or in the presence of failing or failed bone marrow transplantation have not been clearly defined. Although it is prudent to avoid injectable androgens in a population that may be thrombocytopenic, the preponderant use of oral 17α-alkylated androgens in aplastic anemia appears unjustified when non-hepatotoxic oral androgens such as 1-methyl androgens (methenolone, mesterolone) and testosterone undecanoate appear to be equally effective.

15.3.3 Myeloproliferative disorders

The use of androgen therapy in other causes of bone marrow failure has been less extensively studied. One controlled study of 29 patients with *myeloproliferative* disorders randomised patients to treatment with fluoxymesterone (30 mg daily) compared with transfusions alone but was terminated prematurely due to slow recruitment and poor hemoglobin response with only 4/14 achieving an increase of >10 g/l (Brubaker *et al.* 1982). These findings are supported by another randomised study of 56 patients with *myelodysplasia* which found oral methenolone acetate (2.5 mg/kg/day) no better than intravenous cytosine arabinoside or symptomatic maintenance therapy (Najean and Pecking 1979).

15.3.4 Thrombocytopenia

A beneficial effect of androgen therapy in thrombocytopenia due to marrow failure has been suggested by a study in myelodysplasia associated with thrombocytopenia in which 20 patients were randomised to receive either danazol (600 mg daily) or

fluoxymesterone (1 mg/kg/day). Although both groups had an impressive response in termination of clinical bleeding (6/6) and increasing platelet count (11/20), the lack of a placebo group means that the contribution of natural remission could not be evaluated (Wattel *et al.* 1994).

The role of androgen therapy in immune thrombocytopenic purpura (ITP) remains poorly defined in the absence of controlled clinical trials. Two short-term observational studies have reported that danazol increases platelet counts in ITP as well as decreasing prednisone requirement (Ambriz *et al.* 1986) and reducing platelet-reactive IgG (Ahn *et al.* 1983).

15.4 Renal disease

Men with *chronic renal failure* exhibit many features of classical androgen deficiency including gynecomastia, impotence, testicular atrophy, impaired spermatogenesis and infertility as well as somatic disorders of bone, muscle and other androgen-responsive tissues (Handelsman 1985; Handelsman and Dong 1993; Handelsman and Liu 1998). Yet there is little information on androgen replacement therapy in patients with end-stage renal disease, during dialysis or after renal transplantation. Only a single randomised controlled study has examined androgen replacement therapy in uremic men (van Coevorden *et al.* 1986). Nineteen regularly hemodialysed men were randomised to receive either oral *testosterone undecanoate* (240 mg daily) or placebo for 12 weeks. Although libido and sexual activity increased, hemoglobin was unchanged and no other androgen effects on bone, muscle, cognition and well-being were reported. Future studies examining physiological replacement therapy using testosterone patches or gels would be of interest since transdermal testosterone has similar pharmacokinetics in uremic as in hypogonadal men (Singh *et al.* 2001).

Pharmacological androgen therapy has been evaluated in a randomised placebo-controlled trial of nandrolone decanoate in dialysed patients (Johansen *et al.* 1999). Twenty-nine patients were randomised by sequential allocation to nandrolone decanoate (100 mg intramuscularly each week, $n = 14$) or saline placebo ($n = 15$) for 6 months. Lean body mass (measured by DEXA), timed walking and stair-climbing speed were all increased, self-reported fatigue fell but there was no change in handgrip strength. Peak oxygen consumption was also increased at three months, but not significantly so by the end of the sixth month. Larger placebo-controlled clinical studies of longer duration are needed to determine whether the impressive short-term benefits are sustainable and/or improve survival.

15.4.1 Effect of androgens on renal function

Based on the renotrophic effects of androgens (Mooradian *et al.* 1987), it has long been speculated that androgen therapy in patients with chronic renal failure or

nephrotic syndrome might improve or slow the deterioration in underlying renal function. However, clinical evidence for renotrophic effects of androgen therapy has remained ambiguous due to the lack of adequately powered, placebo-controlled studies (Kopera 1976; Kruskemper 1968). The best clinical evidence is derived from a placebo-controlled study of elderly patients without renal disease (Dontas *et al.* 1967) and another evaluating uremic patients but without a control group (Wilkey *et al.* 1960). In the first study (Dontas *et al.* 1967), indices of both glomerular and tubular function improved with nandrolone phenylpropionate (25 mg injections weekly) after ~40 weeks. In the other (Wilkey *et al.* 1960), well-being and biochemical tests of renal function improved but no detailed findings or analysis were presented for the 88 uremic patients treated with various doses of injectable testosterone propionate (50 mg daily) or cypionate (100 mg daily to monthly) and oral fluoxymesterone (5 mg daily) (Wilkey *et al.* 1960). Despite the biological basis for renotrophic effects, the lack of adequate clinical evidence precludes an established role for androgen therapy in the management of chronic renal failure.

More recently, the possibility that androgen therapy may be detrimental to the function of kidney transplants was suggested based on rodent experiments in which androgen therapy hastened, and androgen blockade delayed, *chronic allograft nephropathy* (Antus *et al.* 2001; Muller *et al.* 1999). These effects did not require aromatisation of testosterone nor were they gender-specific (Antus *et al.* 2002). Although no systematic clinical data has been reported, case reports raised concerns about use of androgens in patients with kidney and other transplants (Schofield *et al.* 2002) and warrant further evaluation.

15.4.2 Anemia of end-stage renal failure

The anemia of end-stage renal failure has multiple contributory factors including *EPO* deficiency, toxic inhibitors of EPO action, androgen deficiency, micronutrient deficiency (iron, folate, pyridoxine), blood loss and hemolysis (Neff *et al.* 1985). The effect of androgen therapy on *hemoglobin* involves both increased circulating EPO concentration (Buchwald *et al.* 1977) and augmentation of EPO action (Ballal *et al.* 1991). EPO deficiency is a major factor (Winearls 1995) and androgen therapy probably acts mainly by increasing EPO, since androgen therapy has no effect on hemoglobin after bilateral nephrectomy (von Hartitzsch and Kerr 1976) when the major source of endogenous EPO is removed. Androgen therapy has consistent effects on EPO secretion and hemoglobin concentrations (Navarro and Mora 2001), although circulating EPO is not consistently related to resultant increases in hemoglobin (Teruel *et al.* 1995). Endogenous testosterone is an important physiological determinant of red cell mass in men since blockade of androgen action lowers hemoglobin levels (Teruel *et al.* 1997; Weber *et al.* 1991). Furthermore, post-transplant erythrocytosis may depend on EPO and possibly also endogenous

testosterone (Chan *et al.* 1992). These findings suggest that androgens also have important effects in augmenting EPO effects.

Two randomised placebo-controlled studies have shown that nandrolone treatment increases hemoglobin in patients with end stage renal failure. One randomised 21 men to nandrolone (100 mg weekly) or placebo vehicle injections for 5 months in a cross-over design (Hendler *et al.* 1974) while another randomised 18 patients to nandrolone decanoate (200 mg weekly) for three months (Williams *et al.* 1974). Both found significant increases in mean hemoglobin (15 g/L and 10 g/L, respectively) and one reported a clinically significant decreased transfusion requirement (Hendler *et al.* 1974). A further study confirmed the beneficial effects of nandrolone decanoate (200 mg weekly) compared with placebo vehicle injections for four months (Buchwald *et al.* 1977) whereas three smaller and less well-conducted studies failed to show an increase in hemoglobin (Li Bock *et al.* 1976; Naik *et al.* 1978; van Coevorden *et al.* 1986). A further randomised, controlled clinical study compared four androgen regimens in dialysed patients, finding that testosterone enanthate (4 mg/kg/wk) and nandrolone decanoate (3 mg/kg/wk) were more effective in increasing hematocrit than oxymetholone (1 mg/kg/day) and fluoxymesterone (0.4 mg/kg/day). However, whether these differences reflected different effective androgen doses, the androgen class (17α-alkylated or not) or route of administration (including pharmacokinetics) remains unclear (Neff *et al.* 1981). Future studies examining androgen replacement therapy using transdermal testosterone will be of interest and preliminary studies indicate similar pharmacokinetic profile in uremic as in hypogonadal men (Singh *et al.* 2001).

There is accumulating evidence that androgen or EPO therapies are equally effective in maintaining hemoglobin in patients with chronic renal disease. A retrospective analysis of 84 patients receiving androgen therapy (nandrolone decanoate 200 mg weekly) for 6 months reported that men over 55 years of age had the best hemoglobin responses, and that this response was comparable with those treated with EPO (Teruel *et al.* 1996a). This was subsequently confirmed in two controlled prospective studies which both used either nandrolone decanoate 200 mg/wk or EPO (6000 U/wk) for six months. The first prospective study found very similar hemoglobin responses and safety profiles for 18 men over 50 years of age treated with androgen (nandrolone decanoate 200 mg/wk) compared with six men under 50 years and 16 women receiving EPO (6000 U/wk); however, the lack of randomisation and non-comparability of groups by age and gender limits the interpretation of these findings (Teruel *et al.* 1996b). The second study (Gascon *et al.* 1999) randomised 33 patients over the age of 65 to receive intramuscular nandrolone decanoate 200 mg/wk ($n = 14$) or to continue EPO (mean dose of 6000 units/week, $n = 19$) for six months and found comparable hematological parameters by the end of the study. However, it appears that all seven women were allocated,

rather than randomised to EPO since no woman received nandrolone. Recently, a randomised controlled study in 27 men aged over 50 years reported that nandrolone decanoate (200 mg weekly for 6 months) was equivalent to EPO (initial dose 50 units/kg/week, titrated to maintain hemoglobin between 11 and 13 g/dL) in maintaining hemoglobin (Navarro *et al.* 2002). These studies together suggest that intramuscular nandrolone decanoate (200 mg/week) in dialysed men over 50 years of age is as effective as EPO in maintaining hemoglobin. However, the relative safety of these treatments requires further clarification.

Androgen therapy may also have an adjunctive role to EPO, perhaps as an EPO-sparing agent. This has been examined in two randomised (Berns *et al.* 1992; Gaughan *et al.* 1997) and one nonrandomised (Ballal *et al.* 1991) EPO controlled studies. In the most powerful study (Gaughan *et al.* 1997), 19 dialysed patients were randomised to receive nandrolone (100 mg weekly) plus EPO (4500 U/wk) or EPO alone for 26 weeks. The addition of nandrolone to low dose EPO (approximately equal to 60 U/kg/wk) resulted in a significantly greater rise in hematocrit. Similar significant additional increases in hemoglobin were reported in a small nonrandomised study of eight men choosing to receive nandrolone decanoate (100 mg weekly) plus intermediate dose EPO (6000 U/wk) compared with EPO alone (Ballal *et al.* 1991) for 12 weeks. To the contrary, another small but randomised study employing a higher dose of EPO (120 U/kg/wk) was unable to detect any benefit of nandrolone decanoate (2 mg/kg/wk) for 16 weeks plus EPO compared with the same dose of EPO alone in 12 dialysed patients (Berns *et al.* 1992). Whether these discrepancies are due to study design, age or EPO dose remains to be clarified, although it is possible that androgens have greatest synergism with submaximal EPO dosage, and that the higher EPO dose obviates any additional androgen induced increase in hemoglobin. Randomised prospective studies to examine the use of low-dose subcutaneous EPO with *adjunctive androgen therapy* are needed (Horl 1999), particularly in older men.

A caveat on androgen therapy is the risk of *polycythemia*, which occurs as a rare idiosyncratic reaction among men with normal renal function receiving exogenous testosterone (Drinka *et al.* 1995). Testosterone-induced polycythemia may be more common among older men receiving intramuscular testosterone injections (Hajjar *et al.* 1997) and less common with more steady-state depot testosterone delivery, but has been observed with all forms of exogenous androgen (Jockenhövel *et al.* 1997).

15.4.3 Growth

One small double-blind, placebo-controlled cross-over study examined the effects of *testosterone* on short-term growth in boys with short stature on hemodialysis (Kassmann *et al.* 1992). After an 8-week run-in, eight boys (mean 3.9 SD below

mean height for age) on regular hemodialysis were randomised to start on one of two four-week treatment periods separated by a six-week wash-out period before crossing over to the other treatment. Treatment consisted of 2 g/m^2/day of a transdermal gel corresponding to a topical daily dose of 50 mg/m^2 testosterone or placebo. Although a significant increase in short-term growth velocity (using knemometry) was reported overall, gain of final height was not reported and cannot be predicted from growth velocity. Furthermore, the small sample size and unbalanced randomisation were limitations. Further larger and longer studies would be needed before even low-dose androgen therapy could be considered effective or safe.

15.4.4 Enuresis

Following suggestions from the 1940s that androgen therapy might improve childhood enuresis, a recent controlled clinical trial involving 30 boys aged 6–10 years has claimed a benefit for oral mesterolone treatment compared with placebo (El-Sadr *et al.* 1990). This study may have been flawed as the method of randomisation leading to 20 being treated with mesterolone (20 mg daily for 2 weeks) compared with 10 on placebo (vitamin C) was not explained. The statistically significant increase in cystometric bladder capacity in the mesterolone-treated group was attributable to six boys who had dramatic increases, whereas the remainder did not differ from the ten placebo-treated boys. Although no adverse effects were reported, the well-known potential hazards of androgen therapy in prepubertal children, including premature closure of epiphyses and short stature, precocious sexual maturation and psychological sequelae would require detailed safety evaluation before androgen therapy could be considered acceptable for a benign functional disorder with favourable natural history in otherwise healthy children.

15.5 Neuromuscular disorders

15.5.1 Muscular dystrophies

The effects of androgen therapy on neuromuscular disorders have been best studied by Griggs *et al.* in a series of careful studies of *myotonic dystrophy* (MD), a genetic myopathy due to a trinucleotide (CTG) repeat mutation in the myotonin (protein kinase) gene. MD is associated with testicular atrophy and biochemical androgen deficiency compared with age-matched healthy men or men with other neuromuscular wasting diseases (Griggs *et al.* 1985), although serum testosterone does not correlate with extent of muscle wasting. Since life expectancy in MD is determined by respiratory muscular weakness leading to terminal pneumonia, androgen therapy aiming to improve muscular strength might prolong life. To test this hypothesis, a randomised placebo-controlled study was undertaken in 40 men

with MD who were treated with either *testosterone enanthate* (3 mg/kg) or placebo injections each week for 12 months (Griggs *et al.* 1989). In a well-designed two-site study, muscle mass was increased as indicated by creatinine excretion and total body potassium, but there was no difference in quantitative measures of manual or respiratory muscle strength. Crucially, the lack of improved pulmonary function implies that mortality benefits would be unlikely. Androgen therapy may simply increase the mass of dysfunctional muscle.

The same investigators also examined the effect of androgen therapy in boys with *Duchenne muscular dystrophy* (DMD) in a randomised placebo-controlled study (Fenichel *et al.* 2001) following encouraging results from an uncontrolled pilot study of ten boys treated for three months (Fenichel *et al.* 1997). Boys aged 5–10 years of age with DMD ($n = 51$) were randomly assigned to receive oxandrolone (0.1 mg/kg/day) or placebo for six months. Although the primary endpoint (semi-quantitative average muscle strength score) and timed functional tests of gait were not significantly improved, oxandrolone produced a significant increase in some post-hoc comparisons such as quantitative myometry and in upper limb muscle strength score. The marginal efficacy of oxandrolone was accompanied by proportionate growth and few side effects so that such treatment may find a role before instituting high dose glucocorticoids, which are more effective but also cause more adverse effects including growth retardation and weight gain.

The discrepancy between these findings is puzzling and the precise role of pharmacological androgen therapy in other forms of neurogenetic or degenerative neuromuscular disorders warrants further evaluation.

15.6 Rheumatological diseases

15.6.1 Hereditary angioedema

The efficacy of oral 17α-alkylated androgens in hereditary angioedema was established by a small, double-blind, placebo-controlled randomised cross-over study (Spaulding 1960) in which six members of a single family received multiple periods of treatment or placebo. This study clearly demonstrated the efficacy of oral methyltestosterone in reducing the frequency of attacks well before the disease pathogenesis was understood. Subsequent studies confirmed these observations showing that androgen therapy increases *C1-esterase inhibitor* concentration partially rectifying the underlying biochemical deficiency responsible for the disorder (Sheffer *et al.* 1977). Although other 17α-alkylated oral androgens such as fluoxymesterone, oxymetholone and stanozolol have been used, danazol has become standard prophylactic therapy. This followed a randomised double-blind cross-over study which showed increased blood C1-esterase inhibitor concentration together

with a dramatic decrease (94% vs. 2%) in attack-free 28 day periods using 600 mg danazol daily compared with placebo in 93 courses among nine patients (Gelfand *et al.* 1976). Danazol doses are tapered to minimal levels that maintain adequate control of attack and this dose minimisation may explain the anecdotal impression that such danazol therapy has minimal effects on male fertility although quantitative studies have not been reported. Recent studies suggest that stanozolol (1–2 mg daily) is about as effective as danazol (50–200 mg daily) but, despite their efficacy, hepatotoxicity and female virilisation remain problems (Cicardi *et al.* 1997; Hosea *et al.* 1980). While it is assumed that the beneficial effects of androgen therapy for angioedema are only exhibited by *17α-alkylated androgens*, only very limited studies of non-17α-alkylated androgens such as nandrolone, 1-methyl androgens or testosterone (Spaulding 1960) have been reported. Since angioedema requires lifelong prophylaxis, further studies of non-hepatotoxic androgens should be undertaken. It remains possible that the oral route of administration may constitute a form of liver targeting (via first pass exposure) for high hepatic androgen doses, which might not be feasible or safe for parenteral administration.

15.6.2 Rheumatoid arthritis (RA)

The rationale for androgen therapy in RA is that (a) the lower prevalence in men suggests a protective role for androgens, (b) active disease is associated with reduction in endogenous testosterone production, (c) androgen effects on muscle and bone may improve morbidity in RA and (d) androgen effects (e.g. fibrinolysis) may reduce disease activity.

The best designed and conducted study of androgen therapy involved 107 women with active RA according to American College of Rheumatology (ACR) criteria on stable standard (steroid, NSAID) treatment for at least 3 months who were randomised to treatment with fortnightly injections of either androgen therapy (*testosterone propionate* 50 mg plus progesterone 2.5 mg) or placebo for one year (Booij *et al.* 1996). The inclusion of a very low dose of progesterone, which the authors claim was biologically ineffective, was based on an old clinical practice aiming to reduce virilisation from testosterone. Evaluated on a double-blinded, intention-to-treat basis this study demonstrated significant improvement in the ESR, pain and disability scores and ACR improvement criteria, but not in the numbers of tender or swollen joint or joints requiring intra-articular steroid injections. There was a high dropout rate (39/107), mostly (28/39) due to inefficacy defined as any mid-study increase in anti-rheumatic medication, however, these were evenly distributed between treatment groups. As expected, virilisation was the major adverse effect reported but there were few other side-effects and tolerability was good as most androgen-treated patients (67% vs 37% on placebo) wished to

continue their allocated medication at the end of the study. The significant benefits of androgen therapy over placebo were predominantly in subjective measures rather than objective signs of disease activity. This raises the possibility that androgen therapy may preferentially improve mood or tolerance of disability rather than actually modifying disease impact or natural history. This well designed study is a model for investigation of pharmacological androgen therapy in systemic disease.

Other studies of androgen therapy in RA are small, poorly designed and inconclusive. One uncontrolled study of seven men with RA treated with six months of androgen therapy (oral *testosterone undecanoate* 120 mg daily) observed a decline in disease activity (reduced numbers of tender joints and analgesic usage) together with minor immunological changes that were not correlated with disease activity. The lack of a placebo group in a disease with a remitting natural history renders such observations unconvincing (Cutolo *et al.* 1991). A larger study of 35 men with definite RA randomised them to injections of testosterone enanthate (250 mg monthly) or placebo for nine months (Hall *et al.* 1996). This study noted that overall disease activity (defined by biochemical variables and clinical scales) was not improved by androgen therapy and indeed, significantly more men on testosterone therapy experienced disease 'flare' during the study. The inclusion of men with inactive RA and initial use of an inadequate testosterone dose were limitations of this study. An older double-blind study randomised 40 patients with definite RA on stable NSAID to treatment with stanozolol 10 mg daily or placebo for six months on the basis that androgen therapy might increase fibrinolysis (Belch *et al.* 1986). This study found a significant improvement in the composite Mallaya disease activity index combining objective (ESR, hemoglobin, articular scores) and subjective (pain, morning stiffness) dimensions, despite the failure to influence measurable fibrinolysis. Adverse effects such as hepatotoxicity or virilisation in females were not reported.

Whether androgen therapy in men with RA can truly modify the natural history or whether it only improves mood and toleration of pain and disease remain to be clarified by further well designed studies. No controlled studies examining improved muscle strength, bone density and other androgen-sensitive variables can improve quality of life in RA are yet reported.

15.6.3 Other rheumatological disorders (SLE, Raynauds, systemic sclerosis, and Sjogrens disease, chronic urticaria)

Few well-controlled studies of androgen therapy have been reported in other rheumatological disorders. This is not just due to paucity of cases in the female-preponderant autoimmune diseases as there are no controlled studies of androgen therapy even in ankylosing spondylitis or gout, the male preponderant rheumatological diseases.

In *systemic lupus erythematosus* (SLE), only two small uncontrolled studies (including together five men among 17 patients) using androgen therapy (nandrolone decanoate) have been reported (Hazelton *et al.* 1983; Lahita *et al.* 1992). This information is so limited that no conclusions can be drawn without larger and better-designed studies. Another double-blind study randomised 28 women with mild to moderate SLE to treatment with DHEA (200 mg daily) or placebo for three months. Treatment with this weak androgen precursor did not improve SLE disease activity index, number of flares, prednisone usage or physician overall assessment, although there was an improvement in the patients overall assessment of well-being (Van Vollenhoven *et al.* 1995).

One study has examined the effects of treatment with stanozolol (10 mg daily) or placebo for 24 weeks in *primary Raynaud's phenomenon* and *systemic sclerosis* (Jayson *et al.* 1991). Although 43 patients (19 Raynaud's, 24 systemic sclerosis; including only 4 men) entered, only 28 patients (11 Raynauds, 17 systemic sclerosis) completed the study. Compared with placebo, stanozolol significantly improved ultrasonic Doppler index as well as finger pulp and nail bed temperatures but there was no difference in reported frequency or severity of vasospastic attacks, scleroderma skin score or grip strength. The clinical significance of the changes in digital small vessel function recorded in the absence of vasospasm and without reduction in attack rates is unclear.

A double-blind study randomised 20 women with primary *Sjogren's syndrome* to treatment with androgen (nandrolone decanoate 100 mg fortnightly) or placebo for six months (Drosos *et al.* 1988). Androgen therapy did not produce any significant improvement over placebo in objective validated measures of xerostomia (stimulated parotid flow rate measurements, labial salivary gland histology), xerophthalmia (Schirmer's I test, slit lamp eye examination after rose Bengal staining) or systemic disease (ESR) although the subjective assessment of xerostomia by patients and physicians as well as overall patient's well-being assessment were significantly better on nandrolone. Virilisation was reported in nearly all nandrolone-treated women with this relatively high androgen dose but none discontinued for this reason. Again these studies reinforce the observations that androgen therapy may significantly improve feelings of well-being regardless of the underlying disease activity.

A recent randomised double-blind study examined the role of stanzolol as an adjunct to standard antihistamine therapy for chronic urticaria. Patients (20 men, 30 women) were randomised to treatment with stanazolol 4 mg daily or placebo in addition to antihistamine (cetrizine 10 mg daily) for 12 weeks (Parsad *et al.* 2001). Over 70% improvement in physician and patient scored urticaria was observed in 17/26 patients who received stanazolol, but in only 7/24 patients who received cetrizine alone. This highly statistically significant benefit was observed four weeks

after starting treatment and continued throughout the study. Whether the benefits of stanazolol are gender-specific, or whether other androgens are also effective, has not been established.

15.7 Bone disease

The role of androgens in bone development and disorders is discussed in Chapter 7. Androgen therapy to treat osteoporosis has the advantage for fracture prevention of not only increasing bone mass but possessing potentially synergistic beneficial effects on muscular strength and mental function to prevent falls due to frailty, an independent contributor to osteoporotic fractures. The evidence supporting androgen therapy, however, is limited. For treatment of idiopathic osteoporosis, the largest randomised, placebo-controlled study, involving 327 patients treated for 9 months with 1 year of follow-up, had inadequate power to detect effects of androgen therapy (methandienone 2.5 mg daily) on fracture rates (Inkovaara *et al.* 1983). The only other controlled study randomised 21 men to receive either weekly injections of nandrolone decanoate 50 mg or no treatment for 12 months (Hamdy *et al.* 1998). It remains unclear whether the inconsistent and transient increase in bone density observed were due to the low dose, the minimally aromatised androgen or small sample size. Additionally, an uncontrolled study has claimed striking increase in lumbar (but not hip) bone density in non-androgen deficient men treated with testosterone ester injections 250 mg fortnightly for 6 months (Anderson *et al.* 1997).

An important area for androgen therapy to prevent or ameliorate bone loss and fractures may be *steroid-induced osteoporosis*. High dose glucocorticoid therapy is commonly used for its immunosuppressive or anti-inflammatory effects in autoimmune and chronic inflammatory diseases and in transplantation medicine. Two controlled studies have examined androgen therapy in men taking regular high dose glucocorticoid treatment. The first reported that testosterone may reverse the bone loss due to high-dose glucocorticoid therapy in 15 men with severe asthma (Reid *et al.* 1996). The subjects were randomly allocated to monthly testosterone injections (250 mg mixed testosterone esters) or no treatment for 12 months with the control group crossing over to testosterone treatment for the second 12-month period. After 12 months of testosterone treatment, lumbar spine bone mineral density increased by 5% compared with no change on placebo. However, no benefit was noted in bone density overall or in three other sites. The limitations of this study (unblinded, sub-replacement testosterone dose) are addressed in a larger study randomising 51 men to fortnightly injections of *testosterone esters* 200 mg, nandrolone decanoate 200 mg or matching oil vehicle placebo for 12 months (Crawford *et al.* 2003). This study observed improved muscular strength with both androgens but improved lumbar bone density and bone-specific quality of life only in men treated

with testosterone. This highlights the importance of aromatisation in androgen therapy for bone but not muscle. Larger studies examining fracture outcome as well as earlier studies aimed to prevent the rapid initial bone loss would be most valuable.

15.8 Critical illness, trauma and surgery

Critical illness, trauma, burns, surgery and malnutrition all result in a catabolic state characterised by acute muscle breakdown which is reversed during recovery. These catabolic states are characteristically accompanied by functional hypogonadotrophic, androgen deficiency. This is due to functional partial GnRH deficiency as pulsatile GnRH administration can rescue LH pulsatility and hypoandrogenemia (Aloi *et al.* 1997; van den Berghe *et al.* 2001). This has long led to the hypothesis that androgen therapy might improve mortality or morbidity by pharmacologically enhancing nutritional supplementation and muscle, bone and skin recovery. However, the endocrine response to catabolic states such as critical illness are highly complex involving widespread dysregulation of all pituitary hormonal axes (Van den Berghe 2003) so that restoration of individual anabolic hormones may be inadequate. Nevertheless the success of intensive insulin therapy to regulate hyperglycemia (van den Berghe *et al.* 2001) and the promise of combination pituitary hormonal approaches (Van den Berghe *et al.* 2002) indicates that hormonal regimens are promising. Key outcome variables for evaluating the efficacy of androgen therapy in such catabolic states include (a) muscle mass and strength, (b) bone turnover and wound healing (particularly after burns) as well as (c) health service utilization variable such as duration of in-hospital stay and rate and extent of rehabilitation.

15.8.1 Muscle mass

A number of studies have examined the effects of androgen therapy as an adjunct to elective surgery using improved nitrogen balance as a surrogate for muscle mass for their endpoint. The best designed study randomised 60 patients after colorectal cancer surgery to receive either a single injection of stanozolol (50 mg) or no extra treatment. Participants were also randomised among three types of post-operative, peripheral-vein nutrition (standard dextrose-saline, amino acid supplementation or glucose-amino acid-fat mixture) and stratified by gender (Hansell *et al.* 1989). The primary end-point was cumulative nitrogen balance for the first four post-operative days and this was consistently and significantly influenced only by nutritional supplementation. Stanozolol augmented nitrogen balance only on the third post-operative day in the group receiving amino acid supplements. This was largely attributable to its effects in women and was no improvement over standard

post-operative care on other post-operative days, with other nutritional supplements or had any influence on a wide range of other metabolic variables. Importantly neither convalescence nor complication rates were influenced by androgen or nutritional therapy.

Four other studies have largely confirmed these findings. The first randomised 44 men with tuberculosis requiring pulmonary resection to treatment with either high-dose norethandrolone (50 mg daily) or no extra treatment within strata of different intensity of postoperative hyperalimentation (Webb *et al.* 1960). This showed a modest, transient effect of androgen therapy on positive nitrogen balance restricted to the first three post-operative days which was absent during the second three post-operative days. The second study randomised 36 patients to one injection of stanozolol (50 mg) or placebo one day before surgery with similar outcomes (Blamey *et al.* 1984). A third study randomised 30 men after gastric surgery for duodenal ulcer (vagotomy/pyloroplasty) to a single post-operative injection of nandrolone decanoate (50 or 100 mg), parenteral nutrition, both, or to standard treatment (Tweedle *et al.* 1973). This study reported that the eight day post-operative nitrogen balance was best with the combination of nandrolone plus parenteral nutrition and that each alone was superior to standard treatment but no clinical outcome measures were reported. The fourth study randomised 20 patients recovering from multiple trauma to receive either nandrolone decanoate injections (50 mg on day 3 plus 25 mg on day 6) or no extra treatment. It found that nandrolone plus standard enteral or parenteral nutrition was superior to no extra treatment in nitrogen balance, urinary 3-methyl histidine excretion and amino acid retention for the first ten days of hospitalisation (Hausmann *et al.* 1990). The only clinical outcome measure, however, was six-month survival, which did not differ according to androgen therapy.

Other studies, however, have been unable to detect any clinical benefits. One well-designed study randomised 48 patients requiring hyperalimentation to supplemental treatment with either nandrolone decanoate (50 mg) or placebo injections biweekly aiming to determine whether nitrogen balance could be improved within the first 21 days post-operatively (Lewis *et al.* 1981). No benefit was observed in nitrogen balance, weight gain, creatinine output, and serum albumin or immune function. These negative findings were supported by another study that examined a higher nandrolone dose. This study randomised 24 patients requiring intravenous alimentation to nandrolone decanoate (100 mg before starting and repeated one week later) or no extra treatment and found increased fluid but not nitrogen balance and did not find any clinical benefits (Young *et al.* 1983). Another study has examined the use of oral oxandrolone in a study that randomised 60 patients (including five women) requiring enteral nutrition to oxandrolone 20 mg each day or placebo for no more than 28 days and reported no differences in nitrogen

balance or clinically relevant outcomes such as infection rate or length of stay (Gervasio *et al.* 2000).

15.8.2 Skin healing

One group of investigators has performed two studies examining whether oxandrolone (20 mg per day) can promote skin healing after severe *burn* injury (Demling 1999; Demling and Orgill 2000). The double-blind study randomised 20 patients with severe burns to receive oxandrolone or matching placebo for at least 3 weeks commencing 2–3 days after the injury (Demling and Orgill 2000). Oxandrolone therapy promoted skin healing in the standardised donor site, improved nitrogen balance and reduced weight loss, but did not alter length of hospital stay. Similar results were reported in an earlier non-blinded study of 36 patients randomised to receive growth hormone ($n = 20$) or oxandrolone ($n = 16$) (Demling 1999). However, the non-randomly selected and non-equivalent control group ($n = 16$, with less severe burns) and the lack of blinding limit the interpretation of this study. Whether improved skin healing at the donor site will lead to improved overall recovery and specifically promote the healing of severely burned skin remains unproven.

15.8.3 Rehabilitation

Two studies by one group of investigators have examined whether oxandrolone (20 mg each day) can speed rehabilitation after severe burn injury (Demling 1999; Demling and DeSanti 1997; Demling and DeSanti 2001; Demling and Orgill 2000). The first study randomised 13 patients with severe *burns* receiving usual rehabilitation and high-protein supplementation to also receive oxandrolone or not for at least three weeks during the recovery phase of burn treatment (Demling and DeSanti 1997). The second study additionally stratified 40 patients by age before randomisation (Demling and DeSanti 2001). Although staff performing rehabilitation measurements may not have been blinded, both studies reported significant weight gain (including lean mass) and improved rehabilitation regardless of age, (Demling and DeSanti 1997; Demling and DeSanti 2001).

Two studies have examined whether short-term pharmacological androgen therapy can improve rehabilitation in older men. The first randomised 25 men scheduled for knee replacement to receive weekly doses of 300 mg *testosterone enanthate* or matched placebo during three weeks before surgery (Amory *et al.* 2002). The second randomised 15 men admitted to hospital for general physical rehabilitation to receive weekly injections of 100 mg testosterone enanthate or placebo for 2 months (Bakhshi *et al.* 2000). Small improvements in Functional Independence Measure (FIM) score and strength (hand-grip dynamometry) were reported only in the latter study which, however, suffered from the limitations of small sample

size, non-matching saline placebo, and unbalanced groups despite randomisation (Honkanen and Lesser 2001).

15.9 Immune disease – HIV/AIDS

Androgen therapy for HIV/AIDS has been mainly investigated for its effects on disease-associated morbidity (weight loss, weakness, quality of life) rather than to influence the underlying disease natural history. Indeed, randomised placebo-controlled studies have consistently reported no androgen effect on CD4 count or viral load (Bhasin *et al.* 1998; Bhasin *et al.* 2001; Coodley and Coodley 1997; Dobs *et al.* 1999; Grinspoon *et al.* 1998; Rabkin *et al.* 1999; Sattler *et al.* 1999; Strawford *et al.* 1999) with two exceptions (Berger *et al.* 1996; Grinspoon *et al.* 2000), neither of which showed a consistent decrease in both CD4 count and viral load. One rationale for androgen therapy stems from the observation that body weight loss is an important terminal determinant of survival in AIDS and other fatal diseases (Grunfeld and Feingold 1992). It has been estimated that death occurs when lean body mass reaches 66% of ideal (Kotler *et al.* 1989) leading to the proposition that if androgens (or other agents including megestrol or growth hormone) increased appetite and/or body weight, death may be delayed. Given this hypothesis, the effect of androgen therapy may differ between men with AIDS wasting, and those without weight loss.

15.9.1 AIDS/HIV wasting

A number of randomised placebo-controlled studies of androgen therapy in HIV-positive men with AIDS wasting have reported increased lean mass, but minimal effects on total body weight, possibly due to concomitantly reduced fat mass. In the most comprehensive study (Grinspoon *et al.* 1998; Grinspoon *et al.* 2000), 51 men selected for both weight loss and low serum testosterone concentration were randomised to receive testosterone enanthate 300 mg or oil-based placebo intramuscularly every three weeks for six months. Although total weight, fat mass (DEXA), total body water content (bioimpedance) and physical function were not changed by testosterone therapy, fat-free mass (DEXA), lean mass (total body potassium) and muscle mass (urinary creatinine excretion) were all increased (Grinspoon *et al.* 1998). The increased lean body mass was sustained during the open-label six month extension (Grinspoon *et al.* 1999). In contrast, the other four studies have examined body compositional changes less comprehensively (Batterham and Garsia 2001; Dobs *et al.* 1999) or not at all (Berger *et al.* 1996; Coodley and Coodley 1997). The first randomised 63 HIV seropositive men suffering from wasting and weakness to receive either 15 mg or 5 mg oxandrolone daily or placebo for 16 weeks (Berger *et al.* 1996). Both oxandrolone (but not control) groups demonstrated transient

weight gain within the first month, peaking at the first week. Subsequently, while the high-dose group maintained mean weight gain and the other groups less so, the within-group variance increased, suggesting major within-group heterogeneity in time-course. There was also no clear dose-response relationship. A second placebo-controlled crossover study randomised 39 men with HIV-associated weight loss to receive injections of either *testosterone cypionate* 200 mg or placebo (of unstated type) every fortnight for three months before crossing over to the other treatment (Coodley and Coodley 1997). Although testosterone improved one of five aspects of quality of life (overall well-being), no change in the other components or in weight was detected. However, the null effect could have been due to the lack of washout between treatments. A third study selected men with HIV-associated weight loss with serum testosterone concentrations in the low normal range (Dobs *et al.* 1999). This multi-centre, placebo-controlled study randomised 133 men to receive transscrotal testosterone patch (delivering nominal 6 mg testosterone per day) or matching placebo daily for 12 weeks. Testosterone treatment did not alter weight or lean mass (bioimpedance); however, inconsistent improvements in quality of life were observed. These findings are supported by a study that randomised 15 men to receive nandrolone decanoate (100 mg/fortnight), megestrol acetate (400 mg/day) or dietary advice alone and reported that nandrolone did not increase weight or lean mass (bioimpedance) (Batterham and Garsia 2001).

Recent studies in men with AIDS wasting examining the additional effect of *exercise* have been confirmatory. These studies have examined the effect of intramuscular testosterone therapy with or without exercise in a 2-by-2 factorial design. In both studies, men were selected on the basis of HIV-associated weight loss and exposed to exercise consisting of a progressive resistance programme three times each week throughout the study. In the first study (Bhasin *et al.* 1998), 61 men were randomised to receive testosterone enanthate 100 mg/wk and/or resistance exercise for 16 weeks. Among the 49 evaluable men, testosterone or resistance exercise increased body weight, thigh muscle volume (MRI), muscle strength and lean body mass (deuterium oxide dilution and DEXA) compared with the control (placebo, no exercise) group, but the combination did not promote further gains. Quality of life was not altered. In the other study (Fairfield *et al.* 2001; Grinspoon *et al.* 2000), 50 men were randomised to receive testosterone enanthate 200 mg/wk and/or resistance exercise for 12 weeks. Among the 43 evaluable men, testosterone or resistance exercise increased body weight, lean mass (DEXA) and some components of strength, and reduced fat mass (DEXA) (Grinspoon *et al.* 2000). The effect of the combination over testosterone therapy or exercise alone was not reported. Another study of 24 men with HIV-associated weight loss treated all with progressive resistance exercise and testosterone enanthate 100 mg each week "to suppress endogenous testosterone production" and then randomised half to additionally

receive oxandrolone 20 mg each day or placebo tablets for 8 weeks (Strawford *et al.* 1999). The addition of oxandrolone was reported to increase lean tissue accrual and strength however the lack of a no-treatment control and the concurrent use of two androgens limits interpretation.

15.9.2 HIV without wasting

In HIV-positive men without wasting, androgen induced changes in body composition are more modest. One study of 41 HIV-positive men selected for low-normal serum testosterone concentrations (but not weight loss) and randomised them to 12 months daily transdermal treatment with *testosterone* (delivering 5 mg testosterone daily) or placebo patch (Bhasin *et al.* 1998). Testosterone produced a greater reduction in fat mass (DEXA) but no difference in lean mass, physical function (strength) or quality of life. The additive effect of testosterone with exercise has also been examined in HIV-positive men without weight loss. In this study, all 30 men with stable weight were treated with supraphysiological weekly doses of intramuscular nandrolone decanoate (200 mg for the first dose, 400 mg for the second dose and 600 mg for all subsequent doses) and randomised half to additionally receive progressive resistance exercise 3 times each week or not for 12 weeks (Sattler *et al.* 1999). Although resistance exercise augmented gains in muscular strength and lean body mass (DEXA and bioimpedance), there was no additional effect on body weight. The lack of a no-treatment control and the unblinded exercise intervention limit the interpretation of this study.

15.10 Malignant disease

15.10.1 Effects on morbidity and mortality

Androgen therapy could influence mortality from malignant disease via direct antitumor effects or improve morbidity by maintaining weight, hemoglobin, neutrophil count, muscle mass and bone mass through its known actions. Reduced morbidity may also augment treatment by creating greater tolerance for more aggressive cytotoxic therapy. Despite encouraging results from animal models and uncontrolled clinical reports, human studies are so far less convincing. Although older studies demonstrate a consistent but modest effect of androgen therapy in reducing the magnitude, duration and/or complications from chemotherapy-induced neutropenia, few well-controlled clinical studies have shown unequivocal benefits of androgen therapy. The recent availability of recombinant human G-CSF/GM-CSF with its greater efficacy and better tolerability (albeit expensive) reduces the benefits from androgen-induced prevention of neutropenia to second-line status.

Androgen therapy appears to have morbidity benefits in some but not all studies. One open controlled study randomised 33 patients with lung or other non-hormone responsive solid cancers to standard chemotherapy plus nandrolone

decanoate (200 mg weekly) or no additional treatment. In this study androgen therapy produced better maintenance of body weight, hemoglobin and less transfusion requirement, but no improved survival or physical performance (Spiers *et al.* 1981). Similarly, a cohort of 23 patients with inoperable *lung cancer* requiring palliative chest radiotherapy were randomised to receive or not to receive additional treatment with nandrolone phenylpropionate (loading dose 100 mg followed by 50 mg weekly during hospitalisation). During radiotherapy (4500 cGy), androgen therapy maintained higher hemoglobin and lower transfusion requirements (Evans and Elias 1972). In contrast, two other studies have failed to demonstrate a definite benefit of androgen therapy. In one study of 40 adults (including nine women) who had undergone oesophageal resection for carcinoma, subjects were randomised to receive intramuscular injections of nandrolone decanoate 50 mg or oil-based placebo every three weeks for three months commencing one month after resection (Darnton *et al.* 1999). No treatment effect in weight, appetite or mid-arm circumference was detected, although appetite improved in both groups with time. In the other study, 37 patients with unresectable non-small cell lung cancer requiring standard combination chemotherapy were randomised to receive or not additional treatment with nandrolone decanoate (200 mg weekly for four weeks). Androgen therapy was associated with only a non-significant statistical trend towards improved survival (median 8.2 vs. 5.5 months) and less weight loss but no improvement in marrow function (Chlebowski *et al.* 1986). The subtherapeutic dose employed by the first study (Darnton *et al.* 1999) and the greater myelosuppression resulting from aggressive modern combination chemotherapy in the second study (Chlebowski *et al.* 1986) may have negated any morbidity benefits.

Another study has examined the effect of androgen, progestin or corticosteroid treatment for an indefinite period of time on appetite and weight in 475 men and women with weight loss due to advanced incurable cancer (Loprinzi *et al.* 1999). Subjects were stratified by cancer type, prognosis and degree of weight loss before being randomised to receive fluoxymesterone 20 mg/day or megestrol acetate 800 mg/day or dexamethasone 3 mg/day in a double blind fashion for a median duration of two months. Although survival or quality of life were equivalent between groups, fluoxymesterone at the dose administered was significantly inferior for appetite stimulation and tended to result in less weight gain. Furthermore, hirsutism and virilisation were major problems occurring in about 10% of all women. The role of fluoxymesterone to stimulate appetite is doubtful given the clear superiority of other agents and the dubious quality of life consequences of this indication.

A recent study of 35 men with *hematological malignancies* in complete remission following treatment with cytotoxic chemotherapy evaluated the role of androgen therapy for compensated Leydig cell failure. Men with low-normal circulating testosterone and raised LH concentrations were randomised (single blind) to receive for 12 months transdermal placebo or *testosterone patches* (2.5–5 mg/day) dose

titrated to maintain a serum testosterone concentration of >20 nM (Howell *et al.* 2001). Testosterone treatment did not alter bone turnover markers, hip, spine or forearm bone mineral density (quantitative computed tomography and DEXA), lean mass or fat mass (DEXA), mood (hospital anxiety and depression scale) or sexual function. However, two out of five components of the multi-dimensional fatigue inventory were improved (activity was increased and physical fatigue was reduced). These inconsistent and minor effects were supported by a case control study showing minimal differences based on lower serum testosterone concentrations in similar men (Howell *et al.* 2000) suggesting that androgen replacement therapy offers little objective benefit for men with compensated Leydig cell failure post-cytotoxic therapy.

Pharmacological androgen therapy has also been evaluated for maintenance therapy for *acute non-lymphocytic leukemia* (ANLL) on the basis that enhanced proliferation of residual normal hematopoietic precursors would suppress competitively the growth of the leukemic clones. Among 114/212 patients with newly diagnosed ANLL who obtained complete remission after standard induction chemotherapy, 82 agreed to be randomised to undergo standard maintenance chemotherapy alone or in combination with BCG vaccination, stanozolol (0.1 mg/kg/d) or BCG vaccination plus stanozolol. After three years follow-up, all four arms had similar rates of remission and adverse events (Mandelli *et al.* 1981).

Another study has examined whether androgen ablation may improve *hepatic cell carcinoma* since androgen receptor positivity in these tumours is associated with more rapid disease (Nagasue *et al.* 1995). In this study (Grimaldi *et al.* 1998), 244 subjects with unresectable hepatic cell carcinoma were randomised double-blind using matching placebo into one of four groups: (1) Placebo intramuscular injections monthly and oral nilutamide 300 mg/d for one month then 150 mg/d with (2) Intramuscular long acting GnRH antagonist monthly (either goseriline acetate 3.6 mg or triptorelin 3.75 mg) with oral placebo daily (3) Intramuscular long acting GnRH antagonist monthly (either goseriline acetate 3.6 mg or triptorelin 3.75 mg) with oral nilutamide 300 mg/d for one month then 150 mg/d. Treatment duration was indefinite, and androgen deprivation did not alter survival even after adjusting for known baseline prognostic factors by Cox analysis.

Androgen therapy continues to have an established role in late-stage advanced breast cancer usually as a late option after failure of other hormonal therapies and when the virilising side-effects are less unacceptable. Few recent studies include androgen therapy and it now has a residual but diminishing role relative to modern hormonal and cytotoxic chemotherapy for breast cancer.

There is no good evidence for any direct antitumor effects of androgen therapy and potential indirect androgen effects on quality of life requires further evaluation by well-controlled studies of morbidity end-points.

15.10.2 Cytoprotection

Infertility arising from cancer treatment involving serious bystander damage to the testis from cytotoxic drugs and/or irradiation is distinctive since the timing, dose and nature of the testicular damage are clearly defined. This creates unique possibilities for prevention of male infertility through elective sperm cryostorage, germ cell autotransplantation (Schlatt 2002) and cytoprotective strategies to minimise bystander damage to non-target organs such as the testis. Such cytoprotective strategies might be based on physical measures such as temporary tissue cooling or restricting blood flow during drug exposure or on chemicals such as radioresistance drugs (Adams *et al.* 1976) or hormones that protect against unintended cytotoxic effects.

One of the first approaches proposed to protect the testis was hormonal manipulation to render the testis "quiescent" before and during the administration of the testicular toxin (Morris 1993). The seminal study was the claim that pretreatment with a GnRH analogue reduces cyclophosphamide induced spermatogenic damage in the mouse testis (Glode *et al.* 1981) together with the belief that prepubertal gonads seemed relatively less damaged by chemotherapy treatment for leukaemia compared with their post-pubertal counterparts. However, infancy is not a quiescent period for the testis (Chemes 2001), the clinical impression of prepubertal protection is illusory (Shalet *et al.* 1978) and the initial experimental findings were not reproducible (da Cunha *et al.* 1987), the latter reflecting the known resistance of mice to the GnRH analogue employed (Bex *et al.* 1982). Nevertheless this concept has prompted many better defined and validated experimental animal models showing promise (Meistrich 1993; Morris 1993) and limitations (Crawford *et al.* 1998) as well as several clinical studies to test this hypothesis.

However, the available clinical studies (Brennemann *et al.* 1994; Johnson *et al.* 1985; Kreusser *et al.* 1990; Waxman *et al.* 1987) have not demonstrated that adjuvant GnRH superactive agonists treatment during cancer therapy can promote recovery of spermatogenesis. Nonetheless, these studies were an inadequate test of the hypothesis since (a) superactive GnRH agonists feature an initial boost in, rather than immediate and thorough cessation of, gonadotrophin secretion during the start of cytotoxin exposure which vitiate the hypothesis, (b) only one study was randomised (Waxman *et al.* 1987) while another was uncontrolled (Johnson *et al.* 1985) and (c) the follow-up duration was insufficient to define an improvement given the likely timescale of gonadal recovery. Better designed studies of pure GnRH antagonists would be of interest.

Androgens cause feedback suppression of gonadotrophins and are relatively inexpensive, but are considered impractical as a cytoprotective strategy since their onset of action is too slow to be effective given the imperatives of life-saving cancer treatment which cannot be delayed. Nevertheless, a recent randomised controlled pilot

study reported that androgen administration commencing well before and continuing during cyclophosphamide therapy for nephrotic syndrome could speed recovery of spermatogenesis (Masala *et al.* 1997). However, this potentially important finding warrants cautious interpretation since it was a small study of a single agent cytotoxic drug treatment, which could be delayed. Whether an androgen-based cytoprotective regimen is feasible or effective for the more frequent and intensive combination chemotherapy used for cancer treatment remains unclear.

Meistrich has recently developed an important novel hypothesis regarding the mechanism of the often very slow rate of recovery of spermatogenesis following cytotoxic damage. Noting that stem cells often survive but their differentiation is blocked, he has shown experimentally that high intratesticular testosterone inhibits spermatogonial replication and differentiation. This suggests that a new approach to enhancing recovery from cancer treatment-related spermatogenic damage may be to temporarily depress intratesticular testosterone by the use of GnRH analogs (Shetty *et al.* 2000) or other methods. This interesting but paradoxical claim could provide the basis for a novel and feasible treatment to accelerate recovery of spermatogenesis, although a recent small uncontrolled clinical trial has failed to demonstrate faster recovery (Thomson *et al.* 2002). Evaluation of this hypothesis in well controlled trials is warranted.

Ultimately long-term studies comparing cytoprotection regimens for efficacy, safety and cost-effectiveness compared with standard sperm cryopreservation (Kelleher *et al.* 2001) with or without artificial reproductive technologies will be needed. Potential cytoprotective regimens based on pure GnRH antagonists, with their immediate and complete gonadotrophin suppression, warrant clinical trials. The development of experimental germ cell transplantation to re-establish spermatogenesis in rodents (Brinster and Zimmermann 1994) allowing the restoration of genetic paternity (Brinster and Avarbock 1994) introduces a new and potentially important method of preserving fertility by germ cell autotransplantation in these men (Schlatt 2002). Nuclear transfer cloning may have an unusually acceptable niche if developed for germ cell autotransplantation if the appropriate methodologies are developed.

15.11 Respiratory disease

15.11.1 Chronic obstructive lung disease

Advanced chronic airflow limitation is associated with weight loss and muscle depletion, possibly due to the increased energy requirements required for breathing or reduced serum testosterone concentrations (Kamischke *et al.* 1998). Interventions aimed at improving muscle bulk such as nutrition, exercise or androgens may therefore have an impact on the morbidity and/or mortality of the underlying

respiratory disease. One large well-conducted prospective study demonstrated that short-term low-dose androgen therapy (nandrolone decanoate) augmented the effects of nutritional supplementation in patients with moderate to severe chronic airways disease (Schols *et al.* 1995). From 233 consecutive patients with stable, moderate to severe and bronchodilator-unresponsive pulmonary disease admitted to an intensive pulmonary rehabilitation program, 217 were randomised into three groups. These were to receive eight weeks of treatment with (a) placebo injections, (b) a nutritional supplement (one high fat, high calorie drink daily) plus placebo injections or (c) a nutritional supplement plus androgen injections (nandrolone decanoate [50 mg men, 25 mg women]) with intramuscular injections given fortnightly. Participants were also stratified according to the degree of baseline muscle depletion (body weight <90% and/or lean mass <67% ideal or not) at entry. During the study all patients underwent a standardised exercise program. Both nutrition and androgen therapy increased body weight over placebo, with androgen therapy having more prominent effects on lean body mass and respiratory muscle strength although there was no measurable improvement in submaximal exercise tolerance nor any major adverse effects. The lack of an androgen-alone arm and blinding with respect to nutritional supplementation made it difficult to evaluate the impact of androgen therapy relative to improved nutrition. After 4 years, follow-up of 203 of these men revealed no treatment effect on survival (Schols *et al.* 1998); however in a post hoc analysis, those with larger increases in weight (including 24% of the initial placebo group) had a significantly decreased mortality risk. More recently, a longer term study examining the effect of androgen supplementation without nutritional supplements has been reported (Ferreira *et al.* 1998). In this study, 23 undernourished men with COPD undergoing progressive pulmonary rehabilitation were randomised to receive additionally androgen (testosterone esters 250 mg intramuscularly for one injection followed by stanozolol 12 mg/day) or placebo for 27 weeks. No effect on respiratory muscle strength or endurance exercise capability was detected despite significant increases in lean body mass.

It is important to recognise that improvement in underlying pulmonary disease itself may ameliorate the gonadal dysfunction of systemic disease. In one study of men with chronic obstructive pulmonary disease with severe hypoxia and impotence, long-term oxygen therapy improved total and free testosterone and lowered SHBG (without changes in LH or FSH) in five men who had improved sexual function. The remaining seven who had unimproved sexual function had no changes in circulating hormone concentrations (Aasebo *et al.* 1993).

15.11.2 Obstructive sleep apnea

Sleep apnea has adverse effects on reproductive function (Grunstein *et al.* 1989) which may be precipitated by androgen therapy (Sandblom *et al.* 1983). A

subsequent observational study suggested a general effect of androgen therapy on sleep breathing (Matsumoto *et al.* 1985). Whether this involves central chemoreceptor-mediated regulation (White *et al.* 1985) or increased obstruction of the upper airways (Cistulli *et al.* 1994) remains undecided.

Androgen therapy has been reported to increase sleep arousals from disordered breathing in a randomised crossover study of 11 hypogonadal men receiving testosterone enanthate (200–400 mg per fortnight) or no therapy. This study compared somnography during androgen therapy (3–7 days after a testosterone injection) with a no-treatment group consisting of patients after withdrawal (mean 53 days post-injection) of androgen therapy (Schneider *et al.* 1986). Anatomical and functional evaluation of the upper airway patency in four patients showed no treatment-related difference but this finding is inconclusive due to the small sample size. Another observational study examined the prevalence of obstructive sleep apnea in hemodialysed men and the potential role of testosterone ester injections in its causation (Millman *et al.* 1985). Obstructive sleep apnea symptoms were common (12/29, 41%), particularly in those receiving regular testosterone enanthate injections (250 mg weekly) to stimulate erythropoiesis (9/12, 75%) compared with those not receiving testosterone (6/17, 35%). Withdrawal of testosterone, however, did not alter the signs or symptoms of sleep apnea in the five men studied both during and two months after cessation of testosterone treatment. This suggests that testosterone ester injections may not be a regular precipitant of obstructive sleep apnea. This is corroborated by further surveillance showing that sleep apnoea is common among patients with chronic renal failure even before commencement of dialysis or testosterone treatment (Kimmel *et al.* 1989).

The effect of androgen therapy on sleep and breathing has been examined in only three randomised placebo-controlled studies. In the largest study, among 108 older men (Snyder *et al.* 1999) randomised to receive a dose-titrated testosterone patch (approximately 6 mg/day) or matching placebo for three years, sleep breathing did not deteriorate although the tracking device may lack sensitivity (Portier *et al.* 2000) and sleep architecture was not examined. In a small randomised placebo-controlled study, ten men rendered acutely hypogonadal with leuprolide (Leibenluft *et al.* 1997) were randomised to receive testosterone enanthate 200 mg every fortnight or oil placebo for four weeks. Testosterone did not alter overnight plethysmography-determined sleep parameters (except time slept in stage 4 sleep was lengthened) but the effects on breathing were not reported. Whether the frequent overnight blood sampling may have influenced sleep is not clear. In the only randomised placebo-controlled study to examine both sleep and breathing, 17 community-dwelling healthy men over the age of 60 were randomised to receive three injections of intramuscular testosterone esters at weekly intervals (500 mg, 250 mg and 250 mg) or matching oil-based placebo, and then crossed-over to the other treatment after

eight weeks washout (Liu *et al.* 2003b). Testosterone treatment shortened sleep (~1 hour), worsened sleep apnea (by ~7 events/hour) and increased the duration of hypoxemia (~5 mins/night), but did not worsen function (driving ability and psychomotor performance). These studies together suggest that high-dose administration of testosterone esters may have adverse effects on sleep and breathing in the short-term, however, the effects of longer term use of lower, more physiological testosterone doses remains unknown.

Androgen withdrawal in eugonadal men with OSA had minimal effects on sleep and breathing. A study of eight men with OSA treated initially with the androgen receptor blocker flutamide 750 mg each day for one week and then with placebo for one week after a washout period of two weeks (Stewart *et al.* 1992) found no effect of flutamide on breathing or sleep architecture.

A low frequency of obstructive sleep apnea complicating androgen therapy as an idiosyncratic effect cannot be excluded. Whether this idiosyncratic reaction is related to the pharmacokinetics of the testosterone formulation used, such as the extreme peak serum testosterone following intramuscular injections, has yet to be determined. Whether similar effects would occur with more physiological testosterone formulations remains to be established in properly controlled clinical trials, although the low frequency of such reactions would require very large studies.

15.11.3 Asthma

One small double-blind study of 15 steroid-dependent asthmatic boys randomised to ethylestrenol (0.1 mg/kg/d) or placebo for 12 months reported a significant improvement in peak expiratory flow rate in the androgen group compared with the placebo group (Kerrebijn and Delver 1969). Despite the claim of no acceleration of bone maturation (according to the ratio of bone-age/height velocity) in this older study, the safety of such androgen therapy in boys prior to completion of puberty is very doubtful and androgen therapy has no place in the modern treatment of adolescent asthma.

15.12 Cerebral disease

15.12.1 Headache

The role of androgen withdrawal and therapy in men with cluster headache, an almost exclusively male disorder, has been examined in two controlled studies. In one 60 men with chronic cluster headache were randomised single-blind to treatment with a single dose of a GnRH analog (3.75 mg leuprolide depot) or vehicle injection (Nicolodi *et al.* 1993a). Self-reported frequency, intensity and duration of headache as well as sexual activity declined progressively during three successive ten-day periods after injection compared with pre-injection baseline in those

treated with leuprolide, whereas there was no change in placebo-treated men. The therapeutic response was delayed in onset corresponding temporally to the onset of castrate testosterone concentrations and the benefit persisted in most men for the one month post-treatment follow-up period, while no changes were noted at any stage in the placebo group. As headache is a remitting illness with subjective study end-points, the unmasking of active drug by the regular occurrence of sexual dysfunction in the treated group undermines the validity of the placebo-control group. The surprising absence of a placebo effect in the intended control group reinforces the possibility of an observer bias. Subsequently, another study of 12 men with chronic cluster headache and 12 non-headache controls who underwent treatment with very high dose androgen therapy (testosterone propionate 100 mg daily) for 14 days (Nicolodi *et al.* 1993b). Remarkably, this produced a dramatic increase in self-reported sexual activity in the cluster headache, but not the control, group. These curious findings warrant more rigorous study with a double-blind study design utilising more objective end-points.

15.12.2 Depression

Testosterone has long been considered effective for treatment of depression (Altschule and Tillotson 1948). Recent studies have shown that blood testosterone concentrations are lower in older men with dysthymia or major depression compared with non-depressed controls (Seidman *et al.* 2002). This effect may also be modulated by androgen receptor polymorphisms, since blood testosterone concentrations predict depression, but only in the subgroup of men with shorter *CAG repeats* (Seidman *et al.* 2001a), a putative genetic marker of higher tissue androgen sensitivity (Zitzmann and Nieschlag 2003). So far, however, androgen therapy has not been convincingly shown to improve mood in depressed men. Whether this is due to inappropriate targeting of subjects (blood testosterone concentrations) or dose (Perry *et al.* 2002) is not clear.

Oral mesterolone was the first androgen studied for anti-depressive effects. Laboratory evidence that a single dose of mesterolone (1–25 mg) mimics the effects of tricyclic antidepressants on the electroencephalogram led to a patent predicting that androgens might have beneficial effects on clinical depression (Itil *et al.* 1974). This was, however, refuted in a double-blind clinical trial which randomised 52 depressed men to treatment with mesterolone (150–450 mg daily) or placebo for six weeks (Itil *et al.* 1984). Both groups improved equally in scores for global clinical impression, physician's checklist for depression, self-rating and Hamilton depression rating. There were no differences in electroencephalogram measures or plasma monoamine oxidase levels. Another study of 34 depressed men randomly assigned to receive either mesterolone (150–550 mg/day) or amitriptyline (75–300 mg/day), the two treatments were equally effective in 26 subjects who continued treatment

(Vogel *et al.* 1985). However, the small sample size and lack of a placebo (rather than an active treatment) control limit the interpretation of this study. More recently, intramuscular testosterone has also been examined. In a well controlled study of 30 older depressed men selected on the basis of low-normal blood testosterone concentration, weekly intramuscular injections of 200 mg testosterone enanthate for 6 weeks did not improve mood significantly compared with placebo (Seidman *et al.* 2001b). Observational studies of older men with low-normal blood testosterone concentrations (but not clinically depressed) reported that weekly injections of 100 mg testosterone cypionate had no effect on depression scores in 32 men treated for 12 months (Sih *et al.* 1997) or 14 men treated for three months (Morley *et al.* 1993). However, the lack of proper masking limits the interpretation of these studies.

A potential role for androgen therapy as an adjunct to antidepressant therapy was suggested following a report evaluating potential benefit as salvage therapy when conventional antidepressant therapy is failing (Seidman and Rabkin 1998). After a 1 week run-in period, 22 men with major treatment-resistant depression and low serum testosterone were randomised to receive transdermal testosterone gel (1%, initial dose 10 g/d, downwardly dose-titrated) or placebo gel for eight weeks in addition to their current antidepressant therapy (Pope *et al.* 2003). Testosterone treatment significant improved the Hamilton depression rating and clinical global impression severity score, but not the Beck depression inventory score. Further studies would clearly be of great interest.

15.13 Vascular disease

15.13.1 Arterial disease

The effect of androgen therapy in coronary artery (Alexandersen *et al.* 1996; Barrett-Connor 1996; Liu *et al.* 2003a; Wu and von Eckardstein 2003) and cardiovascular (Liu *et al.* 2003a) disease are reviewed in detail elsewhere. This section will review studies of androgen therapy for peripheral vascular disease.

15.13.2 Venous disease

The use of androgen therapy in acute or chronic venous disease arises from their fibrinolytic effect, which may reduce venous fibrin plugging. One study of chronic venous insufficiency aiming to test whether androgen therapy would reduce the rate of venous ulceration involved 60 patients with venous skin changes but no ulceration being treated with below-knee compression stockings as standard therapy (McMullin *et al.* 1991). They were randomised to receive either stanozolol (10 mg daily) or placebo tablets for six months and androgen therapy produced a significant but modest reduction in the area of venous skin changes but no change

in prospective rate of new ulcers or skin oxygenation. The side-effects comprised mostly virilisation presumably due to stanozolol treatment of women.

Another prospective two-centre study examined the role of androgen therapy in prevention of post-operative *deep venous thrombosis* (DVT). In this study 200 patients scheduled for elective major abdominal surgery were randomised into three groups (Zawilska *et al.* 1990). The first received inhaled heparin (800 units/kg) one day prior to surgery alone, a second group received the same dose of inhaled heparin plus a single injection of nandrolone phenylpropionate (50 mg) and the third group received standard heparin prophylaxis (5000 units twice daily sc). Treatments were from the day before surgery until the fifth post-operative day. Using daily ^{125}I-fibrinogen scanning to detect DVT in 183 evaluable patients, there was no significant difference in post-operative DVT or clinically significant bleeding episodes among the three groups. Unfortunately the study had major between-centre differences and used a suboptimal detection method. It was also underpowered to reliably evaluate the claim that addition of nandrolone to nebulised heparin was as effective as standard heparin but with much lower bleeding risk. Larger and better designed studies of the effects of androgen therapy on venous disease in men seem warranted.

15.14 Body weight

15.14.1 Wasting

Many older studies examined the role of androgen therapy to augment body weight in patients with wasting or cachexia from a variety of underlying medical diseases as well as for cosmetic reasons in otherwise healthy people. For example, one double-blind study treated 28 healthy men and women and 26 male patients with wasting associated with chronic diseases (e.g. tuberculosis, chronic degenerative disorders) with placebo or one of two doses (25 mg or 50 mg daily) of norethandrolone for 12 weeks (Watson *et al.* 1959). The placebo group subsequently also crossed over to active treatment for another 12 weeks. Compared with placebo, both androgen groups had significantly improved body weight gain and reported improved appetite and well being but there was no dose-response relationship. Most patients had abnormal BSP retention and nearly all women experienced some virilisation. Very few other studies, however, were well controlled and the end-point of weight gain has little validity in isolation outside the context of the overall objectives of medical management for specific illnesses (see HIV/AIDS).

15.14.2 Obesity

Few controlled clinical trials of androgen therapy in obesity have been reported. Although massive obesity is associated with lowering of total testosterone, there

have been no controlled studies aiming to rectify any consequent androgen deficiency. A series of studies by Marin has raised interesting questions about the role of pharmacological androgen therapy in obesity. A pilot study reported reduced waist/hip circumference and improved insulin sensitivity following three months' transdermal treatment with *testosterone* (250 mg in 10 g gel daily) in eight men but not with *dihydrotestosterone* (250 mg in 10 g gel daily) in nine men (Marin *et al.* 1992). The study design, lacking placebo controls or any dose finding, did not allow any conclusion as to whether this difference arose from differences in skin bioavailability or androgen type (aromatisable or not) or potency. The same investigators then reported a double-blind study in which 27 middle-aged men with abdominal obesity were randomised to placebo, testosterone or dihydrotestosterone treatment by daily topical application of a transdermal gel (125 mg in 5 g gel daily) for nine months (Marin *et al.* 1995). Testosterone treatment inhibited lipid uptake into adipose tissue triglycerides, decreased lipoprotein lipase activity, reduced visceral fat stores (CT scan) and increased euglycemic clamp insulin sensitivity compared with dihydrotestosterone and placebo groups (Marin 1995). The results of Marin *et al.* were not confirmed by another study which randomised 30 obese middle-aged men into three groups to receive oral oxandrolone (10 mg/day), testosterone enanthate (150 mg) injections fortnightly or placebo treatments for nine months using a double-dummy, double-blinded design (Lovejoy *et al.* 1995). Due to lowering of HDL cholesterol by oral oxandrolone, a monitoring committee required the oxandrolone arm be switched to injections of nandrolone decanoate (30 mg) fortnightly. None of the androgens (oxandrolone, nandrolone, testosterone) had any consistent overall effect on muscle or fat mass but the interim change in study design reduced its power. The discrepancies between these studies require clarification with large sample size, longer duration and more clinically meaningful end-points.

*h*CG has been widely used since the 1960s in ad hoc and unproven low-dose regimens in combination with a low calorie diet to reduce obesity in middle-aged men (Lijesen *et al.* 1995; Young *et al.* 1976). A meta-analysis of controlled studies (Lijesen *et al.* 1995) concurs with the largest available single study (Young *et al.* 1976) that such low-dose hCG therapy is ineffective and has no valid role in the treatment of obesity.

15.15 Dermatological disease

The frequency and cosmetic impact of male pattern balding has, over millennia, led to innumerable attempted "cures". Prompted by a paradoxical claim that topical testosterone could cause hair regrowth, a double-blind, randomised study of 51 balding men showed that topical application of 1% *testosterone propionate* cream daily to one side of the scalp for a median of 4–5 months was no more effective than

placebo applied to the other half of the scalp (Savin 1968). Given the dependence of male pattern balding on masculine levels of androgen exposure after puberty, acceleration of hair loss might have been expected but the study endpoints (investigator and patient subjective global grading of regrowth) were not designed to detect this. More recently controlled studies of a topical 5α-reductase inhibitor have added a selective anti-androgen to the already vast list of baldness cures (Rittmaster 1994).

Vaginal skin atrophy (known by many synonyms including kraurosis vulvae, senile atrophy, vulvar lichen sclerosis, atrophic pruritus vulvae) causes erosions and fissuring resulting in sharp pain, soreness and dyspareunia. Androgen therapy as a topical cream containing testosterone or other androgens is a traditional therapy although no large placebo-controlled clinical studies have been reported. One small open study suggests that testosterone (1 g 2% testosterone propionate petrolatum ointment daily for 4 weeks) is effective at reducing visible skin lesions, vulval pain and itching (Joura *et al.* 1997). Another study reported that 2% DHT is as effective as 2% testosterone propionate in white petrolatum ointment (Paslin 1996). Unfortunately the effects of placebo petrolatum were not studied and virilisation due to systemic absorption was common (Joura *et al.* 1997) so the mechanism of topical androgen therapy remains unclear.

15.16 Key messages

- Androgen replacement therapy aims to replicate but not exceed tissue androgen exposure of eugonadal men and hence is limited to testosterone in physiological doses. Although androgen deficiency may accompany systemic disease, androgen replacement therapy may influence morbidity but is unlikely to improve mortality.
- Pharmacological androgen therapy utilised androgens to maximal efficacy within adequate safety limits without regard to androgen class or dose. Such treatment is judged by the efficacy, safety and cost-effectiveness standards of other drugs. Very few studies of pharmacological androgen therapy fulfil the requirements of adequate study design (randomisation, placebo control, objective end-points, adequate power and duration),
- Pharmacological androgen therapy has not reduced mortality or altered the natural history of any non-gonadal disease.
- Since 17α-alkylated androgens are hepatotoxic, other safer oral and parenteral androgens should be preferred where possible.
- Androgen therapy does not improve mortality or morbidity from acute or chronic alcoholic liver disease. The effects in non-alcoholic liver disease have not been studied.
- Androgen therapy does not improve survival in aplastic anemia but improves morbidity by maintaining hemoglobin and reducing transfusion dependence.
- In anemia of end-stage renal failure, androgen therapy is cheaper than, and augments the effects of, EPO but whether it is equally or less effective remains controversial. Restricting the use of androgen therapy to older men has the most favorable risk-benefit.

- Androgen therapy prevents acute episodes of hereditary angioedema and probably chronic urticaria.
- Many important questions and opportunities remain for androgen therapy in non-gonadal disease but careful clinical trials are essential for proper evaluation.
- Traditional indications for androgen therapy (eg osteoporosis, anemia, advanced breast cancer) persist until more specific and effective treatments become available. Nevertheless newer indications, lower cost and/or equivalent efficacy may still favour androgen therapy in some circumstances.
- The mood-elevating properties of androgen therapy may explain or augment adjuvant effects of androgen therapy on non-gonadal diseases.
- The best opportunities for future evaluation of adjuvant use of androgen therapy in men with non-gonadal disease include steroid-induced osteoporosis, wasting due to AIDS and cancer, chronic respiratory, rheumatological and some neuromuscular diseases. In addition, the role of androgen therapy in recovery and/or rehabilitation after severe catabolic illness such as burns, critical illness or major surgery is promising but requires more detailed evaluation.
- Future studies of adjuvant androgen therapy require high quality clinical data involving randomisation and placebo controls as well as optimal dose finding and real, rather than surrogate, end-points.

15.17 REFERENCES

Aasebo U, Gyltnes A, Bremnes RM, Aakvaag, A Slordal L (1993) Reversal of sexual impotence in male patients with chronic obstructive pulmonary disease and hypoxemia with long term oxygen therapy. J Steroid Biochem Mol Biol 46:799–803

Adams GE, Dische S, Fowler JF, Thomlinson RH (1976) Hypoxic cell sensitisers in radiotherapy. Lancet 1:186–188

Ahn YS, Harrington WJ, Simon SR, Mylvaganam R, Pall LM, So AG (1983) Danazol for the treatment of idiopathic thrombocytopenic purpura. N Engl J Med 308:1396–1399

Alexandersen P, Haarbo J, Christiansen C (1996) The relationship of natural androgens to coronary heart disease in male: a review. Atherosclerosis 125:1–13

Aloi JA, Bergendahl M, Iranmanesh A, Veldhuis JD (1997) Pulsatile intravenous gonadotropin-releasing hormone administration averts fasting-induced hypogonadotropism and hypoandrogenemia in healthy, normal weight men. J Clin Endocrinol Metab 82:1543–1548

Altschule MD, Tillotson KJ (1948) The use of testosterone in the treatment of depressions. N Engl J Med 239:1036–1038

Ambriz R, Pizzuto J, Morales M, Chavez G, Guillen C, Aviles A (1986) Therapeutic effect of danazol on metrorrhagia in patients with idiopathic thrombocyotpenic purpura (ITP). Nouv Rev Fr Hematol 28:275–279

Amory JK, Chansky HA, Chansky KL, Camuso MR, Hoey CT, Anawalt BD, Matsumoto AM, Bremner WJ (2002) Preoperative supraphysiological testosterone in older men undergoing knee replacement surgery. J Am Geriatr Soc 50:1698–1701

Anderson FH, Francis RM, Peaston RT, Wastell HJ (1997) Androgen supplementation in eugonadal men with osteoporosis: effects of six months treatment on bone formation and resorption. J Bone Miner Res 12:472–478

Antus B, Yao Y, Liu S, Song E, Lutz J, Heemann U (2001) Contribution of androgens to chronic allograft nephropathy is mediated by dihydrotestosterone. Kidney Int 60:1955–1963

Antus B, Yao Y, Song E, Liu S, Lutz J, Heemann U (2002) Opposite effects of testosterone and estrogens on chronic allograft nephropathy. Transpl Int 15:494–501

Bacigalupo A, Chaple M, Hows J, Lint MTV, McCann S, Milligan D, Chessells J, Goldstone AH, Ottolander J, Veer ETvt, Comotti B, Coser P, Broccia G, Bosi A, Locasciulli A, Catalano L, Battista R, Arcese W, Carotenuto M, Marmont AM, Gordon Smith EC (1993) Treatment of aplastic anemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. Br J Hematol 83:145–151

Bakhshi V, Elliott M, Gentili A, Godschalk M, Mulligan T (2000) Testosterone improves rehabilitation outcomes in ill older men. J Am Geriatr Soc 48:550–553

Ballal SH, Domoto DT, Polack DC, Marciulonis P, Martin KJ (1991) Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. Am J Kidney Dis 17:29–33

Barrett-Connor E. Testosterone, HDL-cholesterol and cardiovascular disease. In: Bhasin S, Gabelnick HL, Spieler JM, Swerdloff RS, Wang C, Kelly C (eds) Pharmacology, biology, and clinical applications of androgens: current status and future prospect. Wiley-Liss, New York, 1996, pp 215–223

Batterham MJ, Garsia R (2001) A comparison of megestrol acetate, nandrolone decanoate and dietary counselling for HIV associated weight loss. Int J Androl 24:232–240

Belch JJ, Madhok R, McArdle B, McLaughlin K, Kluft C, Forbes CD, Sturrock R (1986) The effect of increasing fibrinolysis in patients with rheumatoid arthritis: a double blind study of stanozolol. Q J Med 58:19–27

Berger JR, Pall L, Hall CD, Simpson DM (1996) Oxandrolone in AIDS-wasting myopathy. AIDS 10:1657–1662

Berns JS, Rudnick MR, Cohen RM (1992) A controlled trial of recombinant human erythropoietin and nandrolone decanoate in the treatment of anemia in patients on chronic hemodialysis. Clin Nephrol 37:264–267

Bex FJ, Corbin A, France E (1982) Resistance of the mouse to the antifertility effects of LHRH agonists. Life Sci 30:1263–1269

Bhasin S, Storer TW, Asbel-Sethi N, Kilbourne A, Hays R, Sinha-Hikim I, Shen R, Arver S, Beall G (1998) Effects of testosterone replacement with a nongenital, transdermal system, Androderm, in human immunodeficiency-virus-infected men with low testosterone levels. J Clin Endocrinol Metab 83:3155–3162

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281:E1172–1181

Blamey SL, Garden OJ, Shenkin A, Carter DC (1984) Modification of postoperative nitrogen balance with preoperative anabolic steroid. Clin Nutr 2:187–192

Bonkovsky HL, Fiellin DA, Smith GS, Slaker DP, Simon D, Galambos JT (1991) A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. I. Short-term effects on liver function. Am J Gastroenterol 86:1200–1208

Booij A, Biewenga-Booij CM, Huber-Bruning O, Cornelis C, Jacobs JW, Bijlsma JW (1996) Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis. Ann Rheum Dis 55:811–815

Branda RF, Amsden TW, Jacob HS (1977) Randomized study of nandrolone therapy for anemia due to bone marrow failure. Arch Intern Med 137:65–69

Brennemann W, Brensing KA, Leipner N, Boldt I, Klingmuller D (1994) Attempted protection of spermatogenesis from irradiation in patients with seminoma by D-Tryptophan-6 luteinizing hormone releasing hormone. Clin Invest Med 72:838–842

Brinster RL, Avarbock MR (1994) Germline transmission of donor haplotype following spermatogonial transplantation. Proc Nat Acad Sci USA 91:11303–11307

Brinster RL, Zimmermann JW (1994) Spermatogenesis following male germ-cell transplantation. Proc Nat Acad Sci USA 91:11298–11302

Brubaker LH, Briere J, Laszlo J, Kraut E, Landaw SA, Peterson P, Goldberg J, Donovan P (1982) Treatment of anemia in myeloproliferative disorders: a randomized study of fluoxymesterone v transfusions only. Arch Intern Med 142:1533–1537

Buchwald D, Argyres S, Easterling RE, Jr FJO, Brewer GJ, Schoomaker EB, Abbrecht PH, Williams GW, Weller JM (1977) Effect of nandrolone decanoate on the anemia of chronic hemodialysis patients. Nephron 18:232–238

Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, Santos G, Gordon-Smith EC, Storb R (1979) A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. Blood 53:504–514

Champlin RE, Ho WG, Feig SA, Winston DJ, Lenarsky C, Gale RP (1985) Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? A prospective randomized trial. Blood 66:184–188

Chan PCK, Wei DCC, Tam SCF, Chan FL, Yeung WC, Cheng IKP (1992) Post-transplant erythrocytosis: role of erythropoietin and male sex hormones. Nephrol Dial Transpl 7: 137–142

Chemes HE (2001) Infancy is not a quiescent period of testicular development. Int J Androl 24:2–7

Chlebowski RT, Herrold J, Ali I, Oktay E, Chlebowski JS, Ponce AT, Heber D, Block JB (1986) Influence of nandrolone decanoate on weight loss in advanced non-small cell lung cancer. Cancer 58:183–186

Cicardi M, Castelli R, Zingale LC, Agostoni A (1997) Side effects of long-term prophylaxis with attenuated androgens in hereditary angioedema: comparison of treated and untreated patients. J Allergy Clin Immunol 99:194–196

Cistulli PA, Grunstein RR, Sullivan CE (1994) Effect of testosterone administration on upper airway collapsibility during sleep. Am J Resp Crit Care Med 149:530–532

Conway AJ, Boylan LM, Howe C, Ross G, Handelsman DJ (1988) A randomised clinical trial of testosterone replacement therapy in hypogonadal men. Int J Androl 11:247–264

Coodley GO, Coodley MK (1997) A trial of testosterone therapy for HIV-associated weight loss. AIDS 11:1347–1352

Cooperative Group for the Study of Aplastic and Refractory Anaemias (1979) Androgen therapy of aplastic anemia: a prospective study of 352 cases. Scand J Haematol 22:343–356

Copenhagen Study Group for Liver Diseases (1986) Testosterone treatment of men with alcoholic cirrhosis: a double-blind study. Hepatology 6:807–813

Crawford BA, Liu PY, Kean M, Bleasel J, Handelsman DJ (2003) Randomised, placebo-controlled trial of androgen effects on bone and muscle in men requiring long-term systemic glucocorticoid therapy. J Clin Endocrinol Metab 88:3167–3176

Crawford BA, Spaliviero JA, Simpson JM, Handelsman DJ (1998) Testing the gonadal regression-cytoprotection hypothesis. Cancer Res 58:5105–5109

Cutolo M, Balleari E, Giusti M, Intra E, Accardo S (1991) Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum 34:1–5

da Cunha MF, Meistrich ML, Nader S (1987) Absence of protection by a GnRH analogue against cyclophosphamide induced testicular cytotoxicity in the mouse. Cancer Res 47: 1093–1097

Darnton SJ, Zgainski B, Grenier I, Allister K, Hiller L, McManus KG, Steyn RS (1999) The use of an anabolic steroid (nandrolone decanoate) to improve nutritional status after esophageal resection for carcinoma. Dis Esophagus 12:283–288

Demling RH (1999) Comparison of the anabolic effects and complications of human growth hormone and the testosterone analog, oxandrolone, after severe burn injury. Burns 25: 215–221

Demling RH, DeSanti L (1997) Oxandrolone, an anabolic steroid, significantly increases the rate of weight gain in the recovery phase after major burns. J Trauma 43:47–51

Demling RH, DeSanti L (2001) The rate of restoration of body weight after burn injury, using the anabolic agent oxandrolone, is not age dependent. Burns 27:46–51

Demling RH, Orgill DP (2000) The anticatabolic and wound healing effects of the testosterone analog oxandrolone after severe burn injury. J Crit Care 15:12–17

Dobs AS, Cofrancesco J, Nolten WE, Danoff A, Anderson R, Hamilton CD, Feinberg J, Seekins D, Yangco B, Rhame F (1999) The use of a transscrotal testosterone delivery system in the treatment of patients with weight loss related to human immunodeficiency virus infection. Am J Med 107:126–132

Dontas AS, Papanicolaou NT, Papanayiotou P, Malamos BK (1967) Long-term effects of anabolic steroids on renal functions in the aged subject. J Gerontol 22:268–273

Drinka PJ, Jochen AL, Cuisinier M, Bloom R, Rudman I, Rudman D (1995) Polycythemia as a complication of testosterone replacement therapy in nursing home men with low testosterone levels. J Am Geriatr Soc 43:899–901

Drosos AA, van Vliet-Dascalopoulos E, Andonopoulos AP, Galanopoulou V, Skopouli FN, Moutsopoulos HM (1988) Nandrolone decanoate (deca-durabolin) in primary Sjogren's syndrome: a double blind study. Clin Exp Rheumatol 6:53–57

El-Sadr A, Sabry AA, Abdel-Rahman M, El-Barnachawy R, Koraitim M (1990) Treatment of primary nocturnal enuresis by oral androgen mesterolone. A clinical and cystometric study. Urology 36:331–335

Evans JT, Elias EG (1972) The erythropoietic response to anabolic therapy in patients receiving radiotherapy. J Clin Pharmacol New Drugs 12:101–104

Fairfield WP, Treat M, Rosenthal DI, Frontera W, Stanley T, Corcoran C, Costello M, Parlman K, Schoenfeld D, Klibanski A, Grinspoon S (2001) Effects of testosterone and exercise on muscle leanness in eugonadal men with AIDS wasting. J Appl Physiol 90:2166–2171

Fenichel G, Pestronk A, Florence J, Robison V, Hemelt VM (1997) A beneficial effect of oxandrolone in the treatment of Duchenne muscular dystrophy: a pilot study. Neurology 48:1225–1226

Fenichel GM, Griggs RC, Kissel J, Kramer TI, Mendell JR, Moxley RT, Pestronk A, Sheng K, Florence J, King WM, Pandya S, Robison VD, Wang H (2001) A randomized efficacy and safety trial of oxandrolone in the treatment of Duchenne dystrophy. Neurology 56: 1075–1079

Fenster LF (1966) The nonefficacy of short-term anabolic steroid therapy in alcoholic liver disease. Ann Intern Med 65:738–744

Ferreira IM, Verreschi IT, Nery LE, Goldstein RS, Zamel N, Brooks D, Jardim JR (1998) The influence of 6 months of oral anabolic steroids on body mass and respiratory muscles in undernourished COPD patients. Chest 114:19–28

Foss GL (1939) Clinical administration of androgens. Lancet: 502–504

Franke WW, Berendonk B (1997) Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. Clin Chem 43:1262–1279

French Cooperative Group for the Study of Aplastic and Refractory Anaemias (1986) Androgen therapy in aplastic anemia: a comparative study of high and low-doses and of 4 different androgens. Scand J Haematol 36:346–352

Gascon A, Belvis JJ, Berisa F, Iglesias E, Estopinan V, Teruel JL (1999) Nandrolone decanoate is a good alternative for the treatment of anemia in elderly male patients on hemodialysis. Geriat Nephrol Urol 9:67–72

Gaughan WJ, Liss KA, Dunn SR, Mangold AM, Buhsmer JP, Michael B, Burke JF (1997) A 6-month study of low-dose recombinant human erythropoietin alone and in combination with androgens for the treatment of anemia in chronic hemodialysis patients. Am J Kidney Dis 30:495–500

Gelfand JA, Sherins RJ, Alling DW, Frank MM (1976) Treatment of hereditary angioedema with danazol: reversal of clinical and biochemical abnormalities. N Engl J Med 295:1444–1448

Gelfand MM, Wiita B (1997) Androgen and estrogen-androgen hormone replacement therapy: a review of the safety literature, 1941 to 1996. Clin Ther 19:383–404; discussion 367–368

Gervasio JM, Dickerson RN, Swearingen J, Yates ME, Yuen C, Fabian TC, Croce MA, Brown RO (2000) Oxandrolone in trauma patients. Pharmacotherapy 20:1328–1334

Gitlin N, Korner P, Yang HM (1999) Liver function in postmenopausal women on estrogen-androgen hormone replacement therapy: a meta-analysis of eight clinical trials. Menopause 6:216–224

Glode LM, Robinson J, Gould SF (1981) Protection from cyclophosphamide-induced testicular damage with an analogue of gonadotrophin-releasing hormone. Lancet 1:1132–1134

Gluud C, Bennett P, Dietrichson O, Johnsen SG, Ranek L, Svendsen LB, Juhl E (1981) Short-term parenteral and peroral testosterone administration in men with alcoholic cirrhosis. Scand J Gastroentero 16:749–755

Gluud C, Bennett P, Svenstrup B, Micic S, Copenhagen Study Group for Liver Diseases (1988a) Effect of oral testosterone treatment on serum concentrations of sex steroids, gonadotrophins and prolactin in alcoholic cirrhotic men. Aliment Pharmacol Ther 2:119–128

Gluud C, Christoffersen P, Eriksen J, Wantzin P, Knudsen BB, Copenhagen Study Group for Liver Diseases (1987a) No effect of long-term oral testosterone treatment on liver morphology in men with alcoholic cirrhosis. Am J Gastroenterol 82:660–664

Gluud C, Dejgard A, Bennett P, Svenstrup B (1987b) Androgens and oestrogens before and following oral testosterone administration in male patients with and without alcoholic cirrhosis. Acta Endocrinol 115:385–391

Gluud C, Henricksen JH, Copenhagen Study Group for Liver Diseases (1987c) Liver hemodynamics and function in alcoholic cirrhosis. J Hepatol 4:168–173

Gluud C, Wantzin P, Eriksen J, Copenhagen Study Group for Liver Diseases (1988b) No effect of oral testosterone treatment on sexual dysfunction in alcoholic cirrhotic men. Gastroenterology 95:1582–1587

Griggs RC, Kingston W, Herr BE, Forbes G, Moxley RT (1985) Lack of relationship of hypogonadism to muscle wasting in myotonic dystrophy. Arch Neurol 42:881–885

Griggs RC, Pandya S, Florence JM, Brooke MH, Kingston W, Miller JP, Chutkow J, Herr BE, Moxley RT (1989) Randomized controlled trial of testosterone in myotonic dystrophy. Neurology 39:219–222

Grimaldi C, Bleiberg H, Gay F, Messner M, Rougier P, Kok TC, Cirera L, Cervantes A, De Greve J, Paillot B, Buset M, Nitti D, Sahmoud T, Duez N, Wils J (1998) Evaluation of antiandrogen therapy in unresectable hepatocellular carcinoma: results of a European Organization for Research and Treatment of Cancer multicentric double-blind trial. J Clin Oncol 16:411–417

Grinspoon S, Corcoran C, Anderson E, Hubbard J, Stanley T, Basgoz N, Klibanski A (1999) Sustained anabolic effects of long-term androgen administration in men with AIDS wasting. Clin Infect Dis 28:634–636

Grinspoon S, Corcoran C, Askari H, Schoenfeld D, Wolf L, Burrows B, Walsh M, Hayden D, Parlman K, Anderson E, Basgoz N, Klibanski A (1998) Effects of androgen administration in men with the AIDS wasting syndrome. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 129:18–26

Grinspoon S, Corcoran C, Parlman K, Costello M, Rosenthal D, Anderson E, Stanley T, Schoenfeld D, Burrows B, Hayden D, Basgoz N, Klibanski A (2000) Effects of testosterone and progressive resistance training in eugonadal men with AIDS wasting. A randomized, controlled trial. Ann Intern Med 133:348–355

Grunfeld C, Feingold KR (1992) Metabolic disturbances and wasting in the Acquired Immunodeficiency Syndrome. N Engl J Med 327:329–337

Grunstein RR, Handelsman DJ, Lawrence SJ, Blackwell C, Caterson ID, Sullivan CE (1989) Hypothalamic dysfunction in sleep apnea: reversal by nasal continuous positive airways pressure. J Clin Endocrinol Metab 68:352–358

Hajjar RR, Kaiser FE, Morley JE (1997) Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. J Clin Endocrinol Metab 82:3793–3796

Hall GM, Larbre JP, Spector TD, Perry LA, Silva JAD (1996) A randomized trial of testosterone therapy in males with rheumatoid arthritis. Br J Rheumatol 35:568–573

Hamdy RC, Moore SW, Whalen KE, Landy C (1998) Nandrolone decanoate for men with osteoporosis. Am J Ther 5:89–95

Hamilton JB (1937) Treatment of sexual underdevelopment with synthetic male hormone substance. Endocrinology 21:649–654

Handelsman DJ (1985) Hypothalamic-pituitary gonadal dysfunction in chronic renal failure, dialysis, and renal transplantation. Endocr Rev 6:151–182

Handelsman DJ. Testicular dysfunction in systemic diseases. In: Nieschlag E, Behre HM (eds) Andrology: Male reproductive health and dysfunction, 2nd ed, Springer-Verlag, Berlin, 2001, pp 241–251

Handelsman DJ, Dong Q (1993) Hypothalamo-pituitary gonadal axis in chronic renal failure. Endocrinol Metab Clin North Am 22:145–161

Handelsman DJ, Liu PY (1998) Androgen therapy in chronic renal failure. Bailliere Clin Endoc 12:485–500

Handelsman DJ, Mackey MA, Howe C, Turner L, Conway AJ (1997) Analysis of testosterone implants for androgen replacement therapy. Clin Endocrinol (Oxf) 47:311–316

Hansell DT, Davies JW, Shenkin A, Garden OJ, Burns HJ, Carter DC (1989) The effects of an anabolic steroid and peripherally administered intravenous nutrition in the early postoperative period. J Pen. J Parenter Enteral Nutr 13:349–358

Hausmann DF, Nutz V, Rommelsheim K, Caspari R, Mosebach KO (1990) Anabolic steroids in polytrauma patients. Influence on renal nitrogen and amino acid losses: a double-blind study. J Pen. J Parenter Enteral Nutr 14:111–114

Hazelton RA, McCruden AB, Sturrock RD, Stimson WH (1983) Hormonal manipulation of the immune response in systemic lupus erythematosus: a drug trial of an anabolic steroid, 19-nortestosterone. Ann Rheum Dis 42:155–157

Hendler ED, Goffinet JA, Ross S, Longnecker RE, Bakovic V (1974) Controlled study of androgen therapy in anemia of patients on maintenance hemodialysis. N Engl J Med 291:1046–1051

Honkanen L, Lesser GT (2001) Testosterone use for rehabilitation of older men. J Am Geriatr Soc 49:339–340

Horl WH (1999) Is there a role for adjuvant therapy in patients being treated with epoetin? Nephrol Dial Transpl 14:50–60

Hosea SW, Santaella ML, Brown EJ, Berger M, Katusha K, Frank MM (1980) Long-term therapy of hereditary angioedema with danazol. Ann Intern Med 93:809–812

Howell SJ, Radford JA, Adams JE, Smets EM, Warburton R, Shalet SM (2001) Randomized placebo-controlled trial of testosterone replacement in men with mild Leydig cell insufficiency following cytotoxic chemotherapy. Clin Endocrinol (Oxf) 55:315–324

Howell SJ, Radford JA, Smets EM, Shalet SM (2000) Fatigue, sexual function and mood following treatment for hematological malignancy: the impact of mild Leydig cell dysfunction. Br J Cancer 82:789–793

Inkovaara J, Gothoni G, Halttula R, Heikinheimo R, Tokola O (1983) Calcium, vitamin D and anabolic steroid in treatment of aged bones: double-blind placebo-controlled long-term clinical trial. Age Ageing 12:124–130

Ishak KG, Zimmerman HJ (1987) Hepatotoxic effects of the anabolic-androgenic steroids. Semin Liver Dis 7:230–236

Itil TM, Cora R, Akpinar S, Herrmann WM, Patterson CJ (1974) "Psychotropic" action of sex hormones: computerized EEG in establishing the immediate CNS effects of steroid hormones. Current Ther Res 16:1147–1170

Itil TM, Michael ST, Shapiro DM, Itil KZ (1984) The effects of mesterolone, a male sex hormone in depressed patients: a double blind controlled study. Methods Find Exp Clin Pharmacol 6:331–337

Jayson MI, Holland CD, Keegan A, Illingworth K, Taylor L (1991) A controlled study of stanozolol in primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis 50:41–47

Jockenhövel F, Vogel E, Reinhardt W, Reinwein D (1997) Effects of various modes of androgen substitution therapy on erythropoiesis. Eur J Med Res 2:293–298

Johansen KL, Mulligan K, Schambelan M (1999) Anabolic effects of nandrolone decanoate in patients receiving dialysis: a randomized controlled trial. JAMA 281:1275–1281

Johnson DH, Linde R, Hainsworth JD, Vale W, Rivier J, Stein R, Flexner J, van Welch R, Greco FA (1985) Effect of a luteinizing hormone releasing hormone agonist given during combination chemotherapy on post-therapy fertility in male patients with lymphoma: preliminary observations. Blood 65:832–836

Joura EA, Zeisler H, Bancher-Todesca D, Sator MO, Schneider B, Gitsch G (1997) Short-term effects of topical testosterone in vulvar lichen sclerosis. Obstet Gynecol 89:297–299

Kaltwasser JP, Dix U, Schalk KP, Vogt H (1998) Effect of androgens on the response to antithymocyte globulin in patients with aplastic anemia. Eur J Hematol 40:111–118

Kamischke A, Kemper DE, Castel MA, Luthke M, Rolf C, Behre HM, Magnussen H, Nieschlag E (1998) Testosterone levels in men with chronic obstructive pulmonary disease with or without glucocorticoid therapy. Eur Respir J 11:41–45

Kassmann K, Rappaport R, Broyer M (1992) The short-term effect of testosterone on growth in boys on hemodialysis. Clin Nephrol 37:148–154

Kelleher S, Wishart SM, Liu PY, Turner L, Di Pierro I, Conway AJ, Handelsman DJ (2001) Long-term outcomes of elective human sperm cryostorage. Hum Reprod 16:2632–2639

Kennedy BJ, Gilbertsen AS (1957) Increased erythropoiesis induced by androgenic hormone therapy. N Engl J Med 256:719–726

Kerrebijn KF, Delver A (1969) Ethylestrenol (Orgabolin): effects on asthmatic children during corticosteroid treatment. Scand J Respir Dis 68:70–77

Kimmel PL, Miller G, Mendelson WB (1989) Sleep apnea syndrome in chronic renal disease. Am J Med 86:308–314

Kopera H. Miscellaneous uses of anabolic steroids. In: Kochakian CD (ed) Anabolic-androgenic steroids, Springer-Verlag, Berlin, 1976, pp 535–625

Kotler DP, Tierney AR, Wang J, Pierson RN (1989) Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. Am J Clin Nutr 50:444–447

Krauss DJ, Taub HA, Lantinga LJ, Dunsky MH, Kelly CM (1991) Risks of blood volume changes in hypogonadal men treated with testosterone enanthate for erectile impotence. J Urol 146: 1566–1570

Kreusser ED, Hetzel WD, Hautmann R, Pfeiffer EF (1990) Reproductive toxicity with and without LHRHa administration during adjuvant chemotherapy in patients with germ cell tumors. Horm Metab Res 22:494–498

Kruskemper HL. Anabolic Steroids. New York: Academic Press, 1968

Lahita RG, Cheng CY, Monder C, Bardin CW (1992) Experience with 19-nortestosterone in the therapy of systemic lupus erythematosus: worsened disease after treatment with 19-nortestosterone in men and lack of improvement in women. J Rheumatol 19:547–555

Leibenluft E, Schmidt PJ, Turner EH, Danaceau MA, Ashman SB, Wehr TA, Rubinow DR (1997) Effects of leuprolide-induced hypogonadism and testosterone replacement on sleep, melatonin, and prolactin secretion in men. J Clin Endocrinol Metab 82:3203–3207

Lewis L, Dahn M, Kirkpatrick JR (1981) Anabolic steroid administration during nutritional support: a therapeutic controvery. J Pen. J Parenter Enteral Nutr 5:64–66

Li Bock E, Fulle HH, Heimpel H, Pribilla W (1976) Die Wirkung von Mesterolon bei Panmyelopathien und renalen Anaemien. Med Klin 71:539–547

Lijesen GK, Theeuwen I, Assendelft WJ, Van Der Wal G (1995) The effect of human chorionic gonadotropin (HCG) in the treatment of obesity by means of the Simeons therapy: a criteria-based meta-analysis. Br J Clin Pharmacol 40:237–243

Liu PY, Death AK, Handelsman DJ (2003a) Androgens and cardiovascular disease. Endocr Rev 24:313–340

Liu PY, Yee BJ, Wishart SM, Jimenez M, Jung DG, Grunstein RR, Handelsman DJ (2003b) The short-term effects of high dose testosterone on sleep, breathing and function in older men. J Clin Endocrinol Metab 88:3605–3613

Loprinzi CL, Kugler JW, Sloan JA, Mailliard JA, Krook JE, Wilwerding MB, Rowland KM, Jr., Camoriano JK, Novotny PJ, Christensen BJ (1999) Randomized comparison of megestrol acetate versus dexamethasone versus fluoxymesterone for the treatment of cancer anorexia/cachexia. J Clin Oncol 17:3299–3306

Lovejoy JC, Bray GA, Greeson CS, Klemperer M, Morris J, Partington C, Tulley R (1995) Oral anabolic steroid treatment, but not parenteral androgen treatment, decreases abdominal fat in obese, older men. Int J Obes 19:614–624

Maddrey WC (1986) Is therapy with testosterone or anabolic-androgenic steroids useful in the treatment of alcoholic liver disease? Hepatology 6:1033–1035

Mandelli F, Amadori S, Dini E, Grignani F, Leoni P, Liso V, Martelli M, Neri A, Petti MC, Ferrini PR (1981) Randomized clinical trial of immunotherapy and androgenotherapy for remission maintenance in acute non-lymphocytic leukemia. Leuk Res 5:447–452

Marin P (1995) Testosterone and regional fat distribution. Obes Res 3 Suppl 4:609S–612S

Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, Lindstedt G, Bjorntorp P (1992) The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes 16:991–997

Marin P, Oden B, Bjorntorp P (1995) Assimilation and mobilization of triglycerides in subcutaneous abdominal and femoral adipose tissue in vivo in men: effects of androgens. J Clin Endocrinol Metab 80:239–243

Masala A, Faedda R, Alagna A, Satta A, Chiarelli G, Rovasio PP, Ivaldi R, Taras MS, Lai E, Bartoli E (1997) Use of testosterone to prevent cyclophosphamide-induced azoospermia. Ann Intern Med 126:292–295

Matsumoto A, Sandblom RE, Schoene RB, Lee KA, Giblin EC, Pierson DJ, Bremner WJ (1985) Testosterone replacement in hypogonadal men: effects on obstructive sleep apnea, respiratory drives and sleep. Clin Endocrinol (Oxf) 22:713–721

McMullin GM, Watkin GT, Coleridge Smith PD, Scurr JH (1991) Efficacy of fibrinolytic enhancement with stanozolol in the treatment of venous insufficiency. Aust NZ J Surg 61:306–309

Meikle AW, Arver S, Dobs AS, Sanders SW, Rajaram L, Mazer NA (1996) Pharmacokinetics and metabolism of a permeation-enhanced testosterone transdermal system in hypogonadal men: influence of application site- a clinical research center study. J Clin Endocrinol Metab 81:1832–1840

Meistrick ML (1993) Effects of chemotherapy and radiotherapy on spermatogenesis. Eur Urol 23:136–141

Mendenhall CL, Anderson S, Garcia-Pont P, Goldberg S, Kiernan T, Seeff LB, Sorrell M, Tamburro C, Weesner R, Zetterman R, Chedid A, Chen T, Rabin L, Veterans Administration Cooperative Study on Alcoholic Hepatitis (1984) Short-term and long-term survival in patients with alcoholic hepatitis treated with oxandrolone and prenisolone. N Engl J Med 311:1464–1470

Mendenhall CL, Moritz TE, Roselle GA, Morgan TR, Nemchausky BA, Tamburro CH, Schiff ER, McClain CJ, Marsano LS, Allen JI (1993) A study of oral nutritional support with oxandrolone in malnourished patients with alcoholic hepatitis. Hepatology 17:564–576

Millman RP, Kimmel PL, Shore ET, Wasserstein AG (1985) Sleep apnea in hemodialysis patients: the lack of testosterone effect on its pathogenesis. Nephron 40:407–410

Mooradian AD, Morley JE, Korenman SG (1987) Biological actions of androgens. Endocr Rev 8:1–28

Morley JE, Perry HM, Kaiser FE, Kraengle D, Jensen J, Houston K, Mattammal M, Perry HM (1993) Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. J Am Geriatr Soc 41:149–152

Morris ID (1993) Protection against cytotoxic-induced testis damage – experimental approaches. Eur Urol 23:143–147

Muller V, Szabo A, Viklicky O, Gaul I, Portl S, Philipp T, Heemann UW (1999) Sex hormones and gender-related differences: their influence on chronic renal allograft rejection. Kidney Int 55:2011–2020

Nagasue N, Yu L, Yukaya H, Kohno H, Nakamura T (1995) Androgen and oestrogen receptors in hepatocellular carcinoma and surrounding liver parenchyma: impact on intrahepatic recurrence after hepatic resection. Br J Surg 82:542–547

Naik RB, Gibbons AR, Gyde OH, Harris BR, Robinson BH (1978) Androgen trial in renal anemia. Proc Eur Dial Transplant Assoc 15:136–143

Nejean Y, Joint Group for the Study of Aplastic and Refractory Anaemias (1981) Long-term follow-up in patients with aplastic anemia. A study of 137 androgen-treated patients surviving more than two years. Am J Med 71:543–551

Najean Y, Pecking A (1979) Refractory anemia with excess of blast cells: prognostic factors and effect of treatment with androgens or cytosine arabinoside. Results of a prospective trial in 58 patients. Cooperative Group for the Study of Aplastic and Refractory Anaemias. Cancer 44:1976–1982

Navarro JF, Mora C (2001) In-depth review effect of androgens on anemia and malnutrition in renal failure: implications for patients on peritoneal dialysis. Perit Dial Int 21:14–24

Navarro JF, Mora C, Macia M, Garcia J (2002) Randomized prospective comparison between erythropoietin and androgens in CAPD patients. Kidney Int 61:1537–1544

Neff MS, Goldberg J, Slifkin RF, Eiser AR, Calamia V, Kaplan M, Baez A, Gupta S, Mattoo N (1981) A comparison of androgens for anemia in patients on hemodialysis. N Engl J Med 304:871–875

Neff MS, Goldberg J, Slifkin RF, Eiser AR, Calamia V, Kaplan M, Baez A, Gupta S, Mattoo N (1985) Anemia in chronic renal failure. Acta Endocrinol suppl 271:80–86

Nicolodi M, Sicuteri F, Poggioni M (1993a) Hypothalamic modulation of nociception and reproduction in cluster headache. I. Therapeutic trials of leuprolide. Cephalgia 13:253–257

Nicolodi M, Sicuteri F, Poggioni M (1993b) Hypothalamic modulation of nociception and reproduction in cluster headache II. Testosterone-induced increase of sexual activity in males with cluster headache. Cephalgia 13:258–260

Nieschlag E, Cuppers HJ, Wickings EJ (1977) Influence of sex, testicular development and liver function on the bioavailability of oral testosterone. Eur J Clin Invest 7:145–147

Nieschlag E, Nieschlag S, Behre HM (1993) Lifespan and testosterone. Nature 366:215

Palacios A, Campfield LA, McClure RD, Steiner B, Swerdloff RS (1983) Effect of testosterone enanthate on hematopoesis in normal men. Fertil Steril 40:100–104

Parsad D, Pandhi R, Juneja A (2001) Stanozolol in chronic urticaria: a double blind, placebo controlled trial. J Dermatol 28:299–302

Paslin D (1996) Androgens in the topical treatment of lichen sclerosis. Int J Dermatol 35:298–301

Perry PJ, Yates WR, Williams RD, Andersen AE, MacIndoe JH, Lund BC, Holman TL (2002) Testosterone therapy in late-life major depression in males. J Clin Psychiatry 63:1096–101

Pope HG, Jr., Cohane GH, Kanayama G, Siegel AJ, Hudson JI (2003) Testosterone gel supplementation for men with refractory depression: a randomized, placebo-controlled trial. Am J Psychiatry 160:105–111

Portier F, Portmann A, Czernichow P, Vascaut L, Devin E, Benhamou D, Cuvelier A, Muir JF (2000) Evaluation of home versus laboratory polysomnography in the diagnosis of sleep apnea syndrome. Am J Respir Crit Care Med 162:814–818

Rabkin JG, Wagner GJ, Rabkin R (1999) Testosterone therapy for human immunodeficiency virus-positive men with and without hypogonadism. J Clin Psychopharmacol 19:19–27

Reid IR, Wattie DJ, Evans MC, Stapleton JP (1996) Testosterone therapy in glucocorticoid-treated men. Arch Intern Med 156:1173–1177

Rittmaster RS (1994) Finasteride. N Engl J Med 330:120–125

Sandblom RE, Matsumoto AM, Scoene RB, Lee KA, Giblin EC, Bremner WJ, Pierson DJ (1983) Obstructive sleep apnea induced by testosterone administration. N Engl J Med 308:508–510

Sattler FR, Jaque SV, Schroeder ET, Olson C, Dube MP, Martinez C, Briggs W, Horton R, Azen S (1999) Effects of pharmacological doses of nandrolone decanoate and progressive resistance training in immunodeficient patients infected with human immunodeficiency virus. J Clin Endocrinol Metab 84:1268–1276

Savin RC (1968) The ineffectiveness of testosterone in male pattern baldness. Arch Dermatol 98:512–514

Schlatt S (2002) Germ cell transplantation. Mol Cell Endocrinol 186:163–167

Schneider BK, Pickett CK, Zwillich CW, Weil JV, McDermott MT, Santen RJ, Varano LA, White DP (1986) Influence of testosterone on breathing during sleep. J Appl Physiol 61:618–623

Schofield RS, Hill JA, McGinn CJ, Aranda JM (2002) Hormone therapy in men and risk of cardiac allograft rejection. J Heart Lung Transplant 21:493–495

Schols AM, Slangen J, Volovics L, Wouters EF (1998) Weight loss is a reversible factor in the prognosis of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 157: 1791–1797

Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF (1995) Physiologic effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo-controlled randomized trial. Am J Respir Crit Care Med 152:1268–1274

Seidman SN, Araujo AB, Roose SP, Davanand DP, Xie S, Cooper TB, McKinlay JB (2002) Low testosterone levels in elderly men with dysthymic disorder. Am J Psychiatry 159:456–459

Seidman SN, Araujo AB, Roose SP, McKinlay JB (2001a) Testosterone level, androgen receptor polymorphism, and depressive symptoms in middle-aged men. Biol Psychiatry 50:371–376

Seidman SN, Rabkin JG (1998) Testosterone replacement therapy for hypogonadal men with SSRI-refractory depression. J Affect Disord 48:157–161

Seidman SN, Spatz E, Rizzo C, Roose SP (2001b) Testosterone replacement therapy for hypogonadal men with major depressive disorder: a randomized, placebo-controlled clinical trial. J Clin Psychiatry 62:406–412

Shalet SM, Beardwell CG, Jacobs HS, Pearson D (1978) Testicular function following irradiation of the human prepubertal testis. Clin Endocrinol (Oxf) 9:483–490

Sheffer AL, Fearon DT, Austen KF (1977) Methyltestosterone therapy in hereditary angioedema. Ann Intern Med 86:306–308

Shetty G, Wilson G, Huhtaniemi I, Shuttlesworth GA, Reissmann T, Meistrich ML (2000) Gonadotropin-releasing hormone analogs stimulate and testosterone inhibits the recovery of spermatogenesis in irradiated rats. Endocrinology 141:1735–1745

Sih R, Morley JE, Kaiser FE, Perry HM, 3rd, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab 82:1661–1667

Simon JA (2001) Safety of estrogen/androgen regimens. J Reprod Med 46:281–290

Singh AB, Norris K, Modi N, Sinha-Hikim I, Shen R, Davidson T, Bhasin S (2001) Pharmacokinetics of a transdermal testosterone system in men with end stage renal disease receiving maintenance hemodialysis and healthy hypogonadal men. J Clin Endocrinol Metab 86:2437–2445

Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL (1999) Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Spaulding WB (1960) Methyltestosterone therapy for hereditary episodic edema (hereditary angioneurotic edema). Ann Intern Med 53:739–745

Spiers ASD, DeVita SF, Allar MJ, Richards S, Sedranak N (1981) Beneficial effects of an anabolic steroid during cytotoxic chemotherapy for metatstatic cancer. J Med 12:433–446

Stewart DA, Grunstein RR, Berthon-Jones M, Handelsman DJ, Sullivan CE (1992) Androgen blockade does not effect sleep disordered breathing or chemosensitivity in men with obstructive sleep apnea. Am Rev Respir Dis 146:1389–1393

Strawford A, Barbieri T, Van Loan M, Parks E, Catlin D, Barton N, Neese R, Christiansen M, King J, Hellerstein MK (1999) Resistance exercise and supraphysiologic androgen therapy in eugonadal men with HIV-related weight loss: a randomized controlled trial. JAMA 281: 1282–1290

Teruel JL, Aguilera A, Marcen R, Antolin JN, Otero GG, Ortuno J (1996a) Androgen therapy for anemia of chronic renal failure. Scand J Urol Nephrol 30:403–408

Teruel JL, Cano T, Marcen R, Villafruela JJ, Rivera M, Fernandez-Juarez G, Ortuno J (1997) Decrease in the hemoglobin level in hemodialysis patients undergoing antiandrogen therapy. Nephrol Dial Transpl 12:1262–1263

Teruel JL, Marcen R, Navarro JF, Villafruela JJ, Fernandez-Lucas M, Liano F, Ortuno J (1995) Evolution of serum erythropoietin after androgen administration to hemodialysis patients: a prospective study. Nephron 70:282–286

Teruel JL, Marcen R, Navarro-Antolin J, Aguilera A, Fernandez-Juarez G, Ortuno J (1996b) Androgen versus erythropoietin for the treatment of anemia in hemodialyzed patients: a prospective study. J Am Soc Nephrol 7:140–144

Thomson AB, Anderson RA, Irvine DS, Kelnar CJ, Sharpe RM, Wallace WH (2002) Investigation of suppression of the hypothalamic-pituitary-gonadal axis to restore spermatogenesis in azoospermic men treated for childhood cancer. Hum Reprod 17:1715–1723

Tweedle D, Walton C, Johnston IDA (1973) The effect of an anabolic steroid on postoperative nitrogen balance. Br J Clin Pract 27:130–132

van Coevorden A, Stolear JC, Dhaene M, Herweghem JLV, Mockel J (1986) Effect of chronic oral testosterone undecanoate administration on the pituitary-testicular axes of hemodialyzed male patients. Clin Nephrol 26:48–54

Van den Berghe G (2003) Endocrine evaluation of patients with critical illness. Endocrinol Metab Clin North Am 32:385–410

Van den Berghe G, Baxter RC, Weekers F, Wouters P, Bowers CY, Iranmanesh A, Veldhuis JD, Bouillon R (2002) The combined administration of GH-releasing peptide-2 (GHRP-2), TRH and GnRH to men with prolonged critical illness evokes superior endocrine and

metabolic effects compared to treatment with GHRP-2 alone. Clin Endocrinol (Oxf) 56:655–669

van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R (2001) Intensive insulin therapy in the surgical intensive care unit. N Engl J Med 345:1359–1367

Van Vollenhoven RF, Engleman EG, McGuire JL (1995) Dehydroepiandrosterone in systemic lupus erythematosus. Arthritis Rheum 38:1826–1831

Victor G, Shanmugasundaram K, Krishnamurthi CA, Rex PM, Nagarajan D (1967) Hemoglobin response to anabolic steroid in iron-deficiency anemia. J Assoc Physicians India 15:177–183

Vogel W, Klaiber EL, Broverman DM (1985) A comparison of the antidepressant effects of a synthetic androgen (mesterolone) and amitriptyline in depressed men. J Clin Psychiatry 46:6–8

von Hartitzsch B, Kerr DNS (1976) Response to parenteral iron with and without androgen therapy in patients undergoing regular hemodialysis. Nephron 17:430–438

Watson RN, Bradley MH, Callahan R, Peters BJ, Kory RC (1959) A six-month evaluation of an anabolic drug, norethandrolone, in underweight persons. Am J Med 26:238–248

Wattel E, Cambier N, Caulier MT, Sautiere D, Bauters F, Fenaux P (1994) Androgen therapy in myelodysplastic syndromes with thrombocytopenia: a report on 20 cases. Br J Hematol 87:205–208

Waxman JH, Ahmed R, Smith D, Wrigley PFM, Gregory W, Shalet S, Crowther D, Rees LH, Besser GM, Malpas JS, Lister TA (1987) Failure to preserve fertility in patients with Hodgkin's disease. Cancer Chemoth Pharm 19:159–162

Webb WR, Doyle RS, Howard HS (1960) Relative metabolic effects of calories, protein, and an anabolic steroid (19-nortestosterone) in early postoperative period. Metabolism 9:1047–1057

Weber JP, Walsh PC, Peters CA, Spivak JL (1991) Effect of reversible androgen deprivation on hemoglobin and serum erythropoietin in men. Am J Hematol 36:190–194

Wells R (1960) Prednisolone and testosterone propionate in cirrhosis of the liver: a controlled trial. Lancet:1416–1419

White DP, Schneider BK, Santen RJ, McDermott M, Pickett CK, Zwillich CW, Weil JV (1985) Influence of testosterone on ventilation and chemosensitivity in male subjects. J Appl Physiol 59:1452–1457

Wilkey JL, Barson LJ, Kest L, Bragagni A (1960) The effect of testosterone on the azotemic patient: an intermediary report. J Urol 83:25–29

Williams JS, Stein JH, Ferris TF (1974) Nandrolone decanoate therapy for patients receiving hemodialysis. A controlled study. Arch Intern Med 134:289–292

Winearls CG (1995) Historical review of the use of recombinant human erythropoietin in chronic renal failure. Nephrol Dial Transpl 10 (suppl 2):3–9

Wu FC, von Eckardstein A (2003) Androgens and coronary artery disease. Endocr Rev 24:183–217

Wu FCW, Farley TMM, Peregoudov A, Waites GMH, WHO Task Force on Methods for the Regulation of Male Fertility (1996) Effects of testosterone enanthate in normal men: experience from a multicenter contraceptive efficacy study. Fertil Steril 65:626–636

Young GA, Yule AG, Hill GL (1983) Effects of an anabolic steroid on plasma amino acids, proteins, and body composition in patients receiving intravenous hyperalimentation. J Pen. J Parenter Enteral Nutr 7:221–225

Young N, Griffith P, Brittain E, Elfenbein G, Gardner F, Huang A, Harmon D, Hewlett J, Fay J, Mangan K (1988) A multicenter trial of antithymocyte globulin in aplastic anemia and related diseases. Blood 72:1861–1869

Young RL, Fuchs RJ, Woltjen MJ (1976) Chorionic gonadotropin in weight control. A double-blind crossover study. JAMA 236:2495–2497

Zawilska K, Tokarz A, Misiak A, Psuja P, Wislawski S, Szymczak P, Meissner J, Karon J, Lewandowski K, Lopaciuk S, Ziemski JM, Sowler J (1990) Nebulised heparin and anabolic steroid in the prevention of postoperative deep venous thrombosis following elective abdominal surgery. Folia Haematologica 117:699–707

Zitzmann M, Nieschlag E (2003) The CAG repeat polymorphism within the androgen receptor gene and maleness. Int J Androl 26:76–83

16

Androgens in male senescence

J.M. Kaufman, G. T'Sjoen and A. Vermeulen

Contents

16.1 Introduction

"Andropause", defined as the male equivalent of the menopause, which in women signals the end of reproductive life and a near total cessation of sex steroid

production by the gonads, does not exist. Indeed, aging in healthy men is normally not accompanied by abrupt or drastic alterations of gonadal function, and androgen production as well as fertility can be largely preserved until very old age.

The limited data available suggests that aging has no major influence on sperm quality and fertilizing capacity (Nieschlag *et al.* 1982; Rolf *et al.* 1996), changes in semen parameters being essentially limited to a decrease of ejaculate volume and sperm motility (Rolf *et al.* 1996). Moreover, a decreased ejaculatory frequency, as observed in elderly men (Rolf *et al.* 1996), might account for at least part of these age-related changes, but may also mask more subtle changes in spermatogenetic activity (Cooper *et al.* 1993). Serum inhibin B, a marker of Sertoli cell function and spermatogenesis, was shown to be relatively well maintained in healthy elderly men, albeit at the cost of clearly increased FSH stimulation that compensates for an age-related regression of Sertoli cell mass and function (Mahmoud *et al.* 2000).

As to hormonal testicular function, it is now well established that mean serum testosterone levels decrease progressively in healthy elderly men, notwithstanding considerable inter-individual variability in the extent of the changes (Vermeulen 1991). Well over 20% of otherwise healthy men over 60 years of age present with subnormal testosterone levels compared to serum levels in young adults. Moreover, this age-dependent decline in androgen production can be accentuated by co-morbidity with transient or more permanent adverse effects on Leydig cell function.

The extent to which a relative hypoandrogenism in the elderly contributes to clinical signs and symptoms of aging remains a largely underexplored issue that certainly deserves further attention as many clinical features of aging in men are reminiscent of those of hypogonadism in younger subjects and the indications for as well as the potential merits of androgen supplementation to aging men are a subject of debate.

16.2 Declining endocrine testicular function in senescence

16.2.1 Testosterone production and serum levels

Early reports of decreased spermatic vein testosterone blood concentrations (Hollander and Hollander 1958) and decreased testosterone blood production rates (Kent and Acone 1966) in elderly men have subsequently been confirmed by several studies performed in the seventies (Baker *et al.* 1977; Giusti *et al.* 1975; Vermeulen *et al.* 1972). However, reduced testosterone blood production rate does not necessarily imply lower testosterone plasma levels. Indeed, the blood production rate is the product of the mean plasma levels and the metabolic clearance rate, and the latter is also reduced in elderly men (Kent and Acone 1966; Vermeulen *et al.* 1972).

Whether aging in healthy men is also associated with decreased serum testosterone concentrations has long been highly controversial. Early reports of decreased

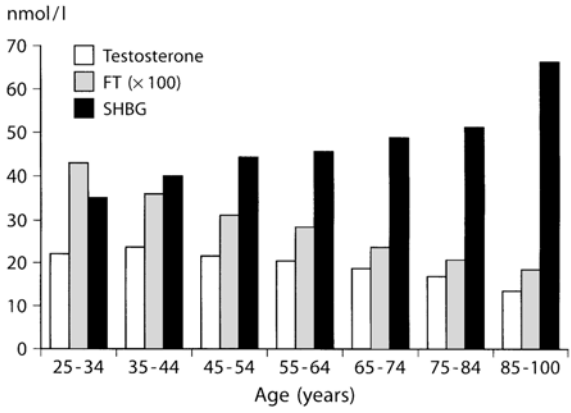


Fig. 16.1 Mean serum levels of testosterone, free testosterone (FT) and sex hormone binding globulin (SHBG) according to age in a cross-sectional study of 300 healthy men (from Vermeulen *et al.* 1996).

mean serum testosterone levels in elderly men, dating from the late sixties and early seventies, were followed by several studies that failed to confirm an age-related decline of testosterone levels. These discrepancies may be explained at least in part by biased selection of the study populations (Gray *et al.* 1991). In some of these studies, reported testosterone serum concentrations in young men were surprisingly low. Moreover, in some studies blood sampling was performed in the late afternoon when, due to the diurnal rhythm of testosterone secretion, serum levels are lower and the effect of age is minimal.

More recent studies including healthy ambulatory young and elderly men sampled in the morning, again confirmed an age-associated decline of serum testosterone levels, albeit of lesser magnitude than reported in the early studies (for review Vermeulen 1991), mean values at age 75 years being about two thirds of those at age 25 years (Fig. 16.1). An age-related decrease of serum testosterone levels has also been documented in longitudinal studies (Feldman *et al.* 2002; Harman *et al.* 2001; Morley *et al.* 1997; Zmuda *et al.* 1997). In fact, the longitudinally assessed decline of serum testosterone tends to be even larger than apparent from cross-sectional analysis (Feldman *et al.* 2002), which might be due to a bias towards healthier subjects in the latter while during longitudinal follow-up more elderly subjects are likely to show a deterioration than an improvement of their general health status.

Whereas mean serum testosterone levels in the adult male population decrease with age, a large inter-individual variability of serum testosterone levels is observed at all ages, with some elderly men having frankly low serum testosterone levels while many others have perfectly preserved testosterone secretion with serum levels well within the normal range for young adults. With advancing age, a progressively

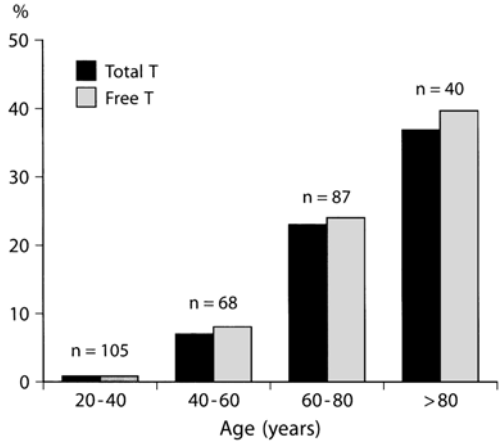


Fig. 16.2 Proportion of healthy men presenting with subnormal serum levels for total testosterone (<11 nmol/l) or free testosterone (<0.18 nmol/l) (from Kaufman and Vermeulen 1997).

larger proportion of men present with subnormal values relative to those in young adults; in a group of 300 healthy men aged 20 to 100 years (Vermeulen *et al.* 1996), we observed a subnormal testosterone level in less than 1% of men below age 40 years but in more than 20% of men older than 60 years (Fig. 16.2).

16.2.2 Sex hormone binding globulin and free testosterone serum levels

Whereas some authors may still argue that total testosterone concentrations are not reduced in perfectly healthy elderly men, all authors agree that the free and non-specifically bound serum testosterone, generally considered to represent the serum testosterone fractions readily available for biological activity, do indeed decrease with age (for review Vermeulen 1991). In healthy ambulatory men, mean serum levels of free testosterone (FT) and of non SHBG-bound or so called "bioavailable" testosterone (i.e. the sum of the free fraction and the fraction loosely bound to albumin) decrease by as much as 50% between age 25 and 75 years (Ferrini *et al.* 1998; Vermeulen *et al.* 1996). The sharper decline of these fractions in comparison with total testosterone is explained by an age-associated increase of sex hormone binding globulin (SHBG) concentrations and has been confirmed in longitudinal studies (Feldman *et al.* 2002; Harman *et al.* 2001; Morley *et al.* 1997). In 300 healthy men aged 25–100 years we observed an approximately log linear decrease of free testosterone levels at a rate of 1.2% per year (Fig. 16.1), while total serum testosterone remained relatively stable up to age 55 years and declined thereafter at a rate of 0.85% per year (Vermeulen *et al.* 1996).

As for total testosterone, there is great inter-individual variability in prevailing free (or bio-available) testosterone levels in elderly men, ranging from markedly

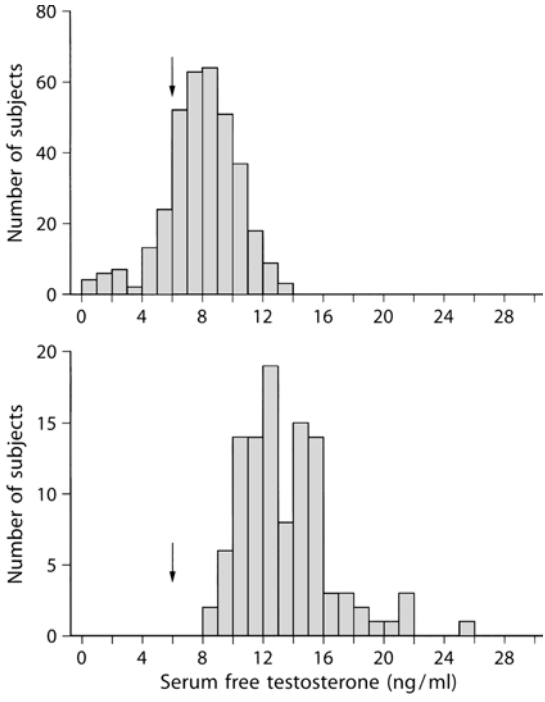


Fig. 16.3 Histogram for the distribution of serum free testosterone in 353 community-dwelling elderly men without major health problems, aged 70 to 85 years (upper panel) and in a younger control population of healthy men aged 23 to 58 years (lower panel); the lower limit for the laboratory normal range is indicated by the arrows.

low levels to levels in the upper normal range for young adults (Fig. 16.3), the proportion of men with subnormal free testosterone levels increasing with age (Fig. 16.2). However, limits of normality are somewhat arbitrary as the sensitivity threshold for androgen action may vary from tissue to tissue and according to age.

16.2.3 Tissue levels and metabolism of androgens

Highest concentrations of androgen receptors (AR) are present in the accessory sex organs, whereas the concentrations in other androgen-sensitive tissues such as the heart and bone are much lower. This concentration of receptors is affected by the androgen levels, and by age (Blondeau *et al.* 1982; Rajfer *et al.* 1989). As both circulating steroids and tissue receptor concentration decrease with age, it is not surprising that tissue androgen concentration also decreases with age (Deslypere and Vermeulen 1981; 1985).

Overall, the influence of the aging process on the metabolism of testosterone manifests itself essentially by a decrease of the metabolic clearance rate (Vermeulen *et al.* 1972), which results from an age-associated decrease of cardiac output and

of hepatic as well as tissue perfusion, and from increased binding of plasma testosterone to SHBG. No data are available concerning the possible influence of aging on blood conversion rates of testosterone to DHT. Cross-sectional studies show no apparent age-related decrease of DHT levels, whereas an increase of serum DHT was recently reported for a longitudinal follow-up study (Feldman *et al.* 2002). In any case, serum DHT is not considered a reliable parameter of tissue DHT formation (Labrie *et al.* 1995).

The conversion of testosterone to estradiol increases with age (Siiteri and MacDonald 1975), which appears to be the consequence of an increase of both aromatase tissue activity and of fat mass in the elderly (Vermeulen *et al.* 1996; 2002). This increasing global aromatase activity compensates for the decreased substrate availability, i.e. the declining testosterone and androstenedione plasma levels, so that serum estradiol levels do not show much change during aging in men (Ferrini *et al.* 1998; Vermeulen *et al.* 1996; 2002). However, there is a decrease with age of the serum testosterone over estradiol ratio and, as a consequence of the age-related increase in SHBG, also a moderate decrease of serum free and bio-available estradiol.

Serum levels of 5α-androstane-3α17β diol-glucuronide (ADG) decrease significantly with age (Deslypere *et al.* 1982), a consequence of the decreased serum concentrations of the precursors (70% testosterone and 30% dehydroepiandrosteronesulfate). In urine there is a decrease of the ratio of 5α to 5β metabolites (Vermeulen *et al.* 1972; Zumoff *et al.* 1976).

16.2.4 Factors affecting serum testosterone levels in elderly men

16.2.4.1 Influence of physiological factors and lifestyle

The physiological basis underlying the large inter-individual variation in serum testosterone levels seen at any age is not yet fully elucidated, but several physiological variables and factors related to lifestyle have been identified accounting for part of the wide range of normal values observed in healthy men (Kaufman and Vermeulen 1999). The apparent inter-individual variability of testosterone levels is not merely artefactual as a result of the cross-sectional design of the clinical studies, as single-point plasma testosterone estimates reflect longer-term androgen status in healthy men fairly well (Vermeulen and Verdonck 1992). The circadian variation of serum testosterone, with highest levels in the early morning and lowest levels in the late afternoon, should not play an important role in the wide range of normal testosterone levels if they are regularly evaluated in the morning (preferably before 10 a.m.). The ultradian pattern of episodic testosterone secretion undoubtedly contributes to the variability of testosterone levels (Naftolin *et al.* 1973; Spratt *et al.* 1988; Veldhuis *et al.* 1987). Therefore, although single time point estimates are a valid approach for clinical studies, the existence of fluctuations in serum

testosterone should be taken into account when assessing the androgen status of individual elderly subjects (Morley *et al.* 2002).

Heredity appears to play an important role, with Meikle *et al.* (1986; 1988) concluding from the study of monozygotic and dizygotic twins that up to 60% of the variability of serum testosterone and up to 30% of the variability of SHBG, after normalization for body surface area, may be attributable to genetic factors. Nevertheless, also according to the same authors, non-genetic factors still have a substantial impact on testosterone serum levels.

The genetic basis for the heredity of serum (free) testosterone and SHBG is presently unknown. Reports of ethnic differences in serum testosterone levels have been inconsistent with small differences tending to disappear if adjustments are made for differences in body composition, especially for abdominal adiposity (Gapstru *et al.* 2002; Heald *et al.* 2003; Winters *et al.* 2001).

Recently there has been considerable interest in a possible role of a polymorphic trinucleotide CAG-repeat contained in exon 1 of the AR (see Chapter 3). It has been suggested that a shorter AR CAG-repeat length may be associated with a more rapid decline of serum (bio-available) testosterone levels in middle-aged men (Krithivas *et al.* 1999), but the same study and other studies failed to establish a relationship between CAG-repeat length and prevalent serum androgen levels in middle-aged or elderly men (Harkonen *et al.* 2003; Van Pottelbergh *et al.* 2001; Zitzmann *et al.* 2001; 2003).

Several metabolic and hormonal factors influence SHBG serum levels. Insulin and insulin-like factor-I (IGF- I) inhibit SHBG production by hepatoma cells in vitro and in clinical studies insulin was found to be inversely correlated with serum SHBG and testosterone levels (Haffner *et al.* 1988; Heald *et al.* 2003; Simon *et al.* 1996; Vermeulen *et al.* 1996).

In clinical studies, body mass index (BMI) emerges as an important determinant of SHBG levels (Demoor and Goossens 1970). For the whole range of BMI values encountered clinically there is a highly significant negative correlation with SHBG and testosterone serum levels, explained at least in part by increased insulin levels (Giagulli *et al.* 1994; Khaw and Barrett-Connor 1992; Plymate *et al.* 1988). In elderly men this inverse relationship between BMI and SHBG levels can be clearly demonstrated notwithstanding the background of an age-related rise of SHBG levels (Vermeulen *et al.* 1996). Similarly, a negative association of serum SHBG and total testosterone with leptin levels has been observed in elderly men (Haffner *et al.* 1997; Van den Saffele *et al.* 1999). Negative associations with serum testosterone levels tend to be most pronounced for indices of abdominal fat (Couillard *et al.* 2000; Haffner *et al.* 1993; Khaw and Barrett-Connor 1992; Vermeulen *et al.* 1999a). Whereas moderate obesity affects mainly total serum testosterone by lowering SHBG binding capacity, in morbid obesity (BMI>35–40) free testosterone

levels are decreased as well as a result of neuroendocrine disturbances (Giagulli *et al.* 1994).

It has long been known that alterations in thyroid hormone levels can have a marked effect on SHBG levels, with thyreotoxicosis resulting in a several-fold increase of SHBG levels and marked increase of total serum testosterone (Vermeulen *et al.* 1971). In this regard, it is interesting to note that more subtle changes in thyroid hormone levels within the "normal range" can also affect SHBG and testosterone levels, with subclinical hyperthyroidism, characterized by suppressed serum levels of thyroid-stimulating hormone without clinical symptoms of hyperthyroidism or elevation of thyroid hormone levels above normal, resulting in a significant increase of SHBG and testosterone levels (Faber *et al.* 1990; Giagulli *et al.* 1992).

As to factors related to lifestyle, reports on the effects of diet on serum testosterone levels do not always agree, but available data suggest that diet influences testosterone levels mainly indirectly through changes in SHBG levels, fiber-rich, vegetarian diets being associated with higher SHBG and testosterone levels than western-type diets and more particularly those diets with high fat content (Adlercreutz 1990; Belanger *et al.* 1989; Key *et al.* 1990; Meikle *et al.* 1990; Reed *et al.* 1987). It is not clear to what extent changes in SHBG related to diet might be mediated through changes in insulin secretion, vegetarians having generally lower fasting insulin levels than omnivores. In men aged 40 to 70 years in the Massachusetts Male Aging Study fiber intake and protein intake but not carbohydrate, fat or total caloric intake were independent positive and negative determinants, respectively, of serum SHBG (Longcope *et al.* 2000).

Smokers tend to have higher testosterone levels than non-smokers (Barrett-Connor and Khaw 1987; Dai *et al.* 1988; Deslypere and Vermeulen 1984; Field *et al.* 1994). This is observed in both young and elderly men, the difference amounting to 5–15% of the levels in non-smokers for both total and free testosterone levels (Vermeulen *et al.* 1996). Alcohol abuse, also in the absence of liver cirrhosis, may accentuate the age-associated decrease of testosterone levels, estradiol serum levels being increased (Cicero 1982; Ida *et al.* 1992; Irwin *et al.* 1988); moderate alcohol consumption has no adverse effect (Longcope *et al.* 2000; Sparrow *et al.* 1980).

Both physical and psychological stress and strenuous physical activity have been shown to result in depressed testosterone levels (Nilsson *et al.* 1995; Opstad 1992; Theorell *et al.* 1990). Acute fasting may transiently affect testosterone production through diminished gonadotropic testicular drive (Cameron *et al.* 1991), although elderly men may be more resistant to the metabolic stress of fasting (Bergendahl *et al.* 1998). Similarly, serum testosterone levels in elderly men were found to be less affected than those in young men during induced hypoglycemia and in the acute phase following myocardial infarction (Deslypere and Vermeulen 1984).

16.2.4.2 Testosterone serum levels in disease

It is now generally accepted that the aging process *per se* adversely affects testosterone production, but it is nevertheless evident that the age-associated decline in testosterone levels may often be accentuated by intercurrent disease (Handelsman 1994; Turner and Wass 1997) (see Chapter 15).

Acute critical illness (Dong *et al.* 1992; Impallomeni *et al.* 1994; Spratt *et al.* 1993; Woolf *et al.* 1985), acute myocardial infarction (Swartz and Young 1987; Wang *et al.* 1978a) and surgical injury (Wang *et al.* 1978b) have been reported to cause profound, but generally transient decreases of (free) testosterone levels. The hypogonadism during acute critical illness involves alterations in all compartments of the hypothalamo-pituitary-testicular axis (Van Den Berghe *et al.* 2001).

A series of chronic diseases can induce more longstanding decreases in testosterone levels. Both testosterone and SHBG levels tend to be decreased in elderly men with diabetes mellitus (Barrett-Connor *et al.* 1990). Impaired glucose tolerance and non insulin-dependent diabetes mellitus (NIDDM), with high prevalence in elderly persons, are associated with decreased testosterone levels (Andersson *et al.* 1994; Chang *et al.* 1994), in agreement with the observations of a negative correlation between insulin levels and testosterone levels.

Coronary atherosclerosis has been reported to be accompanied by lower or similar testosterone levels as compared to controls (Alexandersen *et al.* 1996; Hak *et al.* 2002; Phillips *et al.* 1994) and there have also been several reports of decreased testosterone levels in survivors of myocardial infarction as compared to controls (Lichtenstein *et al.* 1987; Mendoza *et al.* 1983; Poggi *et al.* 1976; Sewdarsen *et al.* 1990; Swartz and Young 1987), although it is not clear whether the decreased testosterone levels represented a consequence of atherosclerosis or rather a pre-existent risk factor for cardiovascular disease. Indeed, serum androgen levels do not predict cardiovascular events in prospective or case-control studies (see Wu and von Eckardstein 2003 for review).

In chronic obstructive pulmonary disease (COPD) and in patients with other hypoxic pulmonary diseases serum testosterone levels are often decreased with inappropriately low gonadotropin levels (Semple *et al.* 1981; 1984), also in the absence of systemic glucocorticoid treatment (Kamischke *et al.* 1998). Sleep apnea syndrome is accompanied by a relative hypogonadotropic hypogonadism (Luboshitzky *et al.* 2002; Veldhuis *et al.* 1993; Worstman *et al.* 1987), with massive obesity often being a contributing factor to the hypogonadism in these patients (Grunstein *et al.* 1989).

Chronic renal failure is often accompanied by hypogonadism with usually increased basal gonadotropin levels, explained at least in part by a decreased plasma clearance, whereas there is also an impaired pulsatile release of pituitary luteinizing hormone (LH) (Handelsman and Dong 1993; Veldhuis *et al.* 1993). In chronic disease of the liver, the decreased (free) testosterone levels are accompanied by an

increase of SHBG, androstenedione and estrone levels (Baker *et al.* 1979; Elewaut *et al.* 1979). Hypogonadism in hemochromatosis is multifactorially determined with a major contribution of pituitary insufficiency (Duranteau *et al.* 1993) besides primary testicular defects, cirrhosis of the liver and diabetes mellitus (Kelly *et al.* 1984).

Moderate impairment of testicular function has been observed in periarteritis nodosa, during acute flares of rheumatoid arthritis and in active ankylosing spondylitis (Gordon *et al.* 1988; Tapia-Serrano *et al.* 1991). In the elderly, as in the young, Leydig cell function may be adversely affected by endocrine diseases such as Cushing's syndrome (Luton *et al.* 1977; McKenna *et al.* 1979) and pituitary tumors, in particular prolactinomas.

Finally, among drugs not uncommonly used in the elderly and that may impair Leydig cell function, special mention should certainly be made of chronic use of glucocorticoids, which often induces a marked suppression of testosterone levels by combined actions at the testicular and at the hypothalamo-pituitary level, and by a decrease of SHBG serum levels (Kamischke *et al.* 1998; MacAdams *et al.* 1986). Opiates can induce a hypogonadotropic hypogonadism (Daniell *et al.* 2002; Finch *et al.* 2000). LH secretion and Leydig cell function may be adversely affected by hyperprolactinemia during chronic use of neuroleptic drugs and related compounds (Bixler *et al.* 1977).

Hormonal treatment of prostate cancer is in its essence aimed at inducing a profound hypogonadism by suppression of LH and testosterone secretion with use of a GnRH analogue and/or by blockade of androgen effects with an anti-androgen (see Chapter 12). As to the use of 5α-reductase inhibitors in benign prostate hypertrophy, under treatment with finasteride testosterone levels are unchanged or modestly elevated (Vermeulen *et al.* 1989b), but the treatment can result in mild symptoms of hypogonadism (Thompson *et al.* 2003) by mitigating androgen effects in those tissues where androgenic effects are largely mediated by DHT.

16.3 Physiopathology of declining testosterone levels in senescence

16.3.1 Primary testicular changes

Primary testicular factors undoubtedly play an important role in the age-associated decline of Leydig cell function as indicated by a reduced absolute secretory response to stimulation with human choriogonadotropin (hCG), albeit the relative testosterone response may be normal (Harman and Tsitouras 1980; Longcope 1973; Nankin *et al.* 1981; Nieschlag *et al.* 1973; 1982; Rubens *et al.* 1974). A diminished testicular secretory capacity in the elderly has also been confirmed for the response to recombinant human LH following down regulation of endogenous LH with leuprolide (Mulligan *et al.* 2001) and for the response to prolonged stimulation of

endogenous LH by a two-week pulsatile gonadotropin-releasing hormone (GnRH) infusion (Mulligan *et al.* 1999). This decrease in testicular reserve for testosterone secretion appears to result from a reduced number of Leydig cells (Harbitz 1973; Neaves *et al.* 1984; Sniffen 1950). There is, moreover, evidence for involvement of vascular changes (Sasano and Ichijo 1969; Suoranta 1971), the deficient oxygen supply inducing changes in testicular steroid metabolism (Pirke *et al.* 1980; Vermeulen and Deslypere 1986). In healthy community dwelling elderly men 70 years of age or older, mean testicular volume is decreased by about 30% as compared to young men (Mahmoud *et al.* 2003).

In apparent agreement with the view of a primary testicular defect are observations of moderate increases of basal gonadotropin levels in elderly men as observed in several, but not all, studies on the age-related decline of testicular function (Tsitouras and Bulat 1995; Vermeulen 1991 for review). Although there have been some discordant findings (Mitchell *et al.* 1995; Urban *et al.* 1988), this increase is not limited to immunoreactive forms of the gonadotropins, as it is also demonstrated for gonadotropin levels measured by bioassay (Kaufman *et al.* 1991; Matzkin *et al.* 1991; Tenover *et al.* 1988).

16.3.2 Altered neuroendocrine regulation

Although the combined observations of a diminished testicular reserve for testosterone secretion and increased basal gonadotropin levels may seem in line with the view that the age-related decline of Leydig cell function results from primary testicular dysfunction, closer examination of the data suggests that other mechanisms must also be involved. Indeed, the observed responses to hCG challenges in elderly men indicate that the secretory reserve of the Leydig cells, albeit diminished, should still be sufficient to allow normalization of plasma testosterone levels, provided the endogenous drive by pituitary LH is adequate. In the face of a persistent status of relative hypoandrogenism, the only modestly increased basal levels of pituitary luteinizing hormone (LH) should be regarded as inappropriately low. Furthermore, in contrast to previous reports of a delayed or diminished LH response upon stimulation with pharmacological doses of GnRH (Nieschlag *et al.* 1982; Rubens *et al.* 1974; Winters and Troen 1982), assessment of pituitary secretory capacity for (immunoreactive as well as bioactive) LH by challenges with small "physiological" doses of GnRH, clearly indicates a well preserved or even increased pituitary secretory reserve in elderly men (Kaufman *et al.* 1991; Mulligan *et al.* 1999).

It can be concluded that elderly men present not only with a primary testicular defect but also with alterations of the neuroendocrine control of Leydig cell function, with failure of the feedback regulatory mechanisms to normalize the testosterone levels, notwithstanding the existence of adequate testosterone and LH secretory reserve capacity (Kaufman *et al.* 1990; Vermeulen and Kaufman 1992;

Winters and Troen 1982). The relative inadequacy of the gonadotropin response to hypoandrogenism in elderly men has also been shown during experimental muting of endogenous testosterone suppression by administration of an anti-androgen (Veldhuis *et al.* 2001).

Several changes in the neuroendocrine control of Leydig cell function have been documented in elderly men. Circadian rhythm of LH and testosterone secretion is clearly blunted in elderly men (Bremner *et al.* 1983; Deslypere and Vermeulen 1984; Plymate *et al.* 1989; Tenover *et al.* 1988). Furthermore, the pulsatile pattern of LH secretion is altered with increased irregularity (Pincus *et al.* 1997) and disruption of synchrony with the secretion of FSH and prolactin as well as with nocturnal penile tumescence and sleep phases (Luboshitzki *et al.* 2003; Veldhuis *et al.* 1999; 2000). The LH pulse frequency remains essentially unchanged (Tenover *et al.* 1987; 1988; Urban *et al.* 1988; Vermeulen *et al.* 1989a; Winters *et al.* 1984), but the mean LH pulse amplitude is decreased as a consequence of reduced numbers of LH pulses with larger amplitude (Veldhuis *et al.* 1992; Vermeulen *et al.* 1989a).

Indirect evidence suggests that the main neuroendocrine changes occur at the level of the hypothalamic GnRH-secreting neuronal system. Indeed, as the responsiveness of the pituitary gonadotrophs to "physiological" doses of GnRH is preserved (Kaufman *et al.* 1999; Mulligan *et al.* 1999), the decreased LH pulse amplitude is most likely due to decreased stimulation by endogenous GnRH, with reduced size of the bolus of the neuropeptide intermittently released into the pituitary portal circulation. Moreover, the LH pulse frequency, governed by the hypothalamic GnRH pulse generator and expected to increase in a state of hypoandrogenism (Plant 1986), has been found by most authors to remain unchanged and thus inappropriately low, an increased LH pulse frequency in elderly men having been reported only by one group (Mulligan *et al.* 1995; Veldhuis *et al.* 1992). Additional evidence of altered hypothalamic regulation of gonadal function in elderly men is provided by the observation of clearly increased sensitivity to the negative feedback effects of sex steroids in comparison to the situation in young adults (Deslypere *et al.* 1987; Winters *et al.* 1984; 1997). Furthermore, the LH response to opioid receptor blockade in elderly men is blunted in comparison to that in young individuals (Mikuma *et al.* 1994; Vermeulen *et al.* 1989a), the receptor blockade failing to produce the expected increase in LH pulse frequency and amplitude observed in the young.

From the latter studies it can be concluded that alteration in LH secretion in elderly men is not due to increased endogenous opioid tone, whereas the possibility of a relative leptin deficiency as underlying cause has also been excluded (Van Den Saffele *et al.* 1999). At present, the mechanisms underlying the apparent deficiency of GnRH secretion in elderly men remain to be fully elucidated. The observed changes in LH secretion with decreased mean LH pulse amplitude can be expected to have

a significant impact on testosterone secretion as there exists a linear correlation between LH pulse amplitude and plasma testosterone levels (Veldhuis *et al.* 1992; Vermeulen *et al.* 1993).

16.3.3 Increase of serum SHBG

The progressive increase of plasma SHBG binding capacity with age should be regarded as a third important aspect of the physiopathological mechanisms that are responsible for the age-related changes in circulating testosterone levels. Indeed, against the background of a relative inability of elderly men to respond to hypoandrogenism by increased testosterone secretion, an independent progressive increase of SHBG binding capacity will result in an even steeper decline of free and not specifically bound (i.e. free and albumin-bound), bioavailable testosterone levels.

The increase of SHBG concentrations in elderly men is remarkable as it occurs in the face of increased fat mass and insulin levels, factors known to be inversely correlated to SHBG levels, but the cause of this increase of SHBG levels remains unclear. It is unlikely that the decreased testosterone levels *per se* are responsible, as the increase in SHBG levels is observed at an earlier age than the decrease of testosterone levels; estradiol serum levels are rather similar in young and elderly men (Vermeulen *et al.* 1996). Serum SHBG and testosterone levels have been reported to be inversely correlated to 24-hour growth hormone and to IGF-I levels (Erfurth *et al.* 1996; Pfeilschifter *et al.* 1996; Vermeulen *et al.* 1996) and it has been proposed that decreased activity of the somatotropic axis may play a role in the age-associated increase of SHBG levels and ensuing decrease of free testosterone levels (Vermeulen *et al.* 1996).

16.4 Clinical relevance of hypoandrogenism of senescence

16.4.1 General background

Many of the clinical features of aging in men are reminiscent of the clinical changes seen in hypogonadism in young men. It seems a reasonable working hypothesis that some of these clinical changes are causally related to declining Leydig cell function. Unfortunately, this possibility has not been fully explored and it is equally plausible that many of the observed changes and the age-related decrease in serum testosterone levels are coincident and independent consequences of aging.

Cross-sectional studies revealed mostly only weak correlations between androgen status and clinical parameters. The studies examining the effects of androgen treatment in elderly men provide precious information, but should be interpreted with caution as beneficial effects of pharmacological intervention do not imply that the corrected clinical symptoms were due to pre-existent androgen deficiency.

Carefully performed longitudinal observational studies, on the other hand, should provide more definitive answers in the future. In any case, the decreased androgen levels can at best contribute to some of the clinical changes in aging men.

16.4.2 Hypoandrogenism of senescence and sexual activity

Aging in men is accompanied by a decrease in libido and sexual activity. Mean coital frequency was reported to be about four times a week at age 20–25 years and decreases to less than twice a month between 75 and 80 years (Masters 1986; Tsitouras-Bulat 1995). Nevertheless, only 15% of men over 60 years old deny any sexual interest (Verwoerdt *et al.* 1969) and 80% of men over 60 years old remain sexually active (Kaiser 1992).

Whereas normal libido requires adequate testosterone levels, as shown by the effect of testosterone withdrawal (Bagatell *et al.* 1994; Basaria *et al.* 2002) the testosterone concentration required to sustain sexual activity and maintain libido appears to be rather low (Gooren 1987; Schiavi 1996), and there is good evidence that healthy adults have substantially higher androgen levels than required for normal sexual behavior (Buena *et al.* 1993; Udry *et al.* 1985).

Several authors reported differences in parameters of sexual desire or activity according to endogenous serum testosterone levels (Davidson *et al.* 1983; Schiavi *et al.* 1988; 1990; Tsitouras *et al.* 1982; Udry *et al.* 1985), but there is a broad overlap of serum testosterone levels in sexually less or more active elderly men in these studies. Moreover, other studies failed to find an association between androgen levels and the perception of sexual functioning (Perry *et al.* 2001; T'Sjoen *et al.* 2003) (see also Chapters 4 and 11). Although potency and nocturnal penile tumescence (NPT) require adequate testosterone levels and although several studies show that hormonal alterations might play some role in 6 to 45% of cases (Morley 1986), most frequently the cause of impotence in elderly males is non-hormonal.

Nocturnal penile tumescence is clearly androgen-dependent, but Schiavi *et al.* (1990) did not observe any correlation between NPT and erectile problems in the elderly, suggesting that their erectile problems are largely non-hormonal in origin. Similarly, several studies failed to find a relationship between erectile dysfunction and serum testosterone levels in elderly men (Feldman *et al.* 1994; Korenman *et al.* 1990; Rhoden *et al.* 2002). This may be explained by a low threshold of serum testosterone required for maintenance of normal erectile function. Among non-hormonal factors that may influence the frequency of impotence in elderly men are:

- the overall health status of both partners, diabetes mellitus being a common cause of impotence at any age;
- boredom with, or loss of attractiveness of the (same) sexual partner, as well as monotony of sexual life;

- low level of sexual activity in young age, the activity of the aging male being strongly correlated with the activity in younger age (Martin 1975; Pfeiffer 1974);
- medications (Tsitouras and Bulat 1995) such as psychotropic drugs (tricyclics; MAO inhibitors; phenothiazines; hypnotics), antihypertensive compounds (β-blockers; guanethidine; prazozine; angiotensin-converting enzyme inhibitors), H_2-antihistaminics, drug abuse (alcohol; heroin; cannabis) (Kligman 1991; Tsitouras and Bulat 1995);
- psychopathology such as stress and depressive states;
- atherosclerosis and cardiovascular disease, being the most frequent cause of erectile dysfunction in the elderly and accounting for about 50% of cases (Kaiser 1992; Virag *et al.* 1985);
- neurological factors with decreased sensory, neural and autonomic functioning, an important cause of impotence in the elderly and together with atherosclerosis the most frequent cause in diabetics.

In conclusion, whereas adequate testosterone levels are required for libido and NPT, it appears that testosterone levels required for normal sexual activity are rather low (below the lower end of the reference range for young men, i.e. below 11 nmol/l or 320 ng/dl for total serum testosterone), and although testosterone codetermines potency, the factors most commonly involved in erectile dysfunction in elderly men are not hormonal. Nevertheless, there have been recent reports of improvements of parameters of sexual functioning during androgen administration to elderly men with low or (low) normal serum testosterone levels (Hajjar *et al.* 1997), although in the setting of controlled studies, the improvements were generally modest and only significant for some of the assessed variables (Kunelius *et al.* 2002; Steidle *et al.* 2003).

16.4.3 Body composition and sarcopenia

Aging in men is associated with a decrease of lean body mass and an increase of fat mass, especially in the upper body and central body regions (Forbes and Reina 1970; Swerdloff and Wang 1993; Tenover 1994; Vermeulen *et al.* 1999a). Fat mass increases from around a mean of 20% of body weight in young men to 30% or more in the elderly, whereas muscle mass may decrease by as much as 35 to 40% from age 20 to 80 years (Bross *et al.* 1999). In a study in community-dwelling healthy men (Vermeulen *et al.* 1999a) we found a mean fat mass of 22.3% of body weight in 61 middle-aged men with mean age of 42 years, as compared to 29.4% in 271 men with mean age of 76 years, BMI being similar in both groups and lean body mass 20% lower in the elderly.

Fat mass, and in particular abdominal fat mass is negatively associated with serum (free) testosterone levels (Van Den Beld *et al.* 2000; Vermeulen *et al.* 1999a; see also section 16.2.4.1). However, the direction of this association remains unclear,

as low testosterone may be a positive determinant of adiposity, whereas conversely adiposity appears to be a negative determinant of serum testosterone. Moreover, altered activity of the somatotropic axis may also play an important role in the age-related changes in body composition. In any case, a negative association of free testosterone with fat mass in elderly men persists after correction for serum IGF-I levels, which are positively correlated to serum (free) testosterone and negatively to fat mass (Vermeulen *et al.* 1999a).

In a majority of controlled trials of several months duration with administration of androgens to elderly men with low or (low) normal serum (bio-available) testosterone, treatment resulted in a modest decrease of total and/or abdominal fat mass (Gruenewald and Matsumoto 2003). These findings were confirmed in recent controlled studies in elderly men with transdermal administration of testosterone (Steidle *et al.* 2003) or DHT (Ly *et al.* 2001), although in this context growth hormone may have a greater effect than testosterone (Münzer *et al.* 2001).

The age-associated loss of muscle mass is accompanied by decreased muscle strength, which occurs regardless of the level of physical activity (Rogers and Evans 1993). Muscle weakness is an important component of frailty in old age, contributing to functional limitation for activities of daily living and related problems such as an increased risk for falls (Bhasin and Tenover 1997; Dutta and Hadley 1995; Guralnik *et al.* 1995; Rubenstein *et al.* 1994; see also Chapter 8). Information on the association of endogenous androgens and muscle mass is scarce. Van Den Beld *et al.* (2000) and Vermeulen *et al.* (1999a) found no association of serum (free or non SHBG-bound) testosterone with lean body mass in sizable populations of ambulant elderly men. Similarly Roy *et al.* (2002) found no association of lean mass with testosterone, independent of age, in men aged 20 to 90 years from the Baltimore Longitudinal Study of Aging. The limited data available suggest the existence of a correlation between serum testosterone levels and muscle function (Abbasi *et al.* 1993), testosterone levels being also correlated with training-induced gain of strength (Häkkinen and Pakarinen 1994). In the study by Roy *et al.* (2002) a free testosterone index (total testosterone over SHBG ratio), albeit not a reliable parameter of free testosterone in men (Vermeulen *et al.* 1999b), was positively associated with muscle strength; Van den Beld *et al.* (2000) observed a positive association of muscle strength with free- and bio-available testosterone.

Several controlled studies of more than three months duration with androgen administration in elderly men with low or (low) normal serum testosterone (see Gruenewald and Matsumoto 2003 for review) have shown increases of lean body mass (Ferrando *et al.* 2002; Kenny *et al.* 2001; Münzer *et al.* 2001; Snyder *et al.* 1999a; Steidle *et al.* 2003). Short-term administration (4 weeks to 3 months) of testosterone to elderly men, aimed at increasing initially low testosterone serum levels to values within the normal range for young men, has been reported to increase lean body

mass (Tenover 1992), muscle strength (Morley *et al.* 1993; Urban *et al.* 1995) and skeletal muscle protein synthesis (Urban *et al.* 1995) However, the latter studies, besides being of short duration also included only limited number of subjects. Sih *et al.* (1997) observed a significant increase in grip strength in the testosterone-treated men over the age of 50 years (mean age 68 years) with low bio-available serum testosterone, in a prospective, randomized, placebo-controlled trial of 12 months' duration; lower extremity muscle strength was not evaluated. Ferrando *et al.* (2002) observed improved leg and arm muscle strength and an increase in muscle net protein balance in a small number of older men treated with testosterone for six months; a positive effect of six months testosterone treatment is seen in older men on net protein balance in the fasted state, but there is no demonstrated additive effect of testosterone when combined with amino acid feedings (Ferrando *et al.* 2003). Muscle strength measured by isokinetic peak torque was increased in flexion of the dominant knee, but not in knee extension or shoulder contraction during three months of transdermal administration of DHT in older men with low normal serum testosterone (Ly *et al.* 2001). In the latter study there was no effect of treatment on tests of gait, balance or mobility; no effect of treatment on muscle strength was observed in the studies by Kenny *et al.* (2001) and Snyder *et al.* (1999a). Crawford *et al.* (2003) reported increased muscle mass and strength during androgen treatment in men with mean age around 60 years under treatment with glucocorticoids.

In conclusion, partial hypoandrogenism might thus play a contributory role in the sarcopenia of older men, but sarcopenia in elderly men is multifactorially determined (Bross *et al.* 1999; Tenover 1994). Data on androgen treatment suggest potentially beneficial effects, but the data on treatment effects on muscle strength in elderly men are still limited and the findings are not all favorable. Moreover, there is no convincing demonstration of functional benefits of androgen treatment, which might in part be due to use of inappropriate methodology (Bhasin and Buckwalter 2001). Considering that we have no information as to the androgen sensitivity of muscle tissue in older men, and that men respond to supraphysiological doses of testosterone by graded increments in muscle mass and force (Bhasin *et al.* 1996; 2001), beneficial effects of androgen treatment on muscle mass and function in elderly men may represent pharmacological effects rather than "physiological" androgen substitution.

16.4.4 Senile osteoporosis

Aging of men is accompanied by progressive bone loss, which persists and may even accelerate in old age. Osteoporosis in men is increasingly being recognized as a significant problem of public health. The age-specific incidence of both hip and vertebral fracture is about half that in women (Van Der Klift *et al.* 2002), and

occurring with a delay of five to six years. One out of four patients suffering a hip fracture is a male and the prognosis of hip fracture, as well as of other major osteoporotic fractures, appears to be worse in men as compared to women in terms of both morbidity and mortality (for review Bilezikian 1999; Kaufman *et al.* 2000; 2001, and Orwoll and Klein 1995; see Chapter 7).

A number of recent studies and clinical observations have demonstrated that estrogens are essential for both bone acquisition and maintenance of adult skeletal integrity in men and that both androgenic and estrogenic input intervenes in the regulation of adult bone metabolism in the male (for review Riggs *et al.* 2002). A preponderant role of estrogen in the regulation of bone metabolism in elderly men has been elegantly demonstrated in a short-term intervention study with selective manipulation of testosterone and estradiol levels (Falahati-Nini *et al.* 2000). In cross-sectional studies in elderly men, associations of bone mineral status or biochemical markers of bone turnover with sex steroid levels have been rather weak, although statistically significant. In several of the latter studies (free or bio-available) estradiol was more strongly associated with bone mineral density and/or bone turnover markers than (free or bio-available) testosterone. Khosla *et al.* (2001) and Van Pottelbergh *et al.* (2003) have observed an inverse relationship in healthy elderly men between serum bio-available estradiol and prospectively assessed bone loss, without independent contribution of serum (bio-available) testosterone in the determination of this loss. In the latter study, changes in bone mineral density, personal clinical fracture history of the subjects and fracture history in their first-degree relatives, were all associated, independently of serum estradiol levels, with a polymorphism of the CYP19 gene that encodes for the aromatase enzyme suggesting indirectly that local aromatization of testosterone in bone tissue might play a role. Barrett-Connor *et al.* (2000) reported an association of vertebral fractures with low serum estradiol levels in elderly men of the Rancho Bernardo Study.

In community-dwelling men over age 70 years, we found no association of bone mineral density or bone metabolism with a CAG repeat polymorphism of the androgen receptor (Van Pottelbergh *et al.* 2001), whereas in another study including younger men 20 to 50 years old such an association was found (Zitzmann *et al.* 2001).

Profound hypogonadism in younger men (Mauras *et al.* 1999; Stepan *et al.* 1989) as well as in older men (Mittan *et al.* 2002; Stoch *et al.* 2001) has been shown to result in accelerated bone loss with high bone turnover and there is indication that testosterone replacement therapy in men with acquired hypogonadism may result in partial recovery of bone density (Behre *et al.* 1997; Devogelaer *et al.* 1992; Finkelstein *et al.* 1989; Katznelson *et al.* 1996). Hypogonadism has also been reported to be a risk factor for hip fracture in elderly men (Boonen *et al.* 1997; Jackson *et al.* 1992;

Stanley *et al.* 1991), but besides low bone mass, other factors related to testosterone, such as muscle weakness may be involved.

Currently available controlled data on the effects of testosterone treatment in elderly men is limited and not conclusive. Observed effects of androgen treatment in elderly men on biochemical indices of bone turnover have been rather inconsistent. Morley *et al.* (1993) observed an increase of serum levels of osteocalcin, a marker of osteoblastic activity, during androgen treatment. Tenover (1992) reported reduced hydroxyprolinuria, a marker of bone resorption, in a small group of elderly men treated with androgen. Orwoll and Oviatt (1992) found no significant effect of androgen treatment on biochemical indices of bone turnover in a larger group of elderly men. No change in serum osteocalcin or alkaline phosphatase was observed in a longer-term study by Sih *et al.* (1997). There were no clear effects on markers of bone turnover in two controlled studies of the effects in the elderly of longer duration transdermal testosterone administration (Kenny *et al.* 2001; Snyder *et al.* 1999a). Also, dehydroepiandrosterone supplementation to older men (90 mg/day for 6 months versus placebo) was without effect on bone turnover markers in men 50 to 80 years of age (Kahn and Halloran 2002). The lack of consistent effect of treatment on biochemical markers of turnover might result in part from methodological problems, as well as from the complexity of differential effects of testosterone and its aromatization product estradiol (Falahati-Nini *et al.* 2000; Riggs *et al.* 2002). Snyder *et al.* (1999a), in a randomized double-blind trial of transdermal administration of testosterone by scrotal patch (6 mg/day) or placebo to men with normal or low serum testosterone ($n = 108$), found that after three years of treatment lumbar spine bone mineral density was increased in the placebo group as well as in the active treatment group, both receiving calcium and vitamin D supplements, without significant testosterone treatment effect; there was no change in bone mineral density at the hip region in either treatment group. These negative findings for testosterone effects on bone were accompanied by significant treatment effects on fat mass and lean body mass. Kenny *et al.* (2001) reported prevention of bone loss at the hip during one-year treatment with transdermal testosterone (5 mg/day by body patch) as compared to placebo in healthy elderly men aged 65 to 87 years, but differences between placebo and active treatment were rather small. Crawford *et al.* (2003) found a beneficial effect of 12 months administration of testosterone, but not of the only minimally aromatizable androgen nandrolone, on bone mineral density in men treated with glucocorticoids who had initially low or low normal serum testosterone levels.

These rather disappointing results, as compared to the findings for bone effects of testosterone administration in younger hypogonadal men, may be explained by the fact that many of the men included in these studies had either normal or near normal initial androgen levels and by the possible existence of a threshold effect

with the testosterone and derived estrogen requirements for near maximal bone effects being situated at the lower end of the physiological range for sex steroid levels. In support of this view is a post hoc analysis of the results of the study by Snyder *et al.* (1999a) which indicates that only those men with initially low serum total testosterone (<300–350 ng/dl) increased their bone mineral density during treatment. For their cohort study in healthy elderly men Khosla *et al.* (2001) have described a threshold for serum bio-available estradiol below which these levels are negatively associated with prospectively assessed bone loss. However, Van Pottelbergh *et al.* (2003) did not detect such a threshold in their cohort study.

In conclusion, evidence for the involvement of (relative) hypoandrogenism in senile osteoporosis in men remains limited, additional studies being needed to clarify this clinically important point and to further evaluate the potential role of hormonal and related treatments for senile osteoporosis.

16.4.5 Additional clinical variables

Aging is associated with a deterioration of multiple aspects of cognitive performance. Hypogonadal men tend to show diminished spatial skills, but the findings of observational studies on the relationship between spatial skills and endogenous androgen levels in elderly men have been inconsistent, whereas in several, but not all intervention studies, androgen administration to elderly men has resulted in improved spatial cognition and working memory, with decreased verbal fluency (Muller *et al.* 2003; Gruenewald and Matsumoto 2003 for review; see also Chapter 4).

Endogenous bio-available testosterone levels were reported to be inversely associated with depressive mood assessed with the Beck Depression Inventory in older men in the Rancho Bernardo Study (Barrett-Connor *et al.* 1999). In a study of selected men aged 50 to 70 years, who participated in a screening program on prostate cancer and 'andropause', there was an inverse correlation between free testosterone and depressive symptoms assessed on the Carroll Rating Scale, but serum free testosterone was not related to the prevalence of a significant score for depression (Delhez *et al.* 2003). In contrast others reported that declining bio-available testosterone levels were associated with lower levels of depressive symptoms on the Hamilton Depression Scale in men 55 to 76 years old (Perry *et al.* 2001).

T'Sjoen *et al.* (2003) failed to observe a relationship between (free or bio-available) testosterone and health-related quality of life as assessed with the SF-36 questionnaire in ambulant community-dwelling men over 70 years, which is in accordance with findings by Dunbar *et al.* (2001). Snyder *et al.* (1999b) found significantly less worsening of the perception of physical functioning according to a sub-score of the SF-36 questionnaire during testosterone treatment as compared

to placebo in elderly men, the largest treatment effect being observed in men with the lowest initial serum testosterone; in the latter study there was no treatment effect for any other sub-score of the SF-36 questionnaire. In studies with androgen administration to elderly men with low or (low) normal serum testosterone, there were no significant effects over placebo for mood and/or overall quality of life (Gruenewald and Matsumoto 2003; Kunelius *et al.* 2002; Ly *et al.* 2001; Steidle *et al.* 2003); improvement of quality of life as measured with a questionnaire intended for patients with osteoporosis and vertebral fracture was reported for testosterone administration in glucocorticoid-treated men (Crawford *et al.* 2003).

16.4.6 Conclusions

The extent of the clinical consequences of the relative hypoandrogenism of elderly men remains unclear. It seems likely that hypoandrogenism intervenes in some aspects of the clinical changes in aging men, but it is also evident that the androgen status is only one of many factors influencing the pace and clinical expression of aging in men. A crucial remaining question is the possible existence of threshold concentrations for androgen action in target tissues and their level in the elderly, whereby local activating and catabolic tissue metabolism of testosterone, variations of androgen receptor concentrations and the possibility of tissue-specific co-activators and co-repressors of the androgen receptor offer almost unlimited possibilities for local diversification and modulation of testosterone action.

16.5 Androgen substitution in the elderly men

16.5.1 Who should be considered for treatment?

The age-associated decrease in serum testosterone levels raises the issue of androgen substitution in elderly males: who should be treated, how and for how long?

As to the first question, in theory androgen administration to elderly men may be either "substitutive" to alleviate symptoms and prevent complications of a partial or more complete androgen deficiency, or rather "pharmacological" administration to elderly men who are not necessarily androgen deficient, but with specific treatment goals such as prevention or treatment of osteoporosis, frailty, or treatment of erectile dysfunction. Clearly, although there have been a few small-scaled studies providing indications of potential treatment benefits (Gruenewald and Matsumoto 2003), for no single indication does the present evidence even approach justifying "pharmacological" androgen treatment in elderly men. Thus we are left with only "substitutive" treatment to be considered at this time.

Albeit systematic studies on the effects of androgen substitution in younger hypogonadal men are few and randomized trials of substantial duration are not available for evident ethical reasons, it is generally accepted that prolonged androgen

deficiency in young men results in symptoms affecting quality of life and carries a risk for longer-term complications; thus intervention to reestablish physiological androgen levels is required unless there is a specific contraindication. As to the elderly, there is no *a priori* medical or moral justification for withholding the benefits of substitutive treatment from symptomatic hypogonadal elderly men, but a prudent approach is advisable in view of the limited data and clinical experience for this population and of the potential for a greater susceptibility for adverse treatment effects in the elderly.

This brings up the key problem of how to diagnose androgen deficiency in elderly men and what their testosterone requirements are (Vermeulen 2001; Vermeulen and Kaufman 2002). If distribution of serum testosterone levels in healthy young men is taken as reference, the question is whether elderly men are equally, less or more sensitive to testosterone action. Any answer to this question is complicated by the fact that, on the one hand, signs and symptoms of androgen deficiency lack specificity, while on the other hand, a useful direct biochemical measure of androgen activity is lacking. Indeed, the more we learn about testosterone action, the more it becomes clear that measures of (total or non-specifically bound) testosterone in the circulation can at best imperfectly reflect the action of testosterone and its bioactive metabolites in the tissues. Moreover, there are clear indications that testosterone requirements for normal functioning may differ widely according to tissue and physiological function, whereas the considerable inter-individual differences in serum testosterone levels seen at all ages may be the expression of individual differences in androgen sensitivity and requirements.

In the absence of a reliable parameter, a pragmatic and sufficiently conservative approach to the diagnosis of androgen deficiency in elderly men should rely on both the clinic and the hormonal levels, diagnosis of androgen deficiency requiring congruent findings of a suggestive clinical picture together with clearly low serum testosterone levels. As to the latter, in the absence of definitive evidence of altered sensitivity to androgens in the elderly, the least arbitrary attitude is to use the same lower normal limit as in young men, i.e. around 11 nmol/l (or 320 ng/dl) for total serum testosterone, around 0.225 nmol/l (or 6.5 ng/dl for serum free testosterone, and around 5 nmol/l (or 145 ng/dl) for bioavailable testosterone. Parameters of the biologically active fraction of serum testosterone, i.e. serum free testosterone and bio-available (i.e. non specifically bound) testosterone, are more appropriate for the evaluation of the androgen status. Their use will result in classification of an even larger proportion of elderly men as being hypoandrogenic, as the age-dependent decrease is steeper than for total testosterone. On the other hand, in a number of situations with low serum SHBG, such as in obesity and during glucocorticoid treatment, these measurements may reveal an androgen status more favorably preserved than that indicated by serum total testosterone. It is advisable to apply the

above cut-off values conservatively and to consider for the diagnosis of deficiency only values that are frankly low. Indeed, whereas reports of decreased tissue concentration of androgen receptors in the elderly (Roehrborn *et al.* 1987) might suggest decreased sensitivity, the only data from functional studies available indicate an increased sensitivity of LH secretion to the negative feedback action of testosterone in older men (Deslypere *et al.* 1987, Winters *et al.* 1984; 1997). Moreover, whereas most studies on administration of testosterone to elderly men have included a large proportion of men with serum testosterone within the lower normal range for young men, probably based on the rationale that these low normal levels might in fact be sub-optimal for many of these particular subjects, the treatment effects were generally disappointing for those men not having clearly low initial serum testosterone levels. As to serum gonadotropin levels, although markedly elevated serum LH certainly adds weight to the finding of decreased serum testosterone and points towards a predominantly testicular factor, elevated serum LH is not a prerequisite for the diagnosis of testosterone deficiency in older men, the age-associated decline of Leydig cell function usually being of mixed testicular and neuroendocrine origin (see section 16.3).

As to the objective signs of relative androgen deficiency, although a decrease of muscle mass and strength and a concomitant increase in central body fat and osteoporosis can most easily be objectified, they are not specific signs. Decreased libido and sexual desire, loss of memory, difficulty in concentration, forgetfulness, insomnia, irritability, depressed mood as well as decreased sense of well-being, are rather subjective feelings or impressions, less easily objectified and certainly difficult to differentiate from hormone-independent aging. Complaints of excessive sweating are not uncommon, whereas true hot flushes do occur in elderly men, although they are mainly prevalent in severe acquired hypogonadism such as under hormonal treatment for prostate cancer.

There exist a number of questionnaires that are being used in clinical or epidemiological settings to help describe and semi-quantify symptoms in different areas that are of relevance to elderly men, such as questionnaires on self-perceived health status, on depressive mood, on urinary symptoms, on erectile function, or on coping with activities of daily living. Morley *et al.* (2000) proposed a dedicated instrument, the "ADAM" screening questionnaire for androgen deficiency in aging males. The available information suggests that this questionnaire, although relatively sensitive to detect men with decreased free or bio-available testosterone, lacks the required specificity to be a valid instrument for diagnosis in the individual subject (Delhez *et al.* 2003). The "Aging Males' Symptoms Scale" (AMS) was developed by Heinemann *et al.* (1999) in Germany to help describe and quantify the clinical syndrome of 'andropause', but was not intended to screen for low serum testosterone and was not validated by the authors against serum androgen levels. Others have

reported that this 17 item-questionnaire, which was subsequently linguistically and culturally adapted in several languages, does not allow androgen serum levels in elderly men to be predicted (Dunbar *et al.* 2001; T'Sjoen *et al.* 2003). Smith *et al.* (2000) developed a self-administered 8 item-screener for testosterone deficiency in aging men. Whereas this screener performs better than chance in identifying men with low serum testosterone, mainly it addresses issues of co-morbidity and again lacks the specificity required for a performing clinical tool. From data presently available it does not appear that, albeit helpful in describing symptoms, these questionnaires contribute significantly to the diagnosis of androgen deficiency. Nor is it presently established whether they might serve as a prescreening instrument to select patients for blood sampling; neither is it clear whether screening for low serum testosterone is in itself presently desirable. Indeed, taken the high prevalence in older men of non-specific symptoms loosely associated with hypoandrogenism, spontaneous active reporting of complaints may have the merit of a higher specificity, whereas soliciting complaints with screening questionnaires might lead to over-diagnosis and over-treatment.

In conclusion, according to the present state of the art, androgen supplementation should probably only be considered in the presence of androgen serum levels clearly below the lower normal limit for young men, together with unequivocal signs and symptoms of androgen deficiency, after having excluded reversible causes of low serum androgen and after careful screening for contra-indications. Indeed, the lack of reliable data on the long-term risk-benefit ratio imposes a critical and conservative attitude in accordance with a basic principle of clinical practice, i.e. *primum non nocere.*

16.5.2 Potential benefits

From the discussion in the preceding sections of this chapter and from literature reviews (Bhasin and Buckwalter 2001; Gruenewald and Matsumoto 2003), it emerges that testosterone administration to elderly men can induce potentially beneficial effects, but the results are often mitigated and there usually is no demonstrated impact on endpoints that are directly relevant for the clinic. Several studies have shown improvement of lean body mass and sometimes also of muscle strength, but whether these changes are sufficient to make a difference in terms of functionality is still unclear. Positive effects on bone mineral density are seen only in men with frankly low serum testosterone and we have no information on the effect of treatment on fracture rates. Abdominal fat may decrease and the insulin sensitivity may improve, whereas high dose testosterone may have direct beneficial effects on heart and arteries, but we have no indication of gains in terms of hard cardiovascular endpoints. There have been reports of favorable effects on mood, cognition an general well being, but the findings are not always consistent and we have no

data indicating that treatment may prevent or help treat depression, or have substantial longer-term effects on cognition and quality of life. Sexual functioning can improve, but the treatment effects are rather small and usually significant for only a few of the several assessed indices of sexual function.

A major limitation is the scarcity of controlled data available. No more than a few hundred elderly men in total have been included in controlled trials and among these we have counted a total of only 275 men included in a trial of at least one year duration; less than half these men received active treatment with testosterone. Moreover, a majority of trials have included a substantial proportion of elderly men with initially (low) normal serum testosterone.

16.5.3 Potential risks

As to the risks of androgen replacement therapy in elderly men, we consider here only effects of "physiological" doses of testosterone and not those of massive "pharmacological" doses, as used by body builders. Traditionally, it has been a matter of concern that prolonged treatment with androgen may increase the risk of cardiovascular disease. The complex relationship between endogenous and exogenous androgens and cardiovascular risk is discussed in Chapter 10. With the evidence currently available, improving cardiovascular risk can certainly not be considered an indication for androgen treatment, but there is also no suggestion that treatment with moderate, close to physiological doses carries an unacceptable risk that should deter initiating otherwise indicated androgen treatment. It should be reminded that no conclusive data is currently available on the effects of androgen treatment on cadiovascular morbidity or mortality in elderly men.

Androgen treatment results in a significant increase of hematocrit and blood hemoglobin level (Gruenewald and Matsumoto 2003). Polycythemia is not uncommon and may necessitate dose reduction, temporary interruption of treatment, or alternative measures such as phlebotomy (see Chapter 13). Whereas a moderate increase in hematocrit in elderly males is probably beneficial, Hajjar *et al.* (1997) observed that out of 27 elderly hypogonadal males receiving 200 mg of testosterone enanthate or cypionate every two weeks, 11 (24%) developed polycythemia sufficient to require phlebotomy or temporary withholding of testosterone, one third of which occurred less than one year after starting treatment. Sih *et al.* (1997) reported a similarly frequent development of polycythemia. The occurrence of polycythemia appears to be more likely when subjects are exposed to markedly supraphysiological androgen levels, as is often the case with commonly applied treatment regimens, consisting of intramuscular administration of depot preparations of testosterone esters at intervals of two to three weeks (Dobs *et al.* 1999). Significant increases of hematocrit and hemoglobin levels are also seen during transdermal administration of either testosterone (Snyder *et al.* 1999b; Steidle *et al.* 2003) or DHT (Kunelius

et al. 2002; Ly *et al.* 2001), occasionally leading to erythrocytosis. Monitoring for occurrence of exaggerated elevations of hematocrit or hemoglobin concentrations during androgen treatment in elderly men is advisable, keeping in mind that some patients, in particular some with pulmonary disease, can have a high *a priori* risk of erythrocytosis.

Androgens may exacerbate obstructive sleep apnea (Matsumoto *et al.* 1985; Sandblom *et al.* 1983). Therefore, patients should be specifically questionned for symptoms of sleep apnea, and chronic obstructive pulmonary disease, especially in overweight subjects or heavy smokers, who may be regarded as having a relative contra-indication for androgen therapy.

Gynecomastia, related to the conversion of testosterone to estradiol in peripheral tissues, mainly fat tissue, which is relatively increased in elderly men, is a not uncommon but benign side-effect in elderly men, especially in the obese. This side-effect is probably less frequent when avoiding exposure to largely supraphysiological serum levels of testosterone. Testosterone causes some sodium and water retention (Wilson 1988), this cannot cause a problem, except in patients with congestive heart failure, hypertension or renal insufficiency. Hepatotoxicity is very rare when non-oral routes of administration of testosterone are used.

Of greater concern are the possible effects on the prostate, which is an androgen dependent organ (Bhasin *et al.* 2003) (see Chapter 12). As far as benign prostatic hyperplasia (BHP) is concerned, studies to date failed to observe an important growth of the prostate (Behre *et al.* 1994; Wallace *et al.* 1993) and all studies have failed to find any relationship between plasma and BPH tissue levels of testosterone, DHT or estradiol. It appears that tissue levels are determined by the enzyme activity in the tissue itself, rather than by surrounding plasma androgen levels. Treatment does not seem to result in increased voiding symptoms or postvoid residual volume (Gruenewald and Matsumoto 2003), and only in cases of severe lower urinary tract obstructive symptoms is benign prostate disease considered a contraindication for androgen treatment (Bhasin *et al.* 2003; Morales 1999).

Clinical prostate carcinoma undoubtedly is an androgen sensitive tumor (Goldenberg *et al.* 1995): hence presence of a clinical prostate carcinoma is an absolute contraindication to testosterone supplementation. Subclinical carcinoma, only detectable on histology but undetectable by biochemical or clinical procedures, is found in a majority of men over 70 years old. Only a small minority of these subclinical carcinomas will develop further into a clinical carcinoma. It is not known whether testosterone treatment will stimulate the progression of subclinical carcinoma and so far no available data indicate that testosterone substitution will activate subclinical carcinoma (Schröder 1996; Jackson *et al.* 1989, see also Chapter 12). However, all studies so far concern only small numbers of carefully selected elderly males treated for short periods of time. In any case, before starting

testosterone supplementation careful exclusion of the presence of a prostate carcinoma by rectal examination and serum PSA, and, when required, supplemented by ultrasonography, is mandatory. For treated patients it is advised to perform controls of rectal examination, PSA and a symptom questionnaire for benign prostatic hyperplasia after three, six and twelve months, thereafter yearly controls (Bhasin *et al.* 2003; Morales 1999).

16.5.4 Modalities of androgen substitution

As discussed in section 16.5.1, in the absence of convincing evidence that androgen requirements change with age, it can be proposed to aim for the physiological levels in young men. There is no evidence that it is clinically important to mimic the diurnal variations as found in young adults. Nevertheless, it should be appreciated that constant levels in the upper normal range will result in 24-hour mean levels that are supraphysiological as compared to the situation in young men subject to diurnal variations of serum testosterone.

Taken that the hypothalamo-pituitary-testicular axis is very sensitive to negative feedback, and even more so in elderly males (Deslypere *et al.* 1987; Winters *et al.* 1984; 1997), it is important to ascertain that the dose administered increases the testosterone levels up to the physiological range and does not merely suppress LH secretion with only replacement of the deficient testosterone production by an inadequate dose of exogenous testosterone. In practical terms, full replacement doses are usually required.

Considering the fact that metabolization of testosterone to DHT and estradiol is important for the regulation and full expression of testosterone effects, treatment with testosterone is the most physiological approach and the preferable option with the currently available evidence, but the debate is certainly not closed in view of data obtained with alternative treatments such as transdermal DHT and in view of ongoing research aimed at the development of "selective androgen receptor modulators" (SARMs) with tissue-specific properties. (The pharmacology and practical aspects of testosterone replacement are discussed in detail in Chapter 13.)

16.6 Key messages

- Mean total serum testosterone decreases progressively in healthy men over the age of 55 years (30% decrease between age 25 and 75 years). Age-associated decrease of the bio-available fractions of serum testosterone is steeper as a consequence of an age-related increase of serum SHBG (50% decrease of free or bio-available testosterone between age 25 and 75 years).
- There is great inter-individual variability of prevailing androgen levels in the elderly, ranging from perfectly preserved to frankly hypogonadal. Part of the inter-individual variability in serum testosterone levels is explained by heredity, physiological factors and lifestyle-related factors.

- The proportion of men with "subnormal" testosterone relative to the levels in young men increases with age (>20% after age 60 years); whether androgen requirements change in aging men remains to be established.
- The age-related decline in Leydig cell function can transiently or more permanently be accentuated by co-morbidity and medication.
- The age-related decline of testosterone production is the result of primary testicular changes as well as of failure of the hypothalamic regulatory mechanisms to respond adequately to the hypoandrogenic state.
- Many of the clinical features of aging in men are reminiscent of the clinical changes seen in hypogonadism in younger men; relative hypoandrogenism may be involved in some, but certainly not all clinical changes.
- Testosterone levels required for normal sexual activity are rather low and although testosterone levels codetermine potency, the factors most commonly involved in sexual dysfunction in elderly men are not hormonal.
- Hypoandrogenism may be involved in the sarcopenia of elderly men.
- The role of hypoandrogenism in male senile osteoporosis remains to be confirmed; recent data indicates that aromatization of testosterone to estradiol plays an important role in the regulation of bone metabolism in elderly men.
- In the present state of the art, androgen supplementation should only be considered in the presence of androgen serum levels clearly below the lower normal limit for younger men, together with unequivocal signs and symptoms of androgen deficiency, in the absence of other reversible causes of decreased androgen levels and after screening for contra-indications. The longer term risk-benefit ratio for androgen administration to elderly men is unknown.
- Available questionnaires assessing aging male symptomatology do not predict decreased serum testosterone in elderly men; their use for screening purpose should not be encouraged.
- Possible benefits of the treatment include an improved sense of general well-being, of libido and of muscle strength, with increase of lean body mass and limited decrease of fat mass.
- So far, the limited data on safety of testosterone replacement therapy in the elderly has been rather reassuring: larger scale studies of longer duration are still needed to assess safety, in particular at the prostate level; development of erythrocytosis seems to emerge as one of the most troublesome side-effects, which may be less frequent if largely supraphysiological androgen levels are avoided.
- Androgen replacement therapy in the elderly requires careful monitoring by an experienced physician.

16.7 REFERENCES

Abbasi AA, Drinka PJ, Mattson DE, Rudman D (1993) Low circulating levels of insulin-like growth factors and testosterone in chronically institutionalized elderly men. J Am Geriatr Soc 41:975–982

Adlercreutz H (1990) Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scan J Clin Lab Invest 201 (supplement):S3–S23

Alexandersen P, Haarbo J, Christiansen C (1996) The relationship of natural androgens to coronary heart disease in males: a review. Atherosclerosis 125:1–13

Andersson B, Marin P, Lissner L, Vermeulen A, Björntorp P (1994) Testosterone concentration in women and men with NIDDM. Diabetes Care 17:405–411

Aversa A, Isidori AM, Spera G, Lenzi A, Fabbri A (2003) Androgens improve cavernous vasodilation and response to sildenafil in patients with erectile dysfunction. Clin Endocrinol 58:632–638

Bagatell CJ, Heiman JR, Rivier JE, Bremner WJ (1994) Effects of endogenous testosterone and estradiol on sexual behaviour in normal young men. J Clin Endocrinol Metab 78:711–716

Baker HWG, Burger HG, de Kretser DM, Hudson B (1977) Endocrinology of aging: pituitary testicular axis. In: James VHT (ed) Proc of the 5th International Congress of Endocrinology, Excerpta Medica Foundation, Amsterdam, pp 179–183

Baker HWG, Burger HG, de Kretser DM, Dulmanis A, Hudson B, O'Connor S, Paulson CA, Purcell N, Rennie GC, Seah CS, Taft HP, Wang C (1979) A study of the endocrine manifestations of hepatic cirrhosis. Q J Med 177:145–178

Barrett-Connor EL, Khaw KT (1987) Cigarette smoking and increased endogenous estrogen levels in men. Am J Epidemiol 166:178–192

Barrett-Connor EL, Khaw KT, Yen SCC (1990) Endogenous sex hormone levels in older adult men with diabetes mellitus. Am J Epidemiol 132:895–901

Barrett-Connor EL, Von Mühlen DG, Kritz-Silverstein D (1999) Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J Clin Endocrinol Metab 84:573–577

Barrett-Connor EL, Mueller JE, Von Mühlen DG, LaughlinGA, Schneider DL, Sartoris DJ (2000) Low levels of estradiol are associated with vertebral fractures in older men: The Rancho Bernardo Study. J Clin Endocrinol Metab 85:219–223

Basaria S, Lieb II J, Tang AM, DeWeese T, Carducci M, Eisenberger M, Dobs AS (2002) Long-term effects of androgen deprivation therapy in prostate cancer patients. Clin Endocrinol 56:779–786

Behre HM, Bohmeyer J, Nieschlag E (1994) Prostate volume in testosterone-treated and untreated hypogonadal men in comparison to age-matched normal controls. Clin Endocrinol 40:341–349

Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E (1997) Long term effect of testosterone therapy on bone mineral density in hypogonadal men. J Clin Endocrinol Metab 82:2386–2390

Belanger A, Locong A, Noel C, Cusan N, Dupont A, Prevost J, Caron S, Sevigny J (1989) Influence of diet on plasma steroids and plasma binding globulin levels in adult man. J Steroid Biochem 32:829–833

Bergendahl M, Aloi JA, Iranmanesh A, Mulligan TM, Veldhuis JD (1998) Fasting suppresses pulsatile luteinizing hormone (LH) secretion and enhances order lines of LH release in young but not in older men. J Clin Endocrinol Metab 83:1967–1975

Bhasin S, Tenover JS (1997) Age-associated sarcopenia – Issues in the use of testosterone as an anabolic agent in older men. J Clin Endocrinol Metab 82:1659–1660

Bhasin S, Buckwalter JG (2001) Testosterone supplementation in older men: a rational idea whose time has not yet come. J Androl 22:718–731

Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R (1996) The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. N Engl J Med 335:1–7

Bhasin S, Woodhouse L, Casabury R, Singh AB, Bhasin D, Berman N, Chen XH, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen RQ, Storer TW (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 281: E1172–E1181

Bhasin S, Singh AB, Mac RP, Carter B, Lee MI, Cunningham GR (2003) Managing the risk of prostate disease during testosterone replacement therapy in older men: recommendations for a standardized monitoring plan. J Androl 24:299–311

Bixler EO, Santen RJ, Kales A (1977) Inverse effects of thioridazine (Melleril) on serum prolactin and testosterone concentrations in normal men. In: Troen P, Nankin HR (eds) The testis in normal and infertile men. Raven, New York, pp 405–409

Blondeau JP, Baulieu EE, Robel P (1982)Androgen dependent regulation of androgen receptor in the rat ventral prostate. Endocrinology 110:1926–1932

Boonen S, Vanderschueren D, Xiao GC, Verbeke G, Dequeker J, Geusens P, Broos P, Bouillon R (1997) Age related (Type II) femoral neck osteoporosis in men: biochemical evidence for both hypovitaminosis D- and androgen deficiency-induced bone resorption. J Bone Miner Res 12:2119–2126

Bremner WJ, Vitiello MV, Prinz PN (1983) Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab 56:1278–1281

Bross R, Javanbakth M, Bhasin S (1999) Anabolic interventions for aging-associated sarcopenia. J Clin Endocrinol Metab 84:3420–3430

Buena F, Swerdloff RS, Steiner BS, Lutchmansingh P, Peterson MA, Pandian MR, Galmarini M, Bhasin S (1993) Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. Fertil Steril 59:1118–1123

Cameron JM, Weltzin TE, McConaha C, Helmreich DL, Kaye WH (1991) Slowing of pulsatile luteinizing hormone secretion in men after fortyeight hours of fasting. J Clin Endocrinol Metab 73:35–41

Chang TC, Tung CC, Hsiao YL (1994) Hormonal changes in elderly men with non-insulin dependent diabetes mellitus and the hormonal relationships to abdominal adiposity. Gerontology 40:260–267

Cicero TJ (1982) Alcohol induced defects in the hypothalamo-pituitary luteinizing hormone action in the male. Alcoholism 6:207–215

Cooper TG, Keck C, Oberdieck U, Nieschlag E (1993) Effects of multiple ejaculations after extended periods of sexual abstinence on total motile and normal sperm numbers as well as on accessory gland secretions from healthy normal and oligospermic men. Hum Reprod 8:1251–1258

Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Després JP, Bouchard C (2000) Contribution of body fatness and adipose tissue distribution to the age

variation in plasma steroid hormone concentration in men: the HERITAGE family study. J Clin Endocrinol Metab 85:1026–1031

Crawford BAL, Liu PY, Kean MT, Bleasel JF, Handelsman DJ (2003) Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. J Clin Endocrinol Metab 88:3167–3176

Dai WS, Gutai JP, Kuller LH, Cauley JA (1988) Cigarette smoking and serum sex hormones in men. Am J Epidemiol 128:796–805

Daniell HW (2002) Hypogonadism in men consuming sustaines-action oral opioids. J Pain 3:377–384

Davidson JM, Kwan M, Greenleaf WJ (1982) Hormonal replacement and sexuality in men. Clin Endocrinol Metab 11:594–623

Davidson JM, Chen JJ, Crapo L, Gray GD, Greenleaf WJ, Catania JA (1983) Hormonal changes and sexual function in aging men. J Clin Endocrinol Metab 57:71–77

Delhez M, Hansenne M, Legros J-J (2003) Andropause and psychopathology: minor symptoms rather than pathological ones. Psychoneuroendocrinology 28:863–874

de Lignieres B (1993) Transdermal dihydrotestosterone treatment of 'andropause'. Ann Med 25:235–241

Demoor P, Goossens JV (1970) An inverse corelation between body weight and the activity of the steroid binding globulin in human plasma. Steroidologia 1:129–136

Deslypere JP, Vermeulen A (1981) Aging and tissue androgens. J Clin Endocrinol Metab 53:430–434

Deslypere JP, Vermeulen A (1984) Leydig cell function in normal men: effect of age, lifestyle, residence, diet and activity. J Clin Endocrinol Metab 59:955–962

Deslypere JP, Vermeulen A (1985) Influence of age on steroid concentration in skin and striated muscle in women and in cardiac muscle and lung tissue in men. J Clin Endocrinol Metab 60:648–653

Deslypere JP, Sayed A, Punjabi U, Verdonck L, Vermeulen A (1982) Plasma 5α-androstane-3α,17-diol and urinary 5α-androstane, 3α, 17-diol glucuronide, parameters of peripheral androgen action: a comparative study. J Clin Endocrinol Metab 54:386–391

Deslypere JP, Kaufman JM, Vermeulen T, Vogelaers D, Vandalem JL, Vermeulen A (1987) Influence of age on pulsatile luteinizing hormone release and responsiveness of the gonadotrophs to sex hormone feedback in men. J Clin Endocrinol Metab 64:68–73

Devogelaer JP, De Cooman S, Nagant de Deux Chaisnes C (1992) Low bone mass in hypogonadal males. Effect of testosterone substitution therapy, a densitometric study. Maturitas 15: 17–23

Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA (1999) Pharmacokonetics, efficacy, and safety of a permeation-enhanced testosterone transdermal system in comparison with biweekly injections of testosterone enanthate for treatment of hypogonadal men. J Clin Endocrinol Metab 84:3469–3478

Dong Q, Hawker F, Mc William D, Bangah M, Burger H, Handelsman DJ (1992) Circulating immunoreactive inhibin and testosterone levels in men with critical illness. Clin Endocrinol 36:399–404

Dunbar N, Gruman Creisine S, Kenny A (2001) Comparison of two health status measures and their associations with testosterone levels in older men. Aging Male 4:1–7

Duranteau L, Chanson P, Blumberg-Tick J, Thomas G, Brailly S, Lubetzki J, Schaison G, Bouchard P (1993) Non-responsiveness of serum gonadotropins and testosterone to pulsatile GnRH in hemochromatosis suggesting a pituitary defect. Acta Endocrinol 128:351–354

Dutta C, Hadley EC (1995) The significance of sarcopenia in old age. J Gerontol 50A:1–4

Elewaut A, Barbier F, Vermeulen A (1979) Testosterone metabolism in normal males and male cirrhotics. Z Gastroenterol 17:402–405

Erfurth EM, Hagmar LE, Sääf M, Hall K (1996) Serum levels of insulin-like growth factor I and insulin-like growth factor binding protein 1 correlate with serum free testosterone and sex hormone binding globulin in healthy young and middle-aged men. Clin Endocrinol 44:659–664

Faber J, Perrild H, Johansen JS (1990) Bone Gla protein and sex hormone-binding globulin in nontoxic goiter: parameters for metabolic status at the tissue levels. J Clin Endocrinol Metab 70:49–55

Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S (2000) Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 106:1533–1560

Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ. McKinlay JB (1994) Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 151:54–61

Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB (2002) Age trends in the level of serum testosterone and other hormones in middle aged men: longitudinal results from the Massachusetts (Male aging study). J Clin Endocrinol Metab 87:589–598

Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR, Urban RJ (2002) Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. Am J Physiol Metab 282:E601–E607

Ferrando AA, Sheffield-Moore M, Paddon-Jones D, Wolfe RR, Urban RJ (2003) Differential anabolic effects of testosterone and amino acid feeding in older men. J Clin Endocrinol Metab 88:358–362

Ferrini LC, Barrett-Connor EL (1998) Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am J Epidemiol 147:750–754

Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB (1994) The relation of smoking, age, relative weight and dietary intake to serum adrenal steroids, sex hormones and sex hormone binding globulin in middle age men. J Clin Endocrinol Metab 79:1310–1316

Finkelstein JS, Klibanski A, Neer RM, Doppelt SH, Rosenthal DI, Segre RV, Crowley WF (1989) Increase in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 69:776–783

Finch PM, Roberts LJ, Price L, Hadlow NC, Pullan PT (2000) Hypogonadism in patients treated with intrathecal morphine. Clin J Pain 16:251–254

Forbes GB, Reina JC (1970) Adult lean body mass declines with age: some longitudinal observations. Metabolism 19:653–663

Friedl KE, Jones RE, Hannan CJ jr, Plymate SR (1989) The administration of pharmacological doses of testosterone or 19-nortestosterone to normal men is not associated with increased insulin secretion or impaired glucose tolerance. J Clin Endocrinol Metab 68:971–975

Friedl KE, Hannan CJ jr, Jones RE, Plymate SR (1990) High density lipoprotein cholesterol is not decreased if an aromatizable androgen is administered. Metabolism 39:69–74

Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K (2002) Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity and race. The CARDIA male hormone study. Canc Epidemiol Biom Prev 11:1041–1047

Giagulli VA, Vermeulen A (1992) Increased plasma 5α-androstane-3α, 17β-diol glucuronide concentration in clinically euthyroid women with suppressed plasma thyrotropin levels: further evidence for generalized tissue overexposure to thyroid hormones in these subjects. J Clin Endocrinol Metab 74:1465–1467

Giagulli VA, Kaufman JM, Vermeulen A (1994) Pathogenesis of decreased androgen levels in obese men. J Clin Endocrinol Metab 79:997–1000

Giusti G, Gonnelli P, Borreli D, Fiorelli G, Forti G, Pazzagli M, Serio M (1975) Age related secretion of androstenedione, testosterone and dihydrotestosterone by the human testes. Exp Gerontol 10:241–245

Goldenberg SL, Bruchowsy N, Gleave ME, Sullivan LD, Akakura K (1995) Intermittent androgen suppression in the treatment of prostate cancer. Urology 45:839–845

Gooren LJG (1987) Androgen levels and sex functions in testosterone treated hypogonadal men. Arch Sex Beh 16:463–476

Gordon D, Beastall GH, Thomson JA, Sturrock RD (1988) Prolonged hypogonadism in male patients with rheumatoid arthritis during flares in disease activity. Brit J Rheumatol 27:440–444

Gray A, Berlin JA, McKinlay JB, Longcope C (1991) An examination of research design effects on the association of testosterone and male aging. Results of a meta-analysis. J Clin Epidemiol 44:671–684

Gruenewald DA, Matsumoto AM (2003) Testosterone supplementation therapy for older men: potential benefits and risks. J Am Geriatr Soc 51:101–115

Grunstein RR, Handelsman DJ, Lawrence SJ, Blackwell C, Caterson ID, Sullivan CE (1989) Neuroendocrine dysfunction in sleep apnea: reversal by nasal continuous positive airway pressure. J Clin Endocrinol Metab 68:352–358

Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB (1995) Lower-extremity function in persons over 70 years as a predictor of subsequent disability. N Engl J Med 322:556–561

Haffner SM, Katz MS, Stern MP, Dunn JF (1988) The relationship of sex hormones to hyperinsulinemia and hyperglycemia. Metabolism 37:683–688

Haffner SM, Mykkänen L, Valdez RA, Stern MP, Katz MS (1993) Relationship of sex hormones to lipids and lipoproteins in non-diabetic men. J Clin Endocrinol Metab 77:1610–1615

Haffner SM, Valdez RA, Mykkänen L, Stern MP, Katz MS (1994) Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associated with increased glucose and insulin concentrations in non-diabetic men. Metabolism 43:599–603

Haffner SM, Miettinen H, Karhapaa L, Laakso M (1997) Leptin concentrations, sex hormones and cortisol in non diabetic men. J Clin Endocrinol Metab 82:1807–1809

Hajjar RR, Kaiser FE, Morley JE (1997) Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. J Clin Endocrinol Metab 82:3793–3796

Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA (2002) Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. J Clin Endocrinol Metab 87:3632–3639

Häkkinen K, Pakarinen A (1994) Serum hormones and strength development during strength training in middle-aged and elderly males and females. Acta Physiol Scand 150:211–219

Handelsman DJ (1994) Testicular dysfunction in systemic disease. Endocrin Metab Clin 23:839–852

Handelsman DJ, Dong Q (1993) Hypothalamo-pituitary-gonadal axis in chronic renal failure. Endocrin Metab Clin 22:145–161

Harbitz TB (1973) Morphometric studies of Leydig cells in elderly men, with special reference to the histology of the prostate. Acta Pathol Microbiol Scand 81:301–314

Harman SM, Tsitouras PD (1980) Reproductive hormones in aging men I. Measurment of sex steroids, basal luteinizing hormone and Leydig cell response to human chorionic gonadotropin. J Clin Endocrinol Metab 51:35–40

Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR (2001) Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J Clin Endocrinol Metab 86:724–730

Harkonen K, Huhtaniemi I, Makinen J, Hubler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F, Pollanen P (2003) The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 26:187–194

Heald AH, Ivison F, Anderson SG, Cruickshank K, Laing I, Gibson JM (2003) Significant ethnic variation in total and free testosterone concentration. Clin Endocrinol 58:262–266

Heinemann L, Zimmermann T, Vermeulen A, Thiel C, Hummel W (1999) A new 'aging males' symptoms' rating scale. Aging Male 2:105–114

Hollander N, Hollander VP (1958) The microdetermination of testosterone in human spermatic vein blood. J Clin Endocrinol Metab 18:966–970

Ida Y, Tsyjimaru S, Nakamura K, Shirao I, Mukasa H, Egarni H, Nakazawa H (1992) Effect of acute and repeated alcohol ingestion on hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal functioning in normal males. Drug Alcohol Dep 31:57–64

Impallomeni M, Kaufman BM, Palmer AJ (1994) Do acute diseases transiently impair anterior pituitary function in patients over the age of 75? A longitudinal study of the TRH test and basal gonadotrophin levels. Postgrad Med J 70:86–91

Irwin M, Dreyfus E, Baird D S, Smith T, Schuckit M (1988) Testosterone in chronic alcoholic men. Br J Addic 83:949–953

Ishimaru T, Edmiston WA, Pages L, Horton K (1978a) Splanchnic extraction and conversion of testosterone and dihydrotestosterone in man. J Clin Endocrinol Metab 46:528–533

Ishimaru T, Edmiston WA, Pages L, Horton R (1978b) Direct conversion of testosterone in dihydrotestosterone glucuronide in man. J Clin Endocrinol Metab 47:1282–1286

Jackson JA, Waxman J, Spiekerman M (1989)Prostatic implications of testosterone replacement therapy. Arch Intern Med 149:2364–2366

Jackson JA, Riggs MW, Spiekerman M (1992) Testosterone deficiency as a risk factor for hip fracture in men: A case control study. Am J Med Sci 304:4–8

Jain P, Rademaker AW, MC Vary KT (2000) Testosterone supplementation for erectile dysfunction: results of a meta-analysis. J Urol 164:371–375

Kaiser FE (1992) Impotence in the elderly. In: Morley J, Krenman P (eds) Endocrinology and metabolism in the elderly. Cambridge-Blackwell, Cambridge, pp 262–271

Kamischke A, Kemper D, Castel M, Luthke M, Rolf C, Behre HM, Magnussen H, Nieschlag E (1998) Testosterone levels in men with chronic obstructive pulmonary disease with or without glucocorticoid therapy. Eur Resp J 11:41–45

Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A (1996) Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 81:4358–4365

Kaufman JM, Vermeulen A (1997) Declining gonadal function in elderly men. Baillière's Endocrinol Metab 11:289–309

Kaufman JM, Deslypere JP, Giri M, Vermeulen A (1990) Neuroendocrine regulation of pulsatile luteinizing hormone secretion in elderly men. J Steroid Biochem 37:421–430

Kaufman JM, Giri M, Deslypere JP, Thomas G, Vermeulen A (1991) Influence of age on the responsiveness of the gonadotrophs to luteinizing hormone-releasing hormone in males. J Clin Endocrinol Metab 72:1255–1260

Kaufman JM, Johnell O, Abadie E, Adami S, Audran M, Avouac B, Ben Sedrine W, Calvo G, Devogelaer JP, Fuchs V, Kreutz G, Nilsson P, Pols H, Ringe J, Van Haelst L, Reginster JY (2000) Background for studies on the treatment of male osteoporosis: state of the art. Ann Rheum Dis 59:765–772

Kaufman JM, Zmierczak H, Goemaere S (2001) Osteoporosis in the aging population the male perspective. Aging Male 4: 62–73

Kelly TM, Edwards CQ, Meikle AW, Kushner JP (1984) Hypogonadism in haemochromatosis – Reversal with iron depletion. Ann Intern Med 101:629–632

Kenny AM, Prestwood KM, Gruman CA, Marcello KM, Raisz LG (2001) Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci 56: M266–M272

Kent JZ, Acone AB (1966) Plasma androgens and aging. In: Vermeulen A, Exley D (eds) Androgens in normal and pathological conditions. Excerpta Medica Foundation, Amsterdam, pp 31–35

Key T, Roe L, Thorogood M, More JW, Clark MC, Wang DY (1990) Testosterone, sex hormone binding globulin, calculated free testosterone and oestradiol in male vegetarians and omnivores. Br J Nutr 64:111–119

Khaw KT, Barrett-Connor EL (1992) Lower endogenous androgens predict central obesity in men. Ann Epidemiol 2:675–682

Khosla S, Melton LJ III, Atkinson EJ, O'Fallon WM (2001) Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocrinol Metab 86:3555–3561

Kligman EW (1991) Office evaluation of sexual function and complaints. Clin Ger Med 7:25–39

Korenman SG, Morley JE, Mooradian AD, Davis SS, Kaiser FE, Silver AJ, Viosca SP, Garza D (1990) Secondary hypogonadism in older men: its relation to impotence. J Clin Endocrinol Metab 71:963–969

Krithivas K, Yurgalevitch SM, Mohr BA, Wilcox EJ, Batter SJ, Brown M, Longcope C, McKinlay JB, Kantoff PW (1999) Evidence that CAG repeat in the androgen receptor gene is associated with the age related decline in serum androgen levels in men. J Endocrinol 162:137–142

Kunelius P, Lukkarinen O, Hannuksela ML, Itkonen O, Tapanainen JS (2002) The effects of transdermal dihydrotestosterone in the aging male: a prospective, randomized, double blind study. J Clin Endocrinol Metab 87:1467–1472

Labrie F, Bélanger A, Simard J, Van Luu-The, Labrie C (1995) DHEA and peripheral androgen and estrogen formation: intracrinology. Ann NY Acad Sc 774:16–28

Lichtenstein MJ, Yarnell JWG, Elwood PC, Beswick AD, Sweetnam PM,Marks V, Teale D, Riad-Fahmy D (1987) Sex hormones, insulin, lipid and prevalent ischemic heart disease. Am J Epidemiol 126:647–657

Longcope C (1973) The effect of human chorionic gonadotropin on plasma steroid levels in young and old men. Steroids 21:583–592

Longcope C, Feldman HA, McKinlay JB, Araujo AB (2000) Diet and sex hormone-binding globulin. J Clin Endocrinol Metab 85:293–296

Luboshitzky R, Aviv A, Hefetz A, Herer P, Shen-Orr Z, Lavie L, Lavie P 2002 Decreased pituitary-gonadal secretion in men with obstructive sleep apnea. J Clin Endocrinol Metab 87:3394–3398

Luboshitzky, Shen-Orr Z, Herer P (2003) Middle-aged men secrete less testosterone at night than young healthy men. J Clin Endocrinol Metab 88:3160–3166

Lumbroso S, Lobaccaro JM, Vial C, Sassolas G, Ollagnon B, Belon C, Pouget J, Sultan C (1997) Molecular analysis of the androgen receptor gene in Kennedy's disease. Depart of two families and review of the literature. Horm Res 47:23–29

Luria MH, Johnson MW, Pego R, Seuc CA, Manubens SJ, Wieland MR, Wieland RG (1982) Relationship between sex hormones, myocardial infarction and occlusive coronary disease. Arch Intern 142:42–44

Luton JP, Thieblot P, Valcke JC, Mahoudeau JA, Bricaire H (1977) Reversible gonadotropin deficiency in male Cushing's disease. J Clin Endocrinol Metab 45:488–495

Ly LP, Jiminez M, Zhuang TN, Celermajer DS, Conway AJ, Handelsman DJ (2001) A double-blind, placebo-controlled, randomized clinical trial of transdermal dihydrotestosterone gel on muscular strength, mobility, and quality of life in older men with partial androgen deficiency. J Clin Endocrinol Metab 86:4078–4088

MacAdams MR, White RH, Chipps BE (1986) Reduction of serum testosterone levels during chronic glucocorticoid therapy. Ann Intern Med 104:648–651

Mahmoud AM, Goemaere S, De Bacquer D, Comhaire FH, Kaufman JM (2000) Serum inhibin B levels in community-dwelling elderly men. Clin Endocrinol 53:141–147

Mahmoud AM, Goemaere S, El-Garem Y, Van Pottelbergh I, Comhaire FH, Kaufman JM (2003) Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. J Clin Endocrinol Metab 88:179–184

Marin P, Holmäng S, Jönsson L, Sjöström L, Kvist H, Holm G, Lindstedt G, Björntorp P (1992) The effects of testosterone treatment on body composition and metabolism in middle aged men. Int J Obesity 16:991–997

Marin P, Holmäng S, Gustafsson C, Jönsson L, Kvist H, Elander A, Eldh J, Sjöström L, Holm G, Björntorp P (1993) Androgen treatment of abdominally obese men. Obesity Res 1:245–251

Marin P, Lönn L, Anderson B, Oden B, Olbe L, Bengtsson BA, Björntorp P (1996) Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. J Clin Endocrinol Metab 81:1018–1022

Martin CE (1975) Marital and sexual factors in relation to age, disease and longevity. In: Wirdt RD, Winokur G, Ruff M (eds) Life history research in psychopathology, University of Minnesota Press, Minneapolis, pp 326–347

Masters WH (1986) Sex and aging – Expectations and reality. Hosp Pract 15:175–198

Matsumoto AM, Sandblom RE, Schoene RB, Lee KA, Giblin EC, Pierson DJ,Bremner WJ (1985) Testosterone replacement in hypogonadal men: effects on obstructive sleep apnoea, respiratory drives, and sleep. Clin Endocrinol 22:713–721

Matzkin H, Braf Z, Nava D (1991) Does age influence the bioactivity of follicle-stimulating hormone in men. Age and Ageing 20:199–205

Mauras N, Hayes VY, Vieira NE, Yergey AL, O'Brien KO (1999) Profound hypogonadism has significant negative effects on calcium balance in males: a calcium kinetic study. J Bone Miner Res 14:577–582

McKenna TJ, Lorber D, Lacroix A, Rabin D (1979) Testicular activity in Cushing's disease. Acta Endocrinol 91:501–510

Meier DE, Orwoll ES, Keenan EJ, Fagerstrom RM (1987) Marked decline in trabecular bone mineral content in healthy men with age: lack of association with sex steroid levels. J Am Geriatr Soc 35:189–197

Meikle AW, Bishop DT, Stringham JD, West DW (1986) Quantitating genetic and non genetic factors to determine plasma sex steroid variation in normal male twins. Metabolism 35:1090–1095

Meikle AW, Stringham JD, Bishop T, West DW (1988) Quantitation of genetic and non genetic factors influencing androgen productions and clearance rates in men? J Clin Endocrinol Metab 67:104–109

Meikle AW, StringhamJD, Woodward MG, McMurry MP (1990) Effects of fat containing meal on sex hormones in men. Metabolism 39: 943–946

Mendoza SG, Zerpa A, Carrasco H, Colmenares O, Rangel A, Gardside PS, Kashyap ML (1983) Estradiol, testosterone, apolipoproteins, apolipoprotein cholesterol and lipolytic enzymes in men with premature myocardial infarction and angiographically assessed coronary occlusion. Artery 2:1–23

Mikuma N, Kumamoto Y, Maruta H, Nitta T (1994) Role of the hypothalamic opioidergic system in the control of gonadotropin secretion in elderly men. Andrologia 26:39–45

Mitchell R, Hollis S, Rothwell C, Robertsons WR (1995) Age related changes in the pituitary testicular axis in normal men; lower serum testosterone results from decreased bioactive LH drive. Clin Endocrinol 42:501–507

Mittan D, LEE S, Miller E, Perez RC, Basler JW, Bruder JM (2002) Bone loss following hypogonadism in men with prostate cancer treated with GnRH analogs. J Clin Endocrinol Metab 87:3656–3661

Morales A (1999) Andropause, androgen therapy and prostate safety. Aging Male 2:81–86

Morley JE (1986) Impotence. Am J Med 80:897–906

Morley JE, Perry HM, Kaiser FE, Kraenzle D, Jensen J, Houston K, Mattammel M, Perry HM (1993) Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. J Am Geriatr Soc 41:149–152

Morley JE, Kaiser FE, Perry HM III, Patrick P, Morley PM, Stauber PM, Vellas B, Baumgartner RN, Garry PJ (1997) Longitudinal changes in testosterone, luteinizing hormone and follicle stimulating hormone in healthy older men. Metabolism 46:410–413

Morley JE, Charlton E, Patrick P, Kaiser FE, Cadeau P, McCready D, Perry HM III (2000) Validation of a screening questionnaire for androgen deficiency in aging males. Metabolism 49:1239–1242

Morley JE, Patrick P, Perry HM III (2002) Evaluation of assays available to measure free testosterone. Metabolism 51:554–559

Muller M, Grobbee DE, Thijssen JHH, van den Beld AW, van der Schouw YT (2003) Trends Endocrinol Metab 14:289–296

Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD (1995) Amplified nocturnal luteinizing hormone (LH) secretory burst frequency with selective attenuation of pulsatile (but not basal) testosterone secretion in healthy aging men: possible Leydig cell desensitization to endogenous LH signaling-a clinical research center study. J Clin Endocrinol Metab 80:3025–3031

Mulligan T, Iranmanesh A, Kerzner R, Demers LW, Veldhuis JD (1999) Two-week pulsatile gonadotropin releasing hormone infusion unmasks dual (hypothalamic and Leydig cell) defects in the healthy aging male gonadotropic axis. Europ J Endocrinol 141:257–266

Mulligan T, Iranmanesh A, Veldhuis JD (2001) Pulsatile iv infusion of recombinant human LH in leuprolide-suppressed men unmasks impoverished Leydig-cell secretory responsiveness to midphysiological LH drive in the aging male. J Clin Endocrinol Metab 86:5547–5553

Münzer T, Haeman MS, Hees P, Shapiro E, Christmas C, Bellantoni MF, Stevens TE, O'Connor KG, Pabst KM, Clair C Sr, Sorkin JD, Blackman MR (2001) Effects of GH and/or sex steroid administration on abdominal subcutaneous and visceral fat in healthy aged women and men. J Clin Endocrinol Metab 86:3604–3610

Naftolin E, Judd JH, Yen SSC (1973) Pulsatile pattern of gonadotropin and testosterone in men. Effects of clomiphene with and without testosterone. J Clin Endocrinol Metab 36:285–288

Nankin HR, Lin T, Murono EP, Osterman J (1981) The aging Leydig cell III. Gonadotropin stimulation in men. J Androl 2:181–189

Neaves WB, Johnson L, Porter JC, Parker CR, Petty CS (1984) Leydig cell numbers, daily sperm production and gonadotropin levels in aging men. J Clin Endocrinol Metab 59:756–763

Nieschlag E, Kley KH, Wiegelmann W, Solback HG, Krüskemper HL (1973) Lebensalter und endokrine Funktion der Testes des erwachsenen Mannes. Deut Med Wochenschr 98:1281–1284

Nieschlag E, Lammers U, Freischem CW, Langer K, Wickings EJ (1982) Reproductive functions in young fathers and grandfathers. J Clin Endocrinol Metab 55:676–681

Nilsson P, Møller L, Solstad K (1995) Adverse effects of psychosocial stress on gonadal function and insulin levels in middle-aged males. J Intern Med 237:479–486

Opstad PR (1992) The hypothalamo-pituitary regulation of androgen secretion in young men, after prolonged physical stress combined with energy and sleep deprivation. Acta Endocrinol 127:231–236

Orwoll ES, Klein RF (1995) Osteoporosis in men. Endocr Rev 16:87–115

Orwoll ES, Oviatt S (1992) Transdermal testosterone supplementation in normal older men. Program of the 74th Annual Meeting of the Endocrine Society. The Endocrine Society, Bethesda, abstract 1071

Perry PJ, Lund BC, Arndt S, Holman T, Bever-Stille KA, Paulsen J, Demers LM (2001) Bioavailable testosterone as a correlate of cognition, psychological status, quality of life, and sexual function in aging males: implications for testosterone replacement therapy. Ann Clin Psychi 13:75–80

Pfeiffer E (1974) Sexuality in the aging individual. Arch Sex Behaviour 22:481–484

Pfeilschifter J, Scheidt-Nave C, Leidig-Bruckner G, Woitge HW, Blum WF, Wüster C, Haack D, Ziegler R (1996) Relationship between circulating insulin-like growth factor components and sex hormones in a population based sample of 50–80 year old men and women. J Clin Endocrinol Metab 81:2534–2540

Phillips GB (1978) Sex hormones, risk factors and cardiovascular disease. Am J Med 65:7–11

Phillips GB, Pinkernell BJ, Jing TY (1994) The association of hypotestosteronemia with coronary heart disease. Arterioscler Thromb 14:701–706

Pincus SM, Veldhuis JD, Mulligan T, Iranmanesh A, Evans WS (1997) Effects of age on the irregularity of LH and FSH serum concentrations in women and men. Am J Physiol 273:E989–E995

Pirke KM, Sintermann R, Vogt HJ (1980) Testosterone and testosterone precursors in the spermatic vein and in the testicular tissue of old men. Gerontology 26:221–230

Plant M (1986) Gonadal regulation of hypothalamic gonadotropin-releasing hormone release in primates. Endocr Rev 7:75–88

Plymate JR, Marej LA, Jones RE, Friedl KE (1988) Inhibition of sex hormone binding globulin production in human hepatoma (Hep G2) cell-line by insulin and prolactin. J Clin Endocrinol Metab 67:460–467

Plymate SR, Tenover JS, Bremner WJ (1989) Circadian variation in testosterone, sex hormone-binding globulin, and calculated non sex hormone-binding globulin bound testosterone in healthy young and elderly men. J Androl 10:366–371

Poggi UI, Arguelles AE, Rosner J, de Laborde NP, Cassini JH, Volmer MC (1976) Plasma testosterone and serum lipids in male survivors of myocardial infarction. J Steroid Biochem 7:229–231

Rajfer JK, Namkun PC, Petra PH (1989) Identification, partial characterization and age associated changes of a cytoplasmatic androgen receptor in the rat penis. J Steroid Biochem 33:1489–1492

Reed MJ, Cheng RW, Simmonds M, Richmond W, James VHT (1987) Dietary lipids: an additional regulator of plasma levels of sex hormone binding globulin. J Clin Endocrinol Metab 64:1083–1085

Rhoden EL, Teloken C, Mafessoni R, Souto CA (2002) Is there any relation between serum levels of total testosterone and the severity of erectile dysfunction? Int J Impot Res 14:167–171

Riggs BL, Khosla S, Melton LJ III (2002) Sex steroids and the construction and conservation of the adult skeleton. Endocr Reviews 23:279–302

Roehrborn CG, Lange JL, George FW, Wilson JD (1987) Changes in amount and intracellular distribution of androgen receptor in human foreskin as a function of age. J Clin Invest 79:44–47

Rogers MA, Evans WJ (1993) Changes in skeletal muscle with aging: effects of exercise training. Exercise Sports Sci Rev 21:65–102

Rolf C, Behre M, Nieschlag E (1996) Reproductive parameters of older compared to younger men of infertile couples. Int J Androl 19:135–142

Rubens R, Dhont M, Vermeulen A (1974) Further studies on Leydig cell function in old age. J Clin Endocrinol Metab 39:40–45

Rubenstein LZ, Josephson KR, Robbins AS (1994) Falls in the nursing home. Ann Intern Med 121:442–451

Sandblom RE, Matsumoto AM, Scoene RB, Lee KA, Giblin EC, Bremner WJ, Pierson DJ (1983) Obstructive sleep apnea induced by testosterone administration N Engl J Med 308:508–510

Sasano N, Ichijo S (1969) Vascular patterns of the human testes with special reference to its senile changes. Tohoku J Exp Med 99:269–280

Schiavi RC (1996) Androgens and sexual function in men. In: Oddens B, Vermeulen A (eds). Androgens and the aging male. Parthenon Publishing Group, New York, pp 111–128

Schiavi RC, Schreiner-Engel P, White D, Mandeli J (1988) Pituitary-gonadal function during sleep in men with hypoactive sexual desire and in normal controls. Psychosom Med 50:304–318

Schiavi RC, Schreiner-Engel P, Mandeli J, Schanzer H, Cohen E (1990) Healthy aging and male sexual function. Am J Psychiatry 147: 776–771

Schröder FH (1996) The prostate and androgens: the risk of supplementation. In: Oddens B, Vermeulen A (eds) Androgens and the aging male. Parthenon Publishing Group, New York, pp 223–26

Semple PD, Beastall GH, Watson WS, Hume R (1981) Hypothalamic-pituitary dysfunction in respiratory hypoxia. Thorax 36:605–609

Semple PD, Beastall GH, Brown TM, Stirling KW, Mills RJ, Watson WS (1984) Sex-Hormone Suppression and Sexual Impotence in Hypoxic Pulmonary Fibrosis. Thorax 39:46–51

Sewdarsen M, Vythilingum S, Jiadal I, Desai RK, Becker P (1990) Abnormalities in sex hormones are a risk factor for premature manifestation of coronary artery disease in South African Indian men. Atherosclerosis 83:111–117

Siiteri PK, MacDonald PC (1975) Role of extraglandular estrogen in human endocrinology. In: Greep RD, Astwood B (eds) Handbook of physiology. Vol II. American Physiological Society, pp 491–508

Sih R, Morley JE, Kaiser FE, Perry HM III, Patrick P, Ross C (1997) Testosterone replacement in older hypogonadal men: a 12 month randomized controlled trial. J Clin Endocrinol Metab 82**:**1661–1667

Simon D, Nahoul K, Charles MA (1996)Sex hormones, ageing, ethnicity and insulin sensitivity in men: an overview of the TELECOM study. In: Oddens B, Vermeulen A (eds) Androgens and the aging male. Parthenon Publishing Group, New York, pp 95–101

Smith KW, Gruman C, Reisine S, Kenny A (2000) Construction and field validation of a self-administered screener for testosterone deficiency (hypogonadism) in aging men. Clin Endocrinol (Oxford) 53:703–711

Sniffen RC (1950) The testes. I. The normal testis. Arch Pathol 50:259–284

Snyder PJ, Lawrence DA (1980) Treatment of hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 51:1335–1339

Snyder PJ, Peachey H, Berlin JA, Hannoush P, Berlin JA, Loh L, Holmes JH, Dlewati A, Staley J, Santanna J, Kapoor SC, Attie MF, Haddad JG Jr, Strom BL (1999a) Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 84:1966–1972

Snyder PJ, Peachey H, Berlin JA, Hannoush P, Berlin JA, Loh L, Lendrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL (1999) Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 84:2647–2653

Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A, Santanna J, Loh L, Lenrow DA, Holmes JH, Kapoor SC, Atkinson LE, Strom BL (2000) Effects of testosterone replacement in hypogonadal men. J Clin Endocrinol Metab 85:2670–2677

Sparrow D, Silbert JE, Rowe JW (1980) The influence of age, alcohol consumption, and body build on gonadal function in men. J Clin Endocrinol Metab 51:508–512

Spratt DI, O'Dea L, Schoenfeld D, Butler J, Narashimha H, Rao P, Crowley WF (1988) Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH and testosterone. Am J Physiol 254:E658–E666

Spratt DI, Cox P, Orav J, Moloney J, Bigos T (1993) Reproductive axis suppression in acute illness is related to disease severity. J Clin Endocrinol Metab 76:1548–1554

Stanley HL, Schmitt BP, Poses RM, Deiss WP (1991) Does hypogonadism contribute to the occurrence of a minimal trauma hip fracture in elderly men? J Am Geriatr Soc 39:766–771

Steidle C, Schwartz S, Jacoby K, Sebree T, Smith T, Bachand R (2003) AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. J Clin Endocrinol Metab 88:2673–2681

Stepan JJ, Lachman M, Zverina J, Pacovsky V, Baylink DJ (1989) Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab 69:523–527

Stoch SA, Parker RA, Chen L, Bubley G, Ko Y-J, Vincelette A, Greenspan SL (2001) Bone loss in men with prostate cancer treated with gonadotropin-releasing hormone agonists. J Clin Endocrinol Metab 86:2787–2791

Suoranta H (1971) Changes in small vessels of the adult testes in relation to age and some pathological conditions. Virchows Arch: Pathological anatomy and histopathology 352:765–781

Swartz CM, Young MA (1987) Low serum testosterone and myocardial infarction in geriatric male patiens. J Am Geriatr Soc 35:39–44

Swerdloff RS, Wang C (1993) Androgens and aging in men. Exp Gerontol 28:435–446

Tapia-Serrano R, Jimenez-Baldera FJ, Murrieta S, Bravo-Gatica C, Guerra R, Mintz G (1991) Testicular function in active ankylosing spondylitis – Therapeutic response to human chorionic gonadotrophin. J Rheumatol 18:841–848

Tenover JS (1992) Effect of testosterone supplementation in the aging male. J Clin Endocrinol Metab 75:1092–1098

Tenover JS (1994) Androgen administration to aging men. Endocrinol Metab Clin 23:877–889

Tenover JS (1996) Effect of androgen supplementation in the aging male in: Oddens B, Vermeulen A (eds) Androgens and the aging male. Parthenon Publish Group pp 191–221

Tenover JS, Matsumoto AM, Plymate SR, Bremner WJ (1987) The effects of aging in normal men on bioavailable testosterone and luteinizing hormone secretion: response to clomiphene citrate; J Clin Endocrinol Metab 65:1118–1126

Tenover JS, Matsumoto AM, Clifton DK, Bremmer WJ (1988) Age related alterations in the circadian rhythms of pulsatile luteinizing hormone and testosterone secretion in healthy men. J Gerontol 43:M163–M169

Theorell T, Karasek RA, Eneroth P (1990) Job strain variation in relation to plasma testosterone in working men – a longitudinal study. J Intern Med 227:31–36

Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA, Jr. (2003) The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224

Tsitouras PD, Martin CE, Harman SM (1982) Relation of serum testosterone to sexual activity in healthy elderly men. J Gerontol 37:288–293

Tsitouras PD and Bulat T (1995) The aging male reproductive system. Endocrinol Metab Clin 24:297–315

T'Sjoen G, Goemaere S, De Meyere M, Kaufman JM (2004) Perception of males' aging symptoms, health and well-being in elderly community-dwelling men is not related to circulating androgen levels. Psychoneuroendocrinology 29: 201–214

Turner HE and Wass JAH (1997) Gonadal function in men with chronic illness. Clin Endocrinol 47:379–403

Udry JR, Billy JO, Morris NM, Groff TR, Raj MH (1985) Serum androgenic hormones motivate normal behavior in adolescent boys. Fertil Steril 43:90–94

Urban RJ, Veldhuis JD, Blizzard R, Dufau ML (1988) Attenuated release of biologically active luteinizing hormone in healthy aging men. J Clin Invest 81:1020–1029

Urban RJ, Bodenburg Y, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, Ferrando A (1995) Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am J Physiol 269:E820–E826

Van den Berghe G, Weekers F, Baxter RC, Wouters P, Iranmanesh, Bouillon R, Veldhuis JD (2001) Five-day pulsatile gonadotropin-releasing hormone administration unveils combined hypothalamic-pituitary-gonadal defects underlying profound hypoandrogenism in men with prolonged critical illness. J Clin Endocrinol Metab 86:3217–3226

Van Den Saffele JK, Goemaere S, De Bacquer D, Kaufman JM (1999) Serum leptin levels in healthy ageing men: are decreased serum testosterone and increased adiposity in elderly men the consequence of leptin deficiency? Clin Endocrinol 51:81–88

Van Der Klift M, De Laet CEDH, McCloskey EV, Hofman A, Pols HAP (2002) The incidence of vertebral fractures in men and women: The Rotterdam Study. J Bone Miner Res 17:1051–1056

Van Pottelbergh I, Lumbroso S, Goemaere S, Sultan C, Kaufman JM (2001) Lack of influence of the androgen receptor gene, CAG repeat polymorphism on sex steroid status and bone metabolism in elderly men. Clin Endocrinol 55:659–666

Van Pottelbergh I, Goemaere S, Kaufman JM (2003) Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J Clin Endocrinol Metab 88:3075–3081

Veldhuis JD, King JC, Urban RJ, Rogol LD, Evans WS, Kolp LA, Johnson ML (1987) Operating characteristics of the male hypothalamo-pituitary-gonadal axis. Pulsatile release of testosterone and follicle stimulating hormone and their temporal coupling with luteinizing hormone. J Clin Endocrinol Metab 68:929–947

Veldhuis JD, Urban RJ, Lizarralde G, Johnson ML, Iranmanesh A (1992) Attenuation of luteinizing hormone secretory burst amplitude as a proximate basis for the hypoandrogenism of healthy aging men. J Clin Endocrinol Metab 75:707–713

Veldhuis JD, Wilkowski MJ, Zwart AD, Urban RJ, Lizarralde G, Iranmanesh A, Bolton WK (1993) Evidence for attenuation of hypothalamic gonadotropin-releasing hormone (GnRH) impulse strength with preservation of GnRH pulse frequency in men with chronic renal failure. J Clin Endocrinol Metab 76:648–654

Veldhuis JD, Iranmanesh A, Mulligan T, Pincus SM (1999) Disruption of the young-adult synchrony between luteinizing hormone release and oscillations in follicle-stimulating hormone, prolactin and nocturnal penile tumescence (NPT) in healthy older men. J Clin Endocrinol Metab 84:3498–3505

Veldhuis JD, Iranmanesh A, Godschalk M, Mulligan T (2000). Older men manifest multifold synchrony disruption of reproductive neurohormone outflow. J Clin Endocrinol Metab 85:1477–1486

Veldhuis JD, Zwart A, Mulligan T, Iranmanesh A (2001) Muting of androgen negative feedback unveils impoverished gonadotropin-releasing hormone/luteinizing hormone secretory reactivity in healthy older men. J Clin Endocrinol Metab 86:529–535

Vermeulen A (1976) Testicular hormonal secretion and aging in males. In: Grayhack St, Wilson J, Scherbenski MJ (eds) Benign prostatic hyperplasia. DHEW Publications (NIH), Bethesda, pp 177–182

Vermeulen A (1991) Androgens in the aging male–Clinical review 24. J Clin Endocrinol Metab 73:221–224

Vermeulen A (2001) Androgen replacement therapy in the aging male – a critical evaluation. J Clin Endocrinol Metab 86: 2380–2390

Vermeulen A, Deslypere JP (1986) Intratesticular unconjugated steroids in elderly men. J Steroid Biochem 24:1079–1083

Vermeulen A, Kaufman JM (1992) Role of the hypothalamo-pituitary function in the hypoandrogenism of healthy aging. J Clin Endocrinol Metab 74:1226A-1226C

Vermeulen A, Kaufman JM (2002) Diagnosis of hypogonadism in the aging male. Aging Male 5:170–176

Vermeulen A, Verdonck G (1992) Representativeness of a single point plasma testosterone level for the long term hormonal milieu in men. J Clin Endocrinol Metab 74:939–942

Vermeulen A, Stoica T, Verdonck L (1971) The apparent free testosterone concentration, an index of androgenicity. J Clin Endocrinol Metab 33:759–767

Vermeulen A, Rubens R, Verdonck L (1972) Testosterone secretion and metabolism in male senescence. J Clin Endocrinol Metab 34:730–735

Vermeulen A, Deslypere JP, Kaufman JM (1989a) Influence of antiopioids on luteinizing hormone pulsatility in aging men. J Clin Endocrinol Metab 68:68–72

Vermeulen A, Giagulli VA, De Schepper P, Buntinx A, Stoner E (1989b) Hormonal effects of an orally active 4-azasteroid inhibitor of 5 alpha-reductase in humans. Prostate 14:45–53

Vermeulen A, Kaufman JM, Deslypere JP, Thomas G (1993) Attenuated LH pulse frequency and its relation to plasma androgens in hypogonadism of obese men. J Clin Endocrinol Metab 76:1140–1146

Vermeulen A, Kaufman JM, Giagulli VA (1996) Influence of some biological indices on sex hormone binding globulin and androgen levels in aging and obese males. J Clin Endocrinol Metab 81:1821–1827

Vermeulen A, Goemaere S, Kaufman JM (1999a) Sex hormones, body composition and aging. The Aging Male 2:8–16

Vermeulen A, Verdonck L, Kaufman JM (1999b) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672

Verwoerdt A, Pfeiffer E, Wangh AS (1969) Sexual behaviour in senescence. Geriatrics 24:137–154

Virag R, Bocully P, Frydman D (1985) Is impotence an arterial disorder? A study of arterial risk factors in 440 impotent men. Lancet i:181–183

Wallace EM, Pye SD, Wild ST, Wu FCW (1993)Prostate specific antigen and prostate gland size in men receiving exogenous testosterone for male contraception. Int J Androl 16:35–40

Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. J Clin Endocrinol Metab 85:2839–2853

Wang C, Chan V, Tse TF, Yeung RTT (1978a) Effect of acute myocardial infarction on pituitary-testicular function. Clin Endocrinol 9:249–253

Wang C, Chan V, Yeung RTT (1978b) Effect of surgical stress on pituitary testicular function. Clin Endocrinol 9:255–266

Wilson JD (1988) Androgen abuse by athletes. Endoc Rev 9:203–208

Winters SJ, Brufsky A, Weissfeld J, Trump DL, Dyky MA, Hadeed V (2001) Testosterone, sex hormone binding globulin and body composition in young adult African, American and Caucasian men. Metabolism 50:1242–1247

Winters SJ, Troen P (1982) Episodic luteinizing hormone (LH) secretion and the response of LH and follicle-stimulating hormone to LH-releasing hormone in aged men: evidence for coexistent primary testicular insufficiency and an impairment in gonadotropin secretion. J Clin Endocrinol Metab 55:560–565

Winters SJ, Sherins RJ, Troen P (1984) The gonadotropin suppressive activity of androgen is increased in elderly men. Metabolism 33:1052–1059

Winters SJ, Atkinson L for the Testoderm Study Group (1997) Serum LH concentrations in hypogonadal men during transdermal testosterone replacement through scrotal skin: further evidence that ageing enhances testosterone negative feedback. Clin Endocrinol 47:317–322

Woolf PD, Hamill RW, McDonald JV, Lee LA, Kelly M (1985) Transient hypogonadotropic hypogonadism caused by critical illness. J Clin Endocrinol Metab 60:444–450

Worstman J, Eagleton LE, Rosner W, Dufau ML (1987) Mechanism for the hypotestosteronemia of the sleep apnea syndrome. Am J Med Sci 293:221–225

Zitzmann M, Nieschlag E (2001) Testosterone levels in healthy men and their relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 144:183–219

Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001) The CAG repeat polymorphism in the AR gene affects bone density and bone metabolism in healthy males. Clin Endocrinol 55:649–657

Zitzmann M, Gromoll J, Von Eckardstein A, Nieschlag E (2003) The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. Diabetologia 46:31–39

Zmuda JM, Cauley JA, Kriska A, Glynn NW, Gutai JP, Kuller LH (1997) Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men: a 13-year follow-up of former multiple risk factor intervention trial participants. Am J Epidemiol 146:609–617

Zumoff B, Bradlow L, Finkelstein J, Boyar RM, Hellman L (1976) The influence of age and sex on the metabolism of testosterone. J Clin Endocrinol Metab 42:703–706

17

The pathobiology of androgens in women

N. Burger and P. Casson

Contents

17.1 Androgen dynamics in women

In women, androgens have been both celebrated and cursed as the hormones of "aggression and anger" and as "fuel for passion". In reality, while the effects of testosterone in men have been widely studied and a clear testosterone deficiency state identified, investigation into the role of testosterone in women is a far more recent venture that is only now yielding fruit. Until recently, circulating androgens in women have simply been considered either by-products of adrenal cortical or ovarian estrogen production, with little inherent clinical relevance. As a result androgen dynamics in women, both in their reproductive and post-reproductive years, are poorly understood. Surprisingly, if one considers the contribution of the adrenal cortex, androgens circulate in levels far exceeding any other steroid hormone in women, as seen in Table 17.1; testosterone itself circulates in levels usually

Table 17.1 Androgens in women: levels, potencies, and bioconversion

Androgen	Serum Level	Potency (rel. to testosterone)
DHEAS	200 μg/dl	0.001
DHEA	500 ng/dl	0.01
Δ^4A	100 ng/dl	0.1
Testosterone	50 ng/dl	1.0
DHT	5 ng/dl	5

exceeding those seen with serum estradiol (E_2), a hormone of undisputed significance in females. The intriguing possibility is thus raised that perhaps androgens serve a fundamental physiologic purpose in women; by extension, a deficiency of these hormones may result in adverse consequences, possibly rectified by testosterone replacement.

The three sources from which androgens in women arise from are the adrenal cortex, the ovarian theca (and to a lesser degree, ovarian stromal cells), and by peripheral bioconversion of circulating androgenic prohormones. The adrenal gland produces about 95% of circulating serum dehydroepiandrosterone (DHEAS, the production rate of which is 19 mg/day in young women) and 50% of dehydroepiandrosterone (DHEA, the production rate of which is 16 mg/day). The rest of circulating DHEA is produced by peripheral conversion of DHEAS (30%) in addition to a small ovarian contribution (20%) (Burger 2002). DHEAS circulates unbound to protein, has virtually no androgenic action, and has a half-life of 10 hours; it serves as a circulating prohormone for production of DHEA and the more potent downstream androgens both in the circulation and in peripheral tissues. Twenty-eight percent of DHEA comes from hydrolysis of DHEAS, and about 31% of DHEA is sulfated to DHEAS (Haning *et al.* 1989; Bird *et al.* 1978). The production of both DHEAS and DHEA is controlled by adrenocortical reticularis cell stimulation by adrenocorticotropin (ACTH) and negative feedback by circulating cortisol, as well as most probably by other as of yet unidentified adrenal androgen stimulating factors.

Androstenedione [Δ^4A] is produced in about equal portions by both the adrenocortical cells and by the thecal cells of the ovary. Additionally, about 40% of Δ^4A is produced by peripheral bioconversion of DHEA (Burger 2002). The ovary produces both Δ^4A and testosterone under tropic stimulation by leutinizing hormone (LH) with negative feedback by serum E_2, with lesser contributions from testosterone and progesterone. The circulating level of Δ^4A is subject to significant short-term variation secondary to the diurnal nature of its adrenal contribution as well as the variation in ovarian contribution over the menstrual cycle.

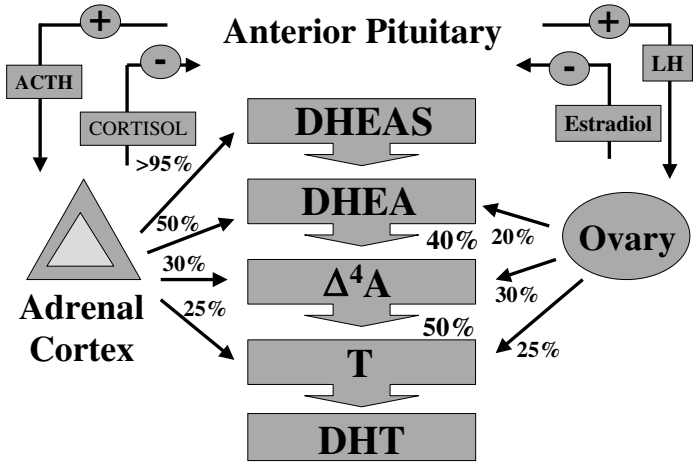


Fig. 17.1 Androgen dynamics in premenopausal women.

Testosterone, the most clinically relevant circulating androgen, has both an adrenal contribution (about 25%) and an ovarian contribution (about 25%), but is mostly produced by peripheral bioconversion from circulating Δ^4A (Burger 2002). By virtue of its relatively large ovarian contribution, serum testosterone is probably the best measure of ovarian androgen production. Dihydrotestosterone (DHT) is produced almost exclusively in target tissues by 5α-reductase action on circulating testosterone; circulating levels are negligible and felt to be largely a reflection of spillover from the primarily intracrine action of this hormone. The circulatory androgen dynamics in premenopausal women are illustrated in Fig. 17.1.

Androgen dynamics in women are subject to three temporal phenomena: ovarian cyclicity, the decline of the adrenal androgens with age (adrenopause), and ovarian follicular depletion with resultant menopausal transition. Throughout the course of the normal ovulatory cycle, changing patterns of LH secretion including a mid-cycle LH surge result in varying ovarian follicular thecal cell stimulation of testosterone and Δ^4A production. In turn, the thecal Δ^4A acts as a substrate for granulosa cell estrogen production. These changes result in a mid-cycle peak in circulating Δ^4A and testosterone production (which parenthetically has been related to a mid-cycle increase in female-initiated sexual activity).

With follicular depletion and the onset of the menopausal transition, ovarian E_2 production declines precipitously, leading to loss of negative feedback at the pituitary and the hypothalamus. In response, circulating serum LH and follicle stimulating hormone (FSH) levels increase dramatically and the circulating LH drives the ovarian theca/stroma to produce increasing amounts of testosterone (Adashi 1994). Thus, the concept that the ovary undergoes endocrine senescence at

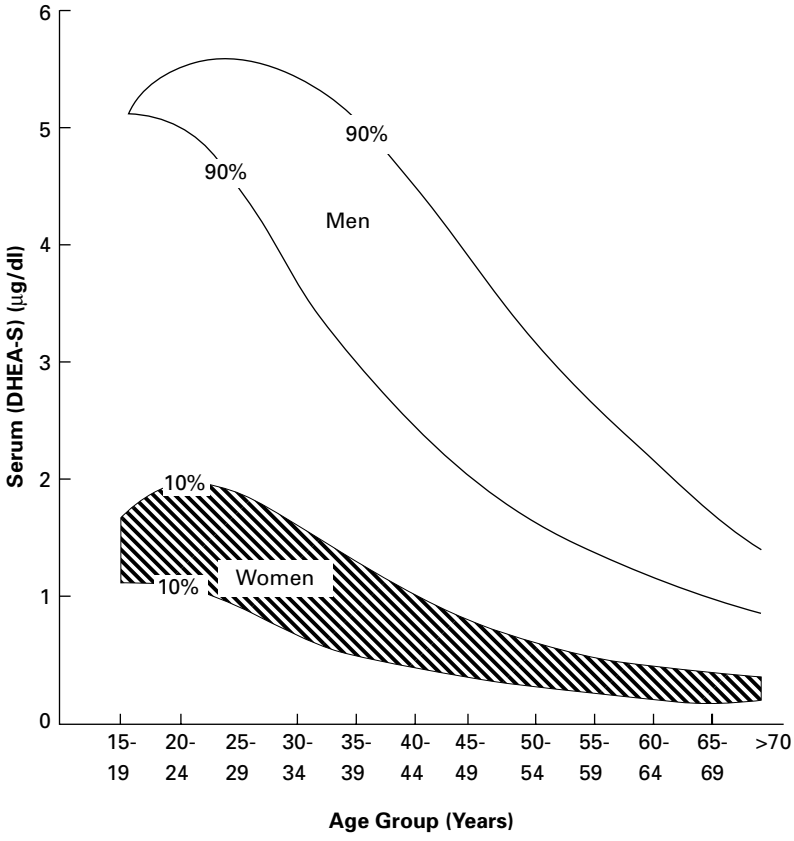


Fig. 17.2 Adrenopause: the senescent, cortisol-independent decline of adrenocortical secretion of androgens (Orientech 1984). Dehydroepiandrosterone sulfate (DHEAS), circulates in amounts far exceeding any other steroid, and after peripheral bioconversion, represents the source of a significant portion of circulating testosterone in women.

the time of menopause is a misperception. Indeed, the ovary actually produces more testosterone after the menopausal transition because of this increased LH-driven androgen secretion. This effect appears to continue well into the postmenopausal years, without attenuation (Meldrum *et al.* 1981).

Superimposed on this androgen tableau is the phenomenon of diminishing adrenocortical androgen production. The adrenal androgens DHEA and DHEAS are produced by the reticularis cells of the adrenal cortex, and in concert with a diminuition of these cells, there is a decline in circulating steroids as well as their responses to ACTH stimulation. Adrenopause is the term coined for this senescent, cortisol-independent decline of adrenocortical secretion of androgens and is illustrated in Fig. 17.2; it occurs in a linear fashion from about the age of 20 or 25 onward, to the point where a woman in her 80s would have about 10% of

circulating DHEAS of that of a woman in early reproductive age (Orentreich *et al.* 1984). Because the adrenal androgens circulate in such high quantities and provide a substantial proportion of circulating Δ^4A and testosterone by virtue of peripheral conversion, adrenopause represents a significant substrate loss for circulating testosterone and thus creates an age-related gradual decline in testosterone levels, independent of menopause.

The cumulative effect of adrenopause and the concurrent increase in ovarian testosterone production over the menopausal transition injects some variability in serum testosterone levels in aging women. Several large cross-sectional studies addressing this issue have demonstrated that circulating testosterone levels do not reproducibly change in relation to the menopausal transition. This is best illustrated by the Melbourne Women's Midlife Health Project, as seen in Fig. 17.2 (Burger 2000). Indeed, because of diminuition of E_2 and a subsequent decline in hepatic production of sex hormone binding globulin (SHBG), the free androgen index, a marker of free testosterone, increases over the menopausal transition. Subsequent testosterone effect may therefore actually increase over the menopausal transition, an explanation for the widely observed clinical phenomenon of menopausal hirsutism. However, there does appear to be a small but significant decline in serum testosterone levels in older reproductive age women probably due to decreased availability of adrenal androgen precursors. A schematic of changes in female androgen dynamics with age is presented in Fig. 17.3.

17.2 Androgen deficiency states in women

A core precept of endocrinology is that of an endocrinopathy, defined as a hormonal deficiency state with clearly defined adverse sequelae. This paradigm is best illustrated by hypothyroidism and subsequent replacement, or by male hypogonadism with testosterone replacement. In the previous section, we have hypothesized that a clear androgen deficiency state does not exist in women undergoing natural menopause, but there are several conditions that are associated with decreased levels of androgens in women. These include the use of postmenopausal hormone replacement therapy (HRT), (particularly orally administered), oral contraceptive use, pre-or postmenopausal oophorectomy, and adrenal suppression. Combined, these iatrogenic causes are prevalent enough to make androgen deficiency in women an extremely common condition.

Several large-scale retrospective and prospective studies have demonstrated that with administration of oral HRT (either estrogen or estrogen-progestin combinations), results in a decrease in serum testosterone (Tazuke *et al.* 1992). In 1997 we illustrated this effect in a series of postmenopausal women receiving 2 mg/day of oral micronized E_2 (Casson *et al.* 1997); as seen in Fig. 17.4, serum testosterone was

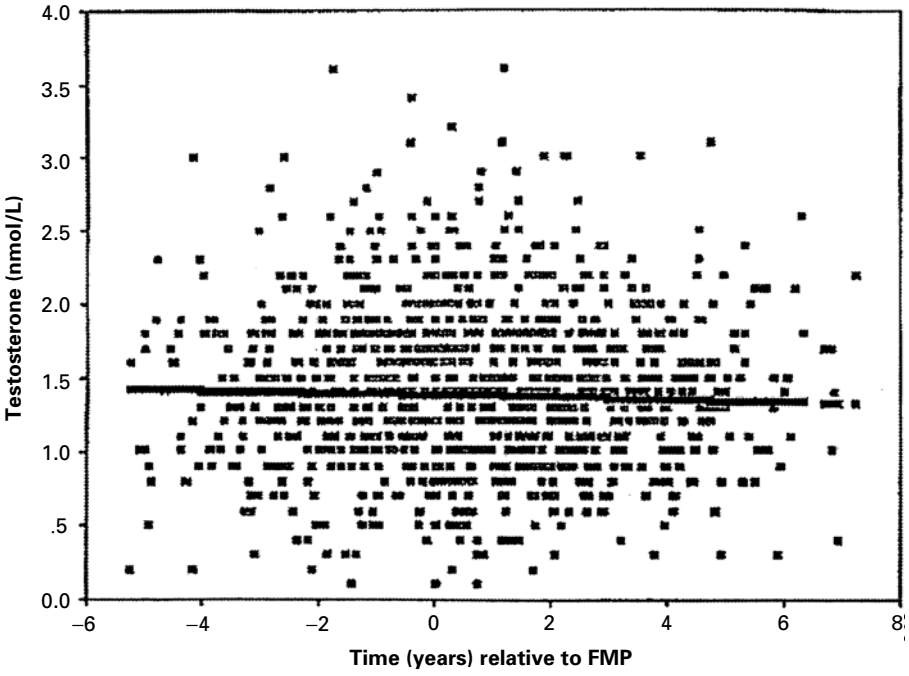


Fig. 17.3 Circulating testosterone levels through the menopausal transition: Melbourne Women's Midlife Health Project (Burger 2000).

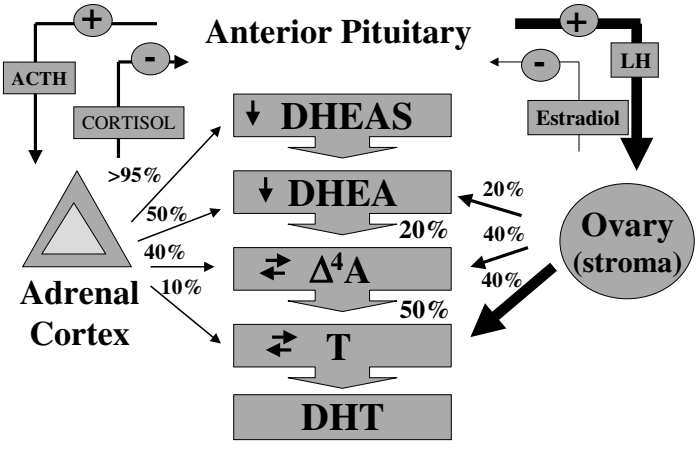


Fig. 17.4 Androgen dynamics in the postmenopausal woman.

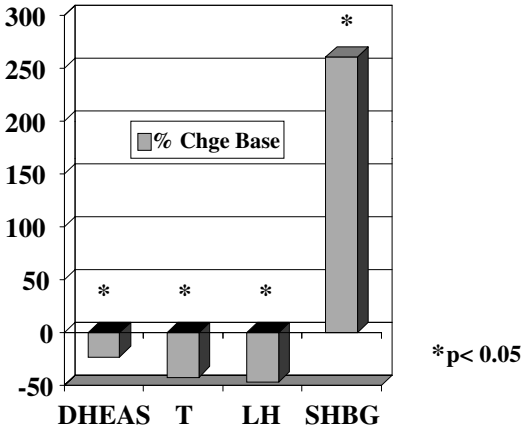


Fig. 17.5 Effect of estrogen replacement therapy on circulating androgens (Casson 1997). Two mg/day of oral micronized estradiol, given over 12 weeks, results in significant decreases in dehydroepiandrosterone, total, and free testosterone.

decreased approximately 40%. This decrease in circulating testosterone is secondary to a decline in serum gonadotropins by about 50%, reducing the postmenopausal LH drive to the ovarian testosterone production.

Fig. 17.4 also illustrates several other concurrent effects of oral estrogen which may accentuate the resultant testosterone deficiency. First, there was a 2.5-fold increase in circulating SHBG that would greatly reduce the amount of unbound or bioavailable testosterone. The HRT-induced increase in serum SHBG is likely due to hepatic first-pass effect and has also been demonstrated with other oral preparations of menopausal HRT as well as oral contraceptives. Of note, it is much less pronounced with transdermal replacement of estrogen (Nachtigall *et al.* 2000). Finally, serum DHEAS was decreased by 30%, representing a significant accentuation of adrenopause. The cause of this augmentation of adrenopause with HRT is less well delineated but has been seen in several other studies. It may be due to estrogenic augmentation of 3ß-hydroxysteroid dehydrogenase activity in the adrenocortical reticularis cells (Casson *et al.* 1996).

Oral contraceptives used in pre and perimenopausal women also significantly decrease total testosterone through similar effects to HRT. This is best illustrated by the work of Thorneycroft *et al.* who in 1999 (Fig. 17.5) demonstrated that oral contraceptive use decreases total testosterone and increases SHBG, resulting in a synergestic decrease in bioavialable testosterone. The effect of oral contraceptives on adrenal androgens is somewhat controversial, but there is some evidence that they may further attenuate secretion of these prohormones for circulating testosterone (Wild *et al.* 1982).

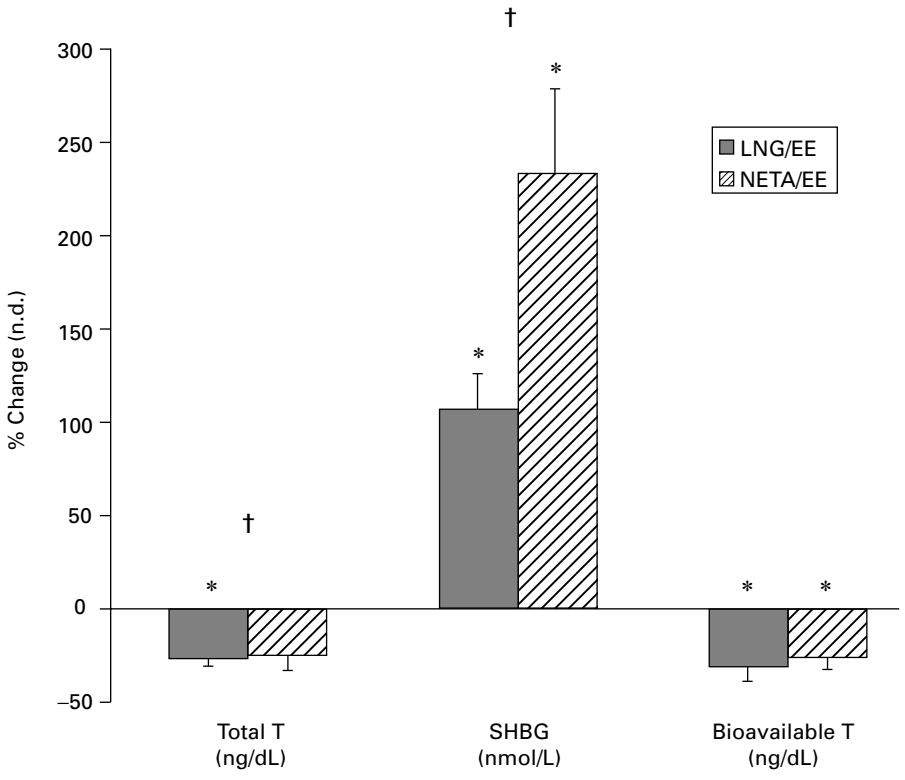


Fig. 17.6 Oral contraceptive use decreases total testosterone and increases SHBG resulting in a synergistic decrease in bioavailable testosterone (adapted from Thorneycroft 1999). NETA/EE and LNG/EE refer to norethindrone acetate/ethinyl estradiol and levonorgestrel/ethinyl estradiol containing oral contraceptives.

Perhaps the most prevalent cause of androgen deficiency in women occurs with surgical menopause. The postmenopausal ovaries clearly remain active androgenic secretory organs; oophorectomy substantially decreases circulating testosterone and Δ^4A by about 50%, as illustrated by Fig. 17.7 (Judd *et al.* 1974).

Hysterectomy with ovarian conservation does not necessarily preclude the possibility of impaired ovarian androgen secretion. One study contends that up to 34% of women who had hysterectomy with ovarian conservation developed menopausal symptoms within the first two years after the procedure (Siddle *et al.* 1987). If hysterectomy with ovarian conservation results in a degree of ovarian devascularization, subsequent ovarian androgen secretory function might well be impaired. This speculation is borne out by Laughlin *et al.* (2000), who published data from the Rancho-Bernardo cohort showing that hysterectomy with ovarian conservation results in bioavailable and total testosterone levels about midway between intact postmenopausal women and surgically menopausal women (Fig. 17.8).

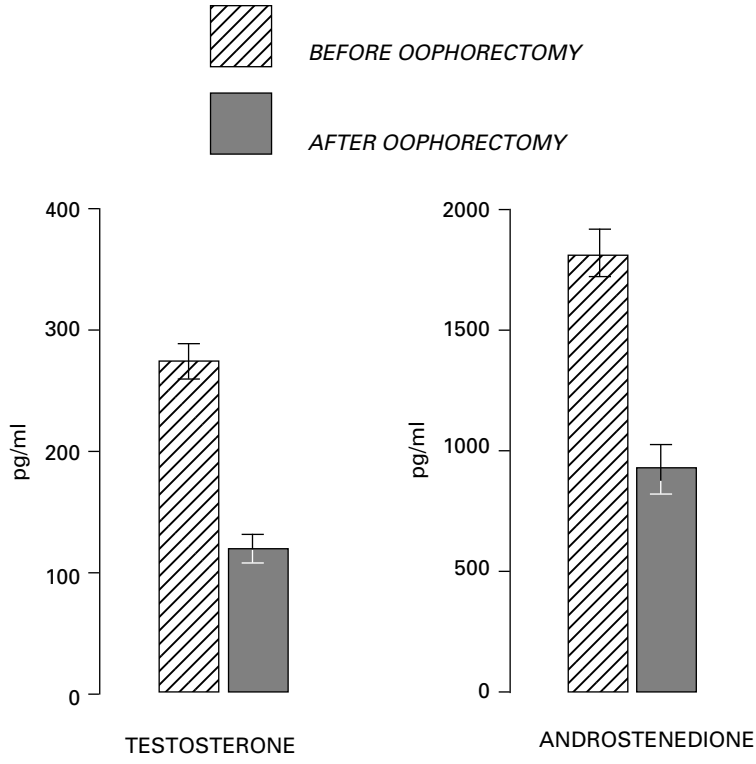


Fig. 17.7 The postmenopausal ovaries remain active androgenic secretory organs as evidenced by the observation that oophorectomy decreases circulating testosterone and Δ^4A by about 50% (adapted from Judd 1974).

Thus, while ovarian conservation at hysterectomy remains a valuable strategy to prevent androgen deficiency in the menopause, it does not necessarily preclude such a phenomenon from occurring. Another potential disadvantage of ovarian conservation is the possible increased incidence of ovarian cancer. The risks/benefit assessment of prophylactic oophorectomy as primary prophylaxis against this lethal condition await more data about the significance of the resultant androgen deficiency.

Adrenal failure or suppression also decreases serum testosterone levels. This is because of decreased zona reticularis production of DHEA and DHEAS, important circulating prohormones for downstream androgens. This effect has been noted with adrenal suppression (Abraham 1974), and more recently by Arlt *et al.* (1999) in patients with adrenal failure. These authors also demonstrated that oral DHEA replacement restituted serum levels of DHEA, DHEAS, Δ^4A and testosterone back to physiologic norms, further illustrating the essential contribution of circulating adrenal androgens to downstream androgens.

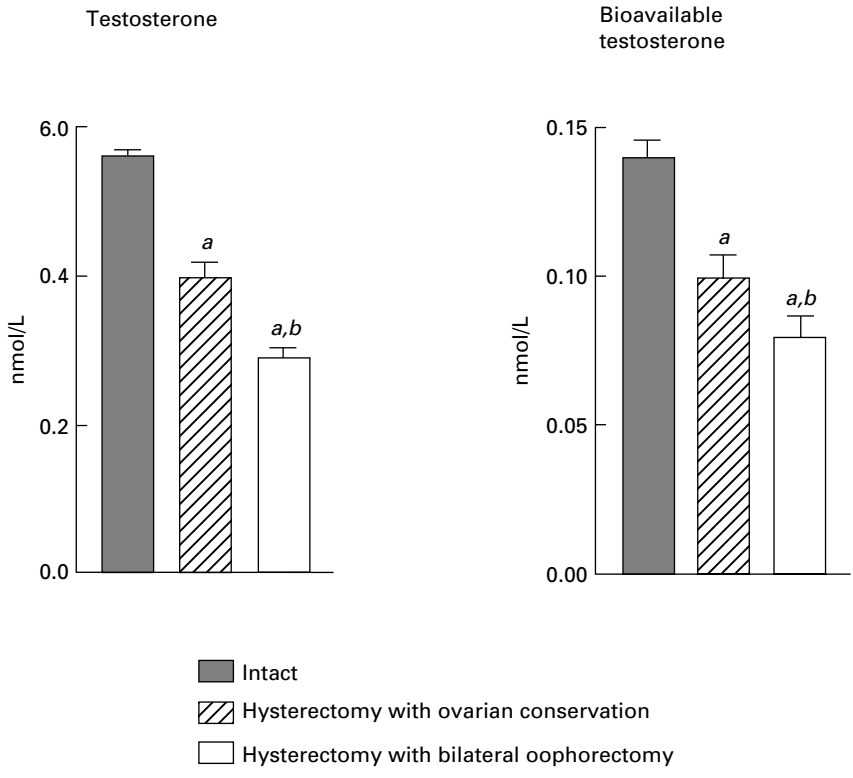


Fig. 17.8 Data from the Rancho-Bernardo cohort showing that hysterectomy with ovarian conservation results in bioavailable and total testosterone levels about midway between intact postmenopausal women and surgically menopausal women. $a = p < 0.05$ from imtact, $b = p < 0.05$ from ovarian conservation (Laughlin 2000).

While natural menopause is not associated with androgen deficiency per se, many of the gynecological interventions associated with menopause do create an iatrogenic androgen deficiency. These include HRT, oophorectomy and subsequent surgical menopause, and gynecologic surgery with ovarian conservation. Additionally, in both reproductive and post-reproductive women, medical interventions such as corticosteroid therapy and most commonly, oral contraceptive use can cause androgen deficiency. Having made the argument that androgen deficiency states do exist in women, at least biochemically, the question is then raised whether such deficiency states are clinically relevant or simply an inconsequential biochemical phenomenon.

Given that in certain conditions an androgen deficiency state may develop in women, how can such a state be defined? A simple measurement of total circulating DHEAS, DHEA, Δ^4A, or testosterone, while a good first step, may not be entirely adequate to define an androgen deficiency because of the cyclic nature of the androgen secretion [Δ^4A, DHEA] or the fact that a significant portion of

circulating steroid (Δ^4A, T, DHT) is rendered biologically inactive by binding to both SHBG and (to a lesser degree) albumin. In the case of another endocrinopathy, menopause, steroid deficiency is defined in terms of a clear symptom complex, but for androgen deficiency little is known in this regard, so defining a clinical complex is more difficult, although attempts have been made (Davis 2001).

Given these limitations, definitions of an androgen deficiency state have been based primarily on serum levels of circulating hormones and are arbitrary – generally considered to be low if they are below the lower third of reproductive age norms. For total testosterone, this would represent a value of less than 15 ng/dl or 1.5 nm/l, and for DHEAS less than 100 μg/dl. Perhaps a better way to look at androgen deficiency would be in terms of free testosterone, and again an arbitrary lower limit of normal would be <1% or 2 pg/ml. Other work in the area of defining androgen deficiency in terms of serum androgen levels centers on calculation of a free testosterone index as determined by total testosterone (nmol/l) multiplied by 100 divided by the SHBG concentration (nmol/L) (Burger 2002b); androgen deficiency is arbitrarily defined as a value less than 4. Ultimately, female androgen deficiency may well be determined on the basis of both a reasonably well defined symptom complex (for instance, decreased lean body mass, bone mass, and sexual function), in conjunction with risk factors (such as previous oophorectomy) and finally, with confirmation by measurements of free testosterone or a related index.

17.3 Do androgens have physiologic relevance in women?

The fundamental question about whether androgens matter in women is still not answered. Speculation that circulating androgens are physiologically relevant in women is based largely on circumstantial evidence. However, this evidence can be used to build a fairly strong case that they are important, certainly to the point where further research is warranted.

It is well established that androgens are not simply reproductive hormones. While they do have multiple reproductive effects both in the fetus and in postnatal life, acting to direct the development of sexual dimorphism and for maintenance of secondary sexual characteristics, they also are multi-system hormones with protean effects on multiple organ systems. Androgens act to enhance bone mass, potentiate certain cognitive behaviors, and enhance erythropoesis. They also modify hepatic protein secretion, stimulate kidney and muscle hypertrophy, and modify patterns of adipose tissue deposition. They are clearly related to skin and appendage function and finally, may have certain immune-enhancing effects.

Further evidence of their potential multi-system role in human physiology is demonstrated by the fact that in human tissues there is a wide distribution of androgen receptor expression (Wilson and Mc Phaul 1996). As seen in Table 17.2, there

Table 17.2 Distribution of androgen receptors in women

Reproductive Tissue	Expression Level	Non-reproductive Tissue	Expression Level
Prostate	1.0	Endometrial carcinoma	0.8
Testis	0.9	Prostate arcinoma	0.5
Seminal vesicles	0.7	Kidney	0.4
Ejaculatory duct	0.4	Thyroid carcinoma	0.4
Endometrium	1.8	Breast	0.3
Ovary	1.5	Colon	0.1
Uterus	1.4	Lung	0.07
Falloian tube	1.0	Adrenal	0.03
Myometrium	0.6		

Adapted from Wilson 1996.

is significant androgen receptor expression in male and female non-reproductive tissues, including kidney, thyroid, breast, colon, lung and adrenal glands. There is also significant expression in female reproductive tissues including endometrium, ovary, uterus, fallopian tube and myometrium.

A speculative line of reasoning that androgens are physiologically important hormones in women is that there might be parallels between female and male androgen deficiency. Testosterone deficiency in men, from either surgical or natural hypogonadism, is a well defined state, and the sequelae are outlined extensively in chapter 13. These men are obese, insulin resistant, at risk for heart disease, have decreased muscle mass and strength, are certainly at risk for osteoporosis, and clearly have diminished sexual function. The question is automatically raised: is there a similar clinical syndrome in women, albeit subtler? We believe what little data does exist in this regard supports this contention.

17.4 Possible benefits of androgen replacement in women

To examine the possible benefits of androgen replacement in women, it may be best to use the complications of the male hypogonadotropic state as a template, reviewing the evidence in that particular area in regards to women. Some provisos need to be noted: the state of the art of androgen replacement in women is rapidly changing, so the existing data is confounded by multiple modalities of androgen replacement, most of which are not physiological regimens. Some studies use surgically menopausal subjects while others do not. Much of the data is also based on cross-sectional studies of endogenous androgen levels and outcomes, with all the limitations of non-randomized epidemiologic data.

17.4.1 Testosterone replacement and sexual function

Multiple studies demonstrate clear evidence that testosterone replacement enhances sexual function in hypogonadal men. In women, there is also strong data in this regard. The best-known study demonstrating a beneficial effect of androgen replacement on sexual function in women was published in 1987 (Sherwin and Gelfand 1987). This trial, although non-randomized and unblinded, did demonstrate increased arousal, fantasy, coitus and orgasm in postmenopausal women given monthly intra-muscular testosterone enanthate (150 mg) and 10 mg of E_2 valerate. However, mean serum testosterone levels noted in this study were well over 200 ng/dl, at least five times the physiological range seen in naturally postmenopausal women. Accordingly, a later study reported that prolonged use of this preparation resulted in virulizing effects in a number of women (Urman *et al.* 1991).

More sexuality data exists with testosterone replacement via subcutaneous (SQ) testosterone pellets, with or without concomitant E_2. These pellets consist of 50 and 100 mg of crystalline testosterone with or without E_2, and are the best characterized form of non-oral testosterone replacement to date. An early series reported a beneficial effect of these implants on sexual function (Cardozo *et al.* 1983), although other data showed no additional benefit of testosterone over E_2 alone (Dow and Hart 1983). As seen in Fig. 17.9, Davis *et al.* demonstrated that placement of the pellets improved multiple parameters of sexual function in postmenopausal women on HRT (Davis 1995). While the circulating testosterone levels attained were again relatively high, no adverse androgenization was reported.

Oral testosterone androgenic preparations have also been assessed in terms of sexual function in postmenopausal women. A carefully designed trial (Myers 1990) demonstrated only a slight improvement in parameters of sexual function (self-stimulating behavior) with the addition of 5 mg methyltestosterone (MT) over estrogen alone. The subjects were not selected for decreased libido. Another study using lower doses of MT did not see an improvement in sexual function (Barrett-Conner *et al.* 1999). In contrast, a more recent study by Lobo *et al.* (2003) and colleagues demonstrated that a much lower dose of MT (1.25 mg/day), in conjuction with esterified estrogens, did improve various parameters of sexual function in postmenopausal women with extant hypoactive sexual desire. Because circulating MT is not easily measured, it is not clear what dose of this compound best mimics physiologic replacement therapy. In the latter study there was an adverse change in serum lipids (HDL) and decreases in SHBG levels seen with MT therapy indicating that the androgenic effect achieved to maintain this level of improvement in sexual function may have been supraphysiologic. Another two-year study with the same preparation of MT and esterified estrogen demonstrated that adverse androgenization indeed frequently occurs (Watts *et al.* 1995).

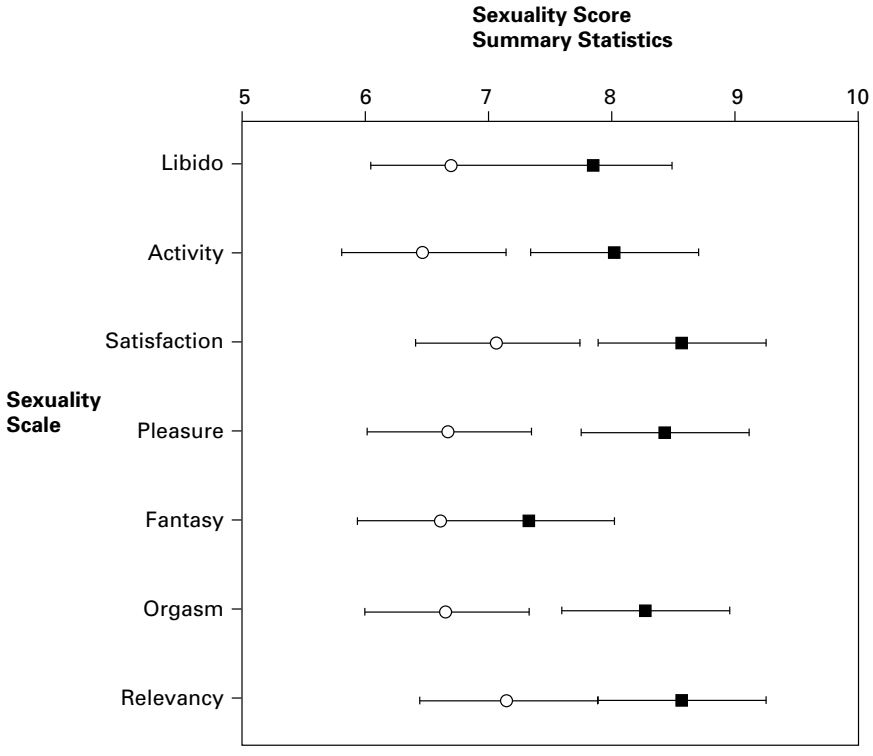


Fig. 17.9 Two years of subcutaneous placement of 50 mg testosterone pellets improves multiple parameters of sexual function in postmenopausal women on HRT (adapted from Davis 1995).

Another oral androgen preparation, testosterone undecanoate, has been assessed in one recent study (Floter *et al.* 2002) in terms of sexual function. This study was well designed, and showed improvements in well-being, self-esteem, and sexual function in these women, compared to estrogen replacement alone.

What about the effect of transdermal testosterone administration and sexual function? Perhaps the best study in this area was published by Shifrin *et al.* (2000). This multi-center, randomized, controlled, and blinded study assessed the effect of 150 and 300 μg transdermal testosterone patches on sexual function in surgically menopausal women who had hypoactive sexual desire. It demonstrated improved sexual function and sense of well-being with restitution of a physiologic serum testosterone milieu. As seen in Fig. 17.10, however, the difference was not dramatic as compared to estrogen patches alone.

In all, the weight of the studies assessing the effect of various forms of androgen replacement on sexual function in women demonstrate a modest benefit in a fashion similar to, but not as pronounced as, that seen in hypogonadal men given testosterone replacement. The fact that improvement in sexual function is not dramatic may point to the relative complexity of sexual response in women, or be

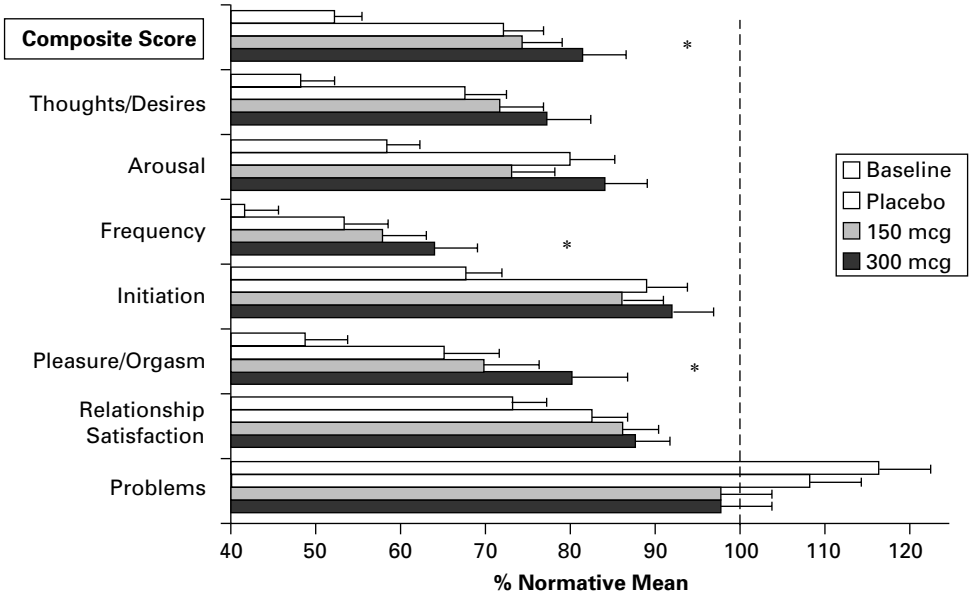


Fig. 17.10 A multi-center, randomized, controlled, and blinded study assessed the effect of 150 and 300 μg transdermal testosterone patches on sexual function in surgically menopausal women who had hypoactive sexual desire, and demonstrated improved sexual function and sense of well being (adapted from Shifren 2000).

a reflection of study limitations, particularly in the measurement of female sexual response.

17.4.2 Testosterone and insulin sensitivity in women

In hypogonadal men, testosterone supplementation certainly does not adversely affect and may mildly enhance parameters of insulin sensitivity. One epidimiologic study has demonstrated an association between low endogenous testosterone levels in men and subsequent development of type 2 diabetes (Oh *et al.* 2002). In contrast, in women, testosterone is commonly thought to increase insulin resistance. This dogma is based primarily on the observation that women with hyperandrogenic anovulation and elevated testosterone levels (polycystic ovarian syndrome, or PCOS) are frequently hyperinsulinemic. Recently, as the thoughts about hyperandrogenic anovulation have shifted, it is now recognized that an antecedent hyperinsulinemia likely results in a secondary elevation of ovarian androgen secretion with resultant hyperandrogenemia. The thought that increased testosterone causes insulin resistance in women has been further eroded by studies demonstrating that intravenous testosterone infusion does not worsen insulin resistance, and that GNRH analog suppression of hyperandrogenemia in PCOS patients does not improve insulin sensitivity (Dunaif *et al.* 1990). Indeed, some tantalizing

data demonstrates that DHEA replacement (with resultant elevations in circulating DHEAS, Δ^4A, and testosterone), may actually improve insulin sensitivity (Casson 1975) (Fig. 17.10).

In female testosterone replacement studies to date, very few measurements of insulin sensitivity have been measured. In a recent testosterone patch study (Schifrin *et al.* 2000) fasting insulin and glucose did not change. The issue of whether physiologic testosterone replacement in an androgen-deficient woman may actually improve insulin sensitivity has not been yet addressed with sensitive insulin resistance endpoints.

17.4.3 Is testosterone cardioprotective in women?

In hypogonadal men, testosterone replacement may indeed be cardioprotective. In normal men, some epidemiologic studies have demonstrated that low testosterone may adversely affect parameters of insulin sensitivity and lipoproteins, both important contributors to cardiovascular disease (Oh *et al.* 2002; Simon 1997). A further possible cardioprotective effect of testosterone in men may also be the observed negative correlation between serum levels and increasing intraabdominal fat (Tsai 2000). Another beneficial effect may be a short-term direct coronary vasodilator effect as demonstrated by Rosano *et al.* (1999). Finally, testosterone may beneficially alter blood viscosity (Basaria *et al.* 2002).

Are androgens actually cardioprotective in women? The few epidemiologic studies that look at correlations between elevated endogenous testosterone levels in women and heart disease show a positive association, but that may be because elevated testosterone is a marker for the metabolic syndrome associated with PCOS. One interesting study may circumferentially address the issue of the possible cardiopretective effect of testosterone in women. In an assessment of the Rancho-Bernardo cohort (Kritz-Silverstein *et al.* 1997) postmenopausal oophorectomy may actually worsen dyslipidemia and insulin resistance, both contributors to increased risk for cardiovascular disease. Intriguingly, the possibility exists that physiologic testosterone replacement in androgen deficiency may not worsen, and possibly protect, against cardiovascular disease. Obviously, given the recent results of the Women's Health Initiative and other studies regarding the effect of HRT on cardiovascular disease on postmenopausal women, such a contention with respect to androgens is highly speculative, but it must be noted that HRT clearly reduces endogenous testosterone levels. (for further discussion also see Chapter 10).

17.4.4 Testosterone replacement and body morphology in women

Multiple studies have demonstrated that testosterone replacement in hypogonadal men positively impacts lean body mass and parameters of muscle strength. Data in

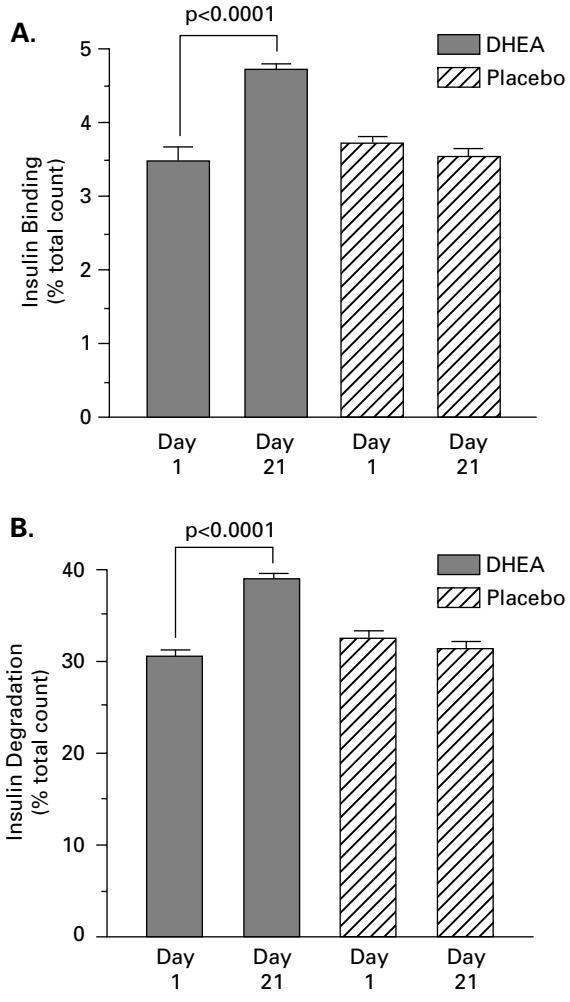


Fig. 17.11 Data demonstrating that three weeks of 50 mg of DHEA replacement (with resultant elevations in circulating DHEAS, Δ^4A, and testosterone), may actually improve insulin sensitivity, as measured by testosterone-lymphocyte insulin binding and degradation (Casson 1995).

this regard with respect to women is scant. If oral DHEA replacement is considered a modified form of androgen replacement in women (a reasonable assumption, given that DHEA elevates serum testosterone), several studies in women using this form of replacement have demonstrated improvements in muscle strength and exercise tolerance. We have recently demonstrated that the $V0_2$ (maximal exercise tolerance) of postmenopausal women increases with DHEA replacement and that this may be independent of cardiac output (Fig. 17.11) (Burger *et al.* 2003). Certainly, several

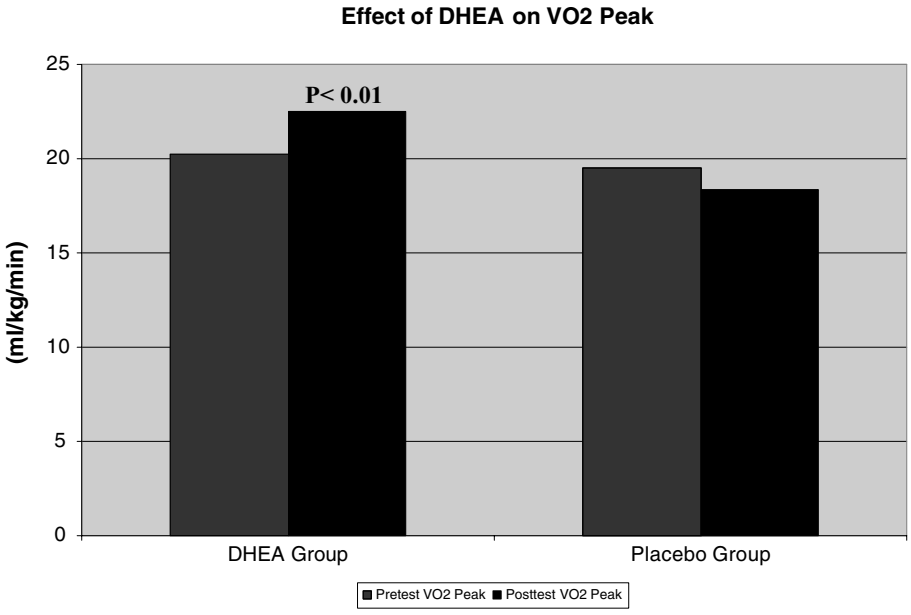


Fig. 17.12 One year of physiologic dehydroepiandrosterone replacement in postmenopausal women increases $V0_2$ (maximal exercise tolerance) independent of cardiac output, compared to placebo (Burger 2003). There was no significant change in weight or lean body mass by DEXA scan.

studies have demonstrated DHEA replacement in women improves lean body mass. The idea that physiologic testosterone replacement in women may improve lean body mass, increase muscle mass and increase exercise tolerance is, of course, an exciting prospect, not without basis, and worthy of further investigation.

17.4.5 Testosterone replacement and bone mass in women

Testosterone replacement clearly increases bone mineral density in hypogonadal men. Does the same effect occur in women who are given testosterone replacement? Two randomized, controlled trials exist addressing this issue. Davis *et al.* (1995) demonstrated a substantial improvement in vertebral and trochanteric bone mineral density in postmenopausal women given E_2-testosterone pellet replacement versus E_2 alone over a period of two years. Additionally, oral MT replacement improves bone mineral density over two years, although the effect seen in this study was not dramatic and at the expense of adverse androgenization, including dyslipidemia (Watts *et al.* 1995). In summary, there is strong evidence that androgen replacement may have a beneficial effect on postmenopausal bone, above that seen with estrogen alone.

The limited data existing regarding the extra reproductive effects of testosterone replacement in women indicates that there are strong parallels between men and women in this area. Testosterone replacement in women, although imperfect, clearly improves sexual function, likely improves bone mineral density (more so than estrogen alone), may improve insulin sensitivity, and finally may have a cardioprotective effect. Certainly, the concept of multi-system benefits of testosterone replacement in women is worthy of further investigation, particularly since the adverse physiologic changes seen with menopause on bone mineral density, cardiovascular disease, and lean body mass and strength significantly impact a large portion of our society.

17.5 Androgen replacement in women: the present state of the art

Another parallel between androgen replacement in men and in women is seen in the evolving technology of testosterone replacement. Hypogonadal men were initially replaced with potent oral anabolic steroids, then by substantial doses of oral MT. In high doses, however, this preparation has significant hepatic problems, including liver dysfunction (Westabay *et al.* 1977), hepatic adenomas (Farrell *et al.* 1975), and possible induction of hepatic carcinoma (Goldfarb 1976) (see also Chapter 14). The focus of androgen replacement in men shifted to depot intramuscular testosterone enanthate (or other esters), to avoid hepatic issues from first-pass metabolism. The intramuscular depot route of administration was a significant improvement over oral synthetic androgens, but still remained an unphysiological replacement modality, with large day-to-day excursions in androgen effect noted. Attention then shifted to transdermal mechanisms to providing a relatively constant release of testosterone. Reservoir and adhesive matrix patches were developed which, while providing a physiologic replacement, were large and ungainly because of the large amount of delivered testosterone needed to effect replacement in men. The most recent advance in male testosterone replacement is the development of transcutaneous testosterone gels, which deliver substantial amounts of testosterone in a relatively constant fashion.

The development of female androgen replacement remains several iterations behind its male counterpart. For many years the most common androgenic preparation for females (at least in North America) was a combination of oral MT and oral estrogens (usually conjugated equine estrogen or esterified estrogen), in levels as high as 5 or 10 mg/day of MT (Greenblatt *et al.* 1950). This has subsequently been replaced by a combination product of oral esterified estrogens 0.625 mg with MT 2.5 or 1.25 mg/day (Estratest®, or Estratest HS®). At these MT doses, which are comparatively low, there have been no cases of hepatic problems (Simon *et al.* 2001). This compound is FDA approved for the treatment of climacteric symptoms

recalcitrant to conventional therapy, although there is a paucity of data to support this contention. This combination has been demonstrated to have beneficial effects on bone homeostasis and sexual function. Problematically, MT is not assayable with conventional testosterone assays and thus, measurement of excessive androgenization must rest on the development of androgenic symptoms alone. At a dose of 1.25 mg/day of MT, one well controlled two-year study (Watts *et al.* 1995) demonstrated a 30% incidence of adverse androgenic effects (hirsutism, acne) as well as adverse effects on lipoproteins, confirmed by others in the more recent past (Lobo *et al.* 2003).

Clearly, oral MT is not an optimal androgen replacement modality for women. Another oral alternative, which is more available outside North America, is testosterone undecanoate, which has recently been shown to have beneficial effects on sexual function and general well-being (Floter *et al.* 2002). This testosterone ester is apparently absorbed by the gut lymphatic system and thus bypasses hepatic first-pass metabolism. It remains to be seen whether this oral preparation will have similar adverse lipoprotein effects.

Following the lead of male testosterone replacement, female replacement is moving away from the oral route of administration. There is an extensive experience with female preparations of depot intramuscular testosterone enanthate 150 mg monthly in conjunction with a 10 mg dose of estradiol valerate (Sherwin and Gelfand 1987). Unfortunately, this results in serum testosterone levels far above those acceptable in women, with concurrent reports of significant virilization (Urman *et al.* 1991). This mode of replacement has largely fallen by the wayside in recent years.

Perhaps the best-characterized method of non-oral androgen replacement in women is the testosterone pellet. This form of replacement, pioneered in both the UK and Australia, results in daily delivery of between 250 and 500 μg of testosterone, a much closer mimic of physiology, where the ovaries produce 250 μg/day. The 50 mg pellet is normally placed every three months, sometimes with concurrent E_2 (40 mg/pellet). It is usually placed in the subcutaneous fat in the right or left lower quadrant of the abdomen, using a specially designed trochar. The delivery of testosterone with this method is well characterized, with demonstrated beneficial effects on bone and sexual function (Davis *et al.* 1995). Despite the fact that supraphysiologic levels of circulating testosterone are attained with the pellet, there appears to be no adverse effects on lipoproteins, in contrast to MT.

More recently, attempts have been made to replace testosterone in women with a transcutaneous gel (Androgel®, 50 mg of testosterone/packet). This preparation is made for male testosterone replacement; data in women are non-existent. Assuming a 10% nominal absorption, even a quarter packet applied per day would result in daily administration of 1,250 mg of testosterone, far in excess of the physiologic

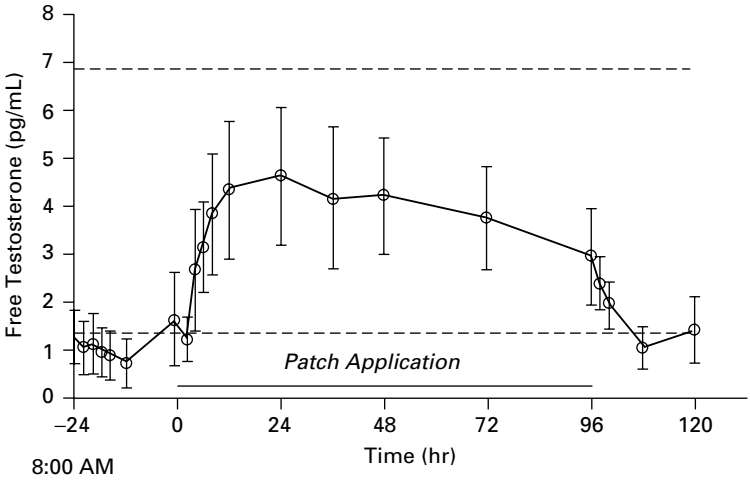


Fig. 17.13 Physiologic serum levels of free testosterone obtained with the investigational testosterone patch, which delivers 150 or 300 μg of testosterone per day, equivalent to female production rates. The patch is changed twice a week and gives serum testosterone levels in the range of 40–60 ng/dl without perturbation of serum E_2 or SHBG levels (Mazer 2002).

production rate. Consequently, serum testosterone levels with administration of this gel, even in that limited dose, are often quite higher than seen with testosterone pellets.

Perhaps the best potential delivery system is the female testosterone patch presently under development. This patch has been well characterized pharmacodynamically and is designed to deliver 150 or 300 μg of testosterone per day, equivalent to female production rates. The patch is changed twice a week and gives serum testosterone levels in the range of 40–60 ng/dl without perturbation of serum E_2 or SHBG levels. The free testosterone levels obtained are quite physiologic, as illustrated in Fig. 17.13 (Mazer 2002). It has been recently tested in a blinded, randomized, controlled trial in surgically menopausal women with decreased libido and found to modestly enhance parameters of sexual function and general well being. Further studies in natural and surgical menopause are presently underway.

As illustrated in Table 17.3, testosterone replacement regimens in women have gradually moved away from oral administration of synthetic androgens towards much more physiologic replacement with transdermal therapies. Only now are these modalities approximating the physiologic androgenic milieu of the naturally post-reproductive woman. Truly, an assessment of the efficacy of testosterone replacement in women can only be made with physiologic replacement after identification of defined deficiency states. Now such studies begin to be possible.

Table 17.3 Current testosterone replacement alternatives for women

Name	Type	Route	Dose	Daily delivery
Ovaries	testosterone	Systemic	250 μg	250 μg
Testes	testosterone	Systemic	7 mg	7 mg
Depot IM testosterone	testosterone enanthate or cypionate	Systemic	200 mg Q 3 mos	2 mg
Methyl-testosterone	MT	(IM)	1.25, 2.5, 5, 10 mg	? 50 mg/day is virilizing
T pellet	testosterone	Oral	50 mg Q 3 mos	250–500 μg
Male testosterone patch	testosterone	Systemic (SQ)	4, 6 mg q daily	5 mg
Female testosterone patch	testosterone	Systemic (TD)		150, 300 μg
testosterone gel	testosterone (?DHT)	Systemic (TD)	50, 75, 100 mg/day	? 250–500 mg

17.6 Summary and future directions

Androgens circulate in appreciable amounts in women. Female serum testosterone levels rely on a complex interplay of hormonal secretion and bioconversion of peripheral prehormones. Testosterone levels are proportional to ovarian and adrenal secretion and peripheral bioconversion of the adrenal androgens DHEAS and DHEA, the predominant circulating androgens. Adrenal androgen secretion attenuates with age in a cortisol-independent fashion due to involution of the reticularis zone of the adrenal cortex. As a result, as women age, less testosterone is produced from peripheral bioconversion of DHEAS and DHEA. With the onset of menopause, while ovarian folliculogenesis ceases, the remaining theca and stroma respond to the elevated, menopausal levels of LH by greatly increasing ovarian testosterone secretion. This compensatory mechanism attenuates the age decline in serum testosterone levels from declining adrenal androgens. The combined effects create a subtle decline in serum testosterone levels with age, with no abrupt decline seen with natural menopause. Indeed, there is a plateauing of testosterone levels because of increased postmenopausal ovarian contribution. When the menopausal estrogen deficiency-related decrease in levels of SHBG are taken into account, there is actually an increase in circulating free testosterone in a woman's post reproductive years.

Most all androgen deficiency in women is not natural, but is iatrogenic. It is secondary to exogenous estrogen supplementation either in the form of oral contraceptives or HRT, particularly if the estrogens are orally administered. With both these therapies, there is a several-fold increase in hepatic production of SHBG, and a subsequent decrease in free testosterone, as well as suppression of pituitary LH secretion, resulting in decreased ovarian drive to produce testosterone. Other causes of testosterone deficiency in women include either adrenal failure or adrenal suppression with corticosteroids. These conditions greatly decrease circulating DHEAS levels and thus remove substrate for a large percentage of circulating testosterone production.

The commonly held axiom that androgens in women are simply steroid byproducts is gradually fading. There is no reason to believe that androgens do not play a physiologic role in women as evidenced by the widespread distribution of androgen receptors in both male and female reproductive and non-reproductive tissues. Careful assessment of the limited evidence available indicates that female androgens, and the replacement thereof, play a role in female sexual function, maintenance of bone mass, maintenance of favorable body morphometric indices, possibly insulin resistance, and cardioprotection.

It must be noted that these studies are limited by the fact that they are small, and involve multiple routes of administration of generally supraphysiologic levels of testosterone. However, modalities of testosterone replacement in women are rapidly evolving towards non-oral, physiologic replacement with the native steroid. With these new, much more appropriate modalities of replacement, more clinical studies need to be performed to assess efficacy in a wide range of end-point parameters. The future in this area is clearly promising.

17.7 Key messages

- Because of the compensatory postmenopausal production of ovarian testosterone, a testosterone deficiency in naturally menopausal women is very uncommon. The vast majority of testosterone deficiency in females is secondary to iatrogenic causes: hormonal replacement, prophylactic oophorectomy, hysterectomy, oral contraceptive use, or corticosteroid replacement.
- A clearly defined constellation of signs and symptoms defining testosterone deficiency in women remains elusive. Biochemical criteria based on testosterone, DHEAS, and SHBG levels are arbitrary at best.
- Testosterone replacement in women, while rapidly evolving, is still several generations behind that of men. Modalities of treatment are hampered by excessive androgenization, use of the non-native steroid, and oral administration with adverse lipoprotein effects. Recent development of a low-dose transdermal testosterone delivery system for women, mimicking physiology, heralds promise for the future.

- The data surrounding efficacy of testosterone replacement in women is hampered by poor study designs, multiple modalities of the testosterone replacement, most of which are supraphysiologic in nature, and lack of a clearly defined deficiency state. However, reasonable data suggests that testosterone replacement at a low dose may enhance sexual function, indices of general well-being, and bone mineral density. Its effects on exercise, muscle mass, lean body mass, insulin sensitivity and cardiovascular risk factors remain to be determined.

17.8 REFERENCES

Abraham G (1974) Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. J Clin Endocrinol Metab 39:340–346

Arlt W, Callies F, Christoph van Vlijmen J, Koehler I, Reincke M, *et al.* (1999) Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 341:1013–1020

Adashi EY (1994) The climacteric ovary as a functional gonadotropin-driven androgen-producing gland. Fertil Steril 62:20–27

Barrett-Conner E, Young RL, Notelovitz M, Sullivan J, Wiita B, Yang HM, Nolan J (1999) A two year double-blind comparison of estrogen-androgen and conjugated estrogens in surgically menopausal women. J Reprod Med 44:1012–1020

Basaria S, Nguyen T, Rosenson RS, Dobbs AS (2002) Effect of methyltestosterone administration on plasma viscosity in postmenopausal women. Clin Endocr 57:209–214

Bird CE, Murphy J, Boroomand K, Finnis W, Dresel D, Clark AF (1978) Dehydroepiandrosterone: kinetics of metabolism in normal men and women. J Clin Endocrinol Metab 47:818

Burger, HG (2002) Androgen production in women. Fertil Steril 77: suppl. 4, S3–S5

Burger HG (2002b) The role of androgen therapy. Best Prac and Res Clin Obstet and Gynec 16:383–393

Burger HG (2000) A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. J Clin Endocrinol Metab 85:2832–2838

Burger NZ, Protas E, Davis W, Buster JE, Carson SA, Casson PR (2003) Effect of physiologic oral dehydroepiandrosterone (DHEA) replacement on exercise tolerance in postmenopausal women. Abstract for poster presentation (accepted), 59th annual meeting of the American Society for Reproductive Medicine, October 11–15 2003, San Antonio, TX

Cardozo L, Gibb DMF, Tuck SM, Thom M, Studd JWW, Cooper DJ (1983) The effects of subcutaneous hormone implants during the climacteric. Maturitas 5:177–184

Casson PR, Faquin LC, Stentz FS, Straughn AB, Andersen RN, Abraham GE, Buster JE (1995) Replacement of dehydroepiandrosterone (DHEA) enhances T-lymphocyte insulin binding in postmenopausal women. Fertil Steril 63:1027–1031

Casson PR, Endoh A, Buster JE, Hornsby PJ (1996) Physiologic-estrogen enhances 3β ol hydroxysteroid dehydrogenase (type 113-HSD) and 17A P450 (P45Ocl7) mRNA in isolated adult human adrenocortical fasciculata and reticularis cells. Oral Presentation (0–1 16) 43rd Annual Meeting of the Society of Gynecologic Investigation, Philadelphia, PA

Casson PR, Elkind-Hirsch KE, Buster JE, Hornsby PJ, Carson SA (1997) Effect of postmenopausal estrogen replacement on circulating androgens. Obstet Gynecol 90:995–998

Davis SR, McCloud P, Strauss BJG, Burger HG (1995) Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. Maturitas 21:227–236

Davis SR (2001) When to expect androgen deficiency other than at menopause. Fertil Steril 77:S68–S71

Dunaif A, Green G, Futterweit W, Dobrjansky A (1990) Suppression of hyperandrogenism does not improve peripheral or hepatic insulin resistance in the polycystic ovary syndrome. J Clin Endocrinol Metab 70:699–704

Dow MGT, Hart DM (1983) Hormonal treatments of sexual unresponsiviness in postmenopausal women: a comparative study. Brit J Obstet Gynecol 90:361–366

Farrell GC, Uren RF, Perkins KW, Joshua DE, Baird PJ, Kronenburg H (1975) Androgen-induced hepatoma. Lancet 7904:430–432

Floter A, Nathorst-Boos J, Carlstrom K, von Schoultz B (2002) Addition of testosterone to estrogen replacement therapy in oophorectomized women: effects on sexuality and well-being. Climacteric 5:357–365

Goldfarb S (1976) Sex hormones and hepatic neoplasia. Cancer Res 36:2584–2588

Greenblatt RB, Barfield WE, Garner JF, Calk GL, Harrod JP Jr. (1950) Evaluation of an estrogen, androgen, estrogen-androgen combination, and a placebo in the treatment of the menopause. J Clin Endocrinol 10:1547–1558

Haning RV Jr, Chabot M, Flood CA, Hackett R, Longcope C (1989) Metabolic clearance rate (MCR) of DHEAS, its metabolism to DHEA, androstenedione, T, and DHT, and the effect of increased plasma DS on DS MCR in normal women. J Clin Endocrinol Metab 69: 1047

Judd HL, Judd GE, Lucas WE, Yen SSC (1974) Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. J Clin Endocrinol Metab 39:1020

Kritz-Silverstein D, Barrett-Connor E, Wingard, DL (1997) Hysterectomy, oophorectomy, and heart disease risk factors in older women. Am J Pub Health, 57:676–680

Laughlin GA, Barrett-Conner E, Kritz-Silverstein D, von Muhlen D (2000) Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the rancho bernardo study. J Clin Endocrinol Metab 85:645–651

Lobo RA, Rosen RC, Yang HM, Block B, Van Der Hoop RG (2003) Comparative effects of oral esterified estrogens with and without methyltestosterone on endocrine profiles and dimensions of sexual function in postmenopausal women with hypoactive sexual desire. Fertil Steril 79:1341–1352

Mazer NA (2002) Testosterone deficiency in women: etiologies, diagnosis, and emerging treatments. Int J Fertil 47:77–86

Myers LS, Dixen J, Morrisette MC, Davidson JM (1989) Effects of estrogen, androgen, and progestin on sexual psychophysiology and behavior in postmenopausal women. J Clin Endocrinol Metab 70:1124–1131

Meldrum DR, Davidson BJ, Tataryn IV, Judd HL (1981) Changes in circulating steroids in postmenopausal women. Obstet Gynecol 57:624–628

Nachtigall LE, Raju U, Banerjee S, Wan L, Levitz M (2000) Serum estradiol-binding profiles in postmenopausal women undergoing three common estrogen replacement therapies: associations with sex hormone-binding globulin, estradiol, and estrone levels. Menopause 7: 243–250

Oh JH, Wedick NM, Barrett-Connor E, Wingard DL (2002) Endogenous sex hormones and the development of type 2 diabetes in older men and women: the rancho bernardo study. Diabetes Care 25:55–60

Orentreich N, Brind JL, Rizer RL, Vogelman JH (1984) Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551–555

Rosano G MC, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E, della Monica PL, Bonfigli B, Volpe M, Chierchia SL (1999) Acute anti-ischemic effect of testosterone in men with coronary artery disease. Circulation 99:1666–1670

Sherwin BB, Gelfand MM (1987) The role of androgen in the maintenance of sexual functioning in postmenopausal women. Psychosom Med 49:397–409

Shifrin JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP *et al.* (2000) Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688

Siddle N, Sarrel P, Whitehead M (1987) The effect of hysterectomy on the age of ovarian failure: identification of a subgroup of women with premature loss of ovarian function and literature review. Fertil Steril 47:94–100

Simon JA (2001) Safety of estrogen/androgen regimens. J Reprod Med 46:281–290

Simon D, Charles MA, Nahoul K, Orssaud G, Kremski J, Hully V, Joubert E, Papoz L, Eschwege E (1997) Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: the telecom study. J Clin Endocrinol Metab 82:682–685

Tazuke S, Shaw KT, Barrett-Conner E (1992) Exogenous estrogens and endogenous sex hormones. Medicine 71:44–50

Thorneycroft IH, Stanczyk FZ, Bradshaw KD, Ballagh SA, Nichols M, Weber ME (1999) Effect of low-dose oral contraceptives on androgenic markers and acne. Contraception 60:255–262

Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY (2000) Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obesi 24:485

Urman B, Pride S, Ho Yuen B (1991) Elevated serum testosterone, hirsutism, and virilism associated with combined androgen-estrogen hormone replacement therapy. Obstet Gynecol 77:595–598

Watts NB, Notelovitz M, Timmonc MC, Addison WA, Wiita B, Downey LJ (1995) Comparison of oral estrogens and estrogens plus androgen on bone mineral densiy, menopausal symptoms, and lipid-lipoptrotein profiles in surgical menopause. Obstet Gynecol 85:529–537

Westabay D, Ogle SJ, Paradinas FJ, Randell JB, Murray-Lyon IM (1977) Liver damage from long term methyltestosterone. Lancet 8032:262–263

Wild RA, Umstot ES, Andersen RN, Givens JR (1982) Adrenal function in hirsutism: effect of an oral contraceptive. J Clin Endocrinol Metab 54:676–681

Wilson CM, McPhaul MJ (1996) A and B forms of the androgen receptor are expressed in a variety of human tissues. Mole Cell Endocri 120:51–57

18

Clinical use of 5α-reductase inhibitors

K.D. Kaufman

Contents

18.1 Role of 5α-reductase in androgen physiology and pathophysiology

18.1.1 Normal androgen metabolism

During the last century, the identification and characterization of the major sex steroids, which include androgens, estrogens, and progestins, helped define their biologic functions. Androgens were demonstrated to be essential for normal male sexual differentiation *in utero* and for development and maintenance of male secondary sexual characteristics, including terminal body hair growth, muscle mass, sexual behavior and fertility. Androgens are steroid hormones and, as such, produce effects by binding to an intracellular receptor, forming a hormone-receptor complex that interacts with DNA to modulate protein transcription (Mainwaring 1977). Testosterone, the major circulating androgen in adult men, was logically suspected to be the hormone responsible for these effects. Observations in 46XY subjects with inborn androgen insensitivity (syndrome of testicular feminization) confirm that the sexual phenotype in humans is predominantly female in the absence of androgen effects (Morris *et al.* 1963, see Chapter 3). Specifically, despite normal circulating levels of testosterone, subjects with androgen insensitivity who have impaired responses to androgens secondary to a dysfunctional androgen receptor manifest female external genitalia (with blind vaginal pouch, cryptorchid testes and infertility) and breast development, no terminal sexual body hair growth, and a pre-pubertal pattern of scalp hair growth. Due to the absence of androgen action, no androgen-related disorders typical of aging men, such as disorders of the prostate or male pattern hair loss over the scalp, are observed. The latter finding is consistent with Hamilton's conclusions regarding the androgen dependence of typical male pattern scalp hair loss, based on observations of eunuchs compared to normal subjects (Hamilton 1942; 1951). Each of these examples suggested that the lack of testosterone, or the lack of its biologic action, was responsible for the observed effects, although effects due to decreased activity of metabolites of testosterone (Fig. 18.1) could not be ruled out. Subsequent observations demonstrated that specific androgens other than testosterone were, in fact, crucial for effecting biologic functions and contributing to the pathophysiology of androgen-related disorders.

18.1.2 Evidence for role of 5α-reductase in pathophysiology of androgen disorders

In the 1970s, remarkable findings, based on observations of kindreds harboring mutations in the gene coding for steroid 5α-reductase (5αR), the enzyme that catalyzes the conversion of testosterone to dihydrotestosterone (DHT), were reported. The presence of 5αR activity had first been identified in the prostate in preclinical species, and DHT had been identified as a potent androgen with greater affinity (approximately 5-fold) for the androgen receptor than testosterone, although

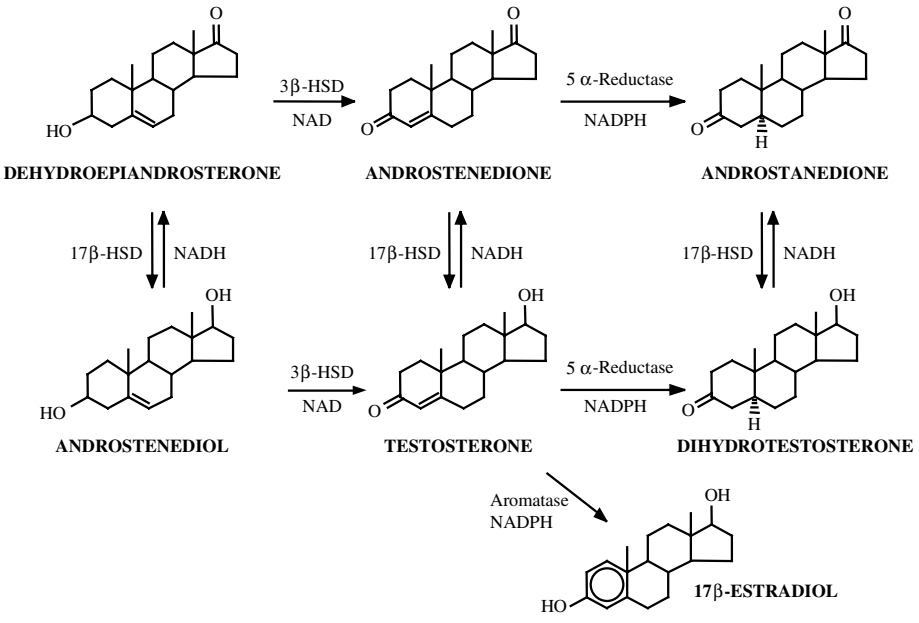


Fig. 18.1 Androgen metabolic pathways in skin (adapted from Kaufman 1996).

its precise role in androgen biology was not fully understood (Anderson and Liao 1968; Fang *et al.* 1969; Mainwaring 1969; Saunders 1963; Wilson 1972; Wilson and Gloyna 1970; Wilson and Lasnitzki 1971). The findings in subjects with genetic deficiency of 5αR led to a significantly greater understanding of how the specific androgen DHT participates in fetal development and sexual maturation and contributes to the pathogenesis of disorders associated with aging men.

18.1.2.1 Genetic 5α-reductase deficiency

In 1974, two independent groups, using classical clinical observation and premolecular biochemical techniques, reported on cohorts of subjects harboring genetic mutations affecting androgen metabolism (Imperato-McGinley *et al.* 1974; 1979; Walsh *et al.* 1974). Subjects with these mutations were characterized by impaired activity of 5αR, with marked reductions in the levels of 5α-reduced steroid metabolites, including decreased levels of DHT, the 5α-reduced metabolite of testosterone. Males homozygous for genetic 5αR deficiency are born with ambiguous genitalia and initially reared as girls; with puberty, virilization occurs, presumably due to high circulating levels of testosterone, along with the transition from a female to a male sexual identity, development of male muscle mass and normal skeletal integrity. As adults, these men are otherwise healthy, with sparse facial and body hair and apparent protection against development of common

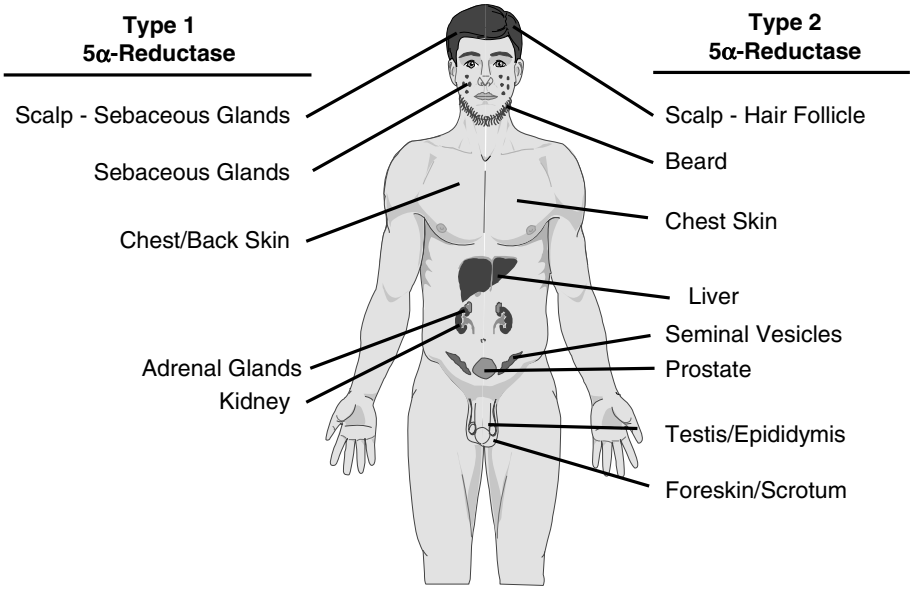


Fig. 18.2 5α-reductase enzyme activity in adult human tissues (adapted from Gormley 1995).

androgen-related disorders associated with aging, including benign prostatic hyperplasia (BPH), prostate cancer and male pattern hair loss, despite normal or supranormal circulating levels of testosterone. Subsequent reports of normal spermatogenesis and fertility in these subjects strengthened the theory that testosterone, rather than DHT, is the key androgen supporting reproductive capacity in men. Females with genetic 5αR deficiency can be detected by biochemical assay and are considered phenotypically normal, although subtle physiological alterations, such as delayed onset of menarche and decreased body hair growth, have been reported (Katz *et al.* 1995).

It was subsequently discovered, and later confirmed through genetic sequencing, that two distinct isoenzymes of 5αR, referred to as types 1 and 2, exist in humans and most other species studied (Andersson *et al.* 1991; Russell and Wilson 1994). DNA sequencing analysis demonstrates that subjects with genetic 5αR deficiency harbor mutations in the gene coding for the type 2 isoenzyme while their type 1 gene is normal. This is consistent with localization studies that subsequently identified differing amounts of the two isoenzymes in specific tissues (Harris *et al.* 1992; Russell and Wilson 1994; Thigpen *et al.* 1993). For example, while both isoenzymes are prominent in the liver, contributing to circulating levels of DHT, type 2 5αR is predominant in the genitourinary tract, including the prostate, while type 1 5αR is predominant in sebaceous glands of the skin (Fig. 18.2). Subsequent genetic studies have failed to identify functional mutations in the gene coding for the

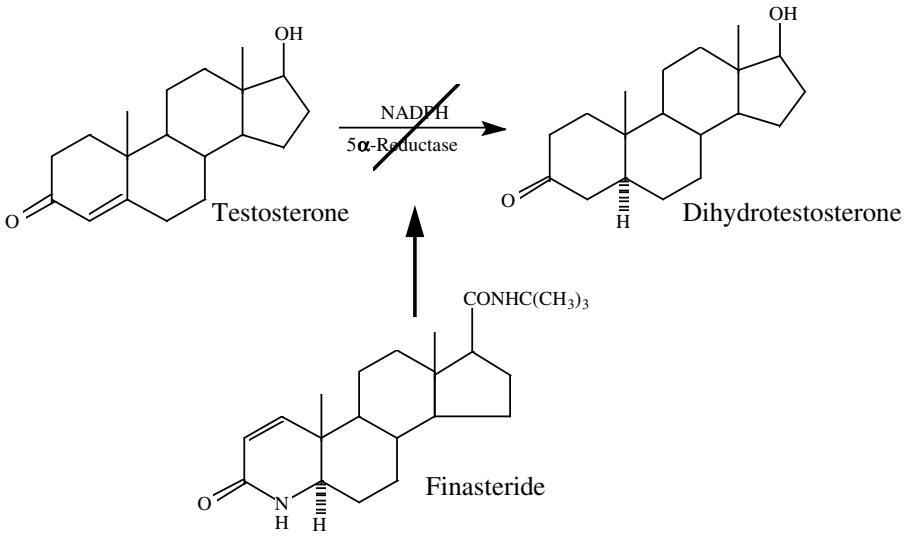


Fig. 18.3 Structure and mechanism of action of finasteride (Gormley 1991).

type 1 5αR enzyme (Russell and Wilson 1994). Taken together, the presence of two distinct 5αR isoenzymes accounts for the fact that suppression of DHT formation is incomplete in subjects with 5αR deficiency, even in those harboring mutations that yield no measurable type 2 enzymatic activity, while the differing amounts of the two isoenzymes in individual tissues provide insight into the observed sequelae of the genetic syndrome.

18.2 Rationale for and development of 5α-reductase inhibitors

The findings in subjects with genetic 5αR deficiency established essential roles for DHT in both male external genital development *in utero* and the pathogenesis of common, androgen-dependent disorders of adult men. Subsequently, several laboratories attempted to synthesize inhibitors of the human 5αR enzyme intended for clinical investigation to test for utility in the treatment of patients afflicted with these disorders, which include BPH, prostate cancer and male pattern hair loss (androgenetic alopecia, AGA).

The first of these inhibitors developed for clinical use was finasteride (N-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), a 4-azasteroid with high affinity for the human 5αR enzyme (Brooks *et al.* 1986; Liang *et al.* 1983, 1984; Rasmusson *et al.* 1983) (Fig. 18.3). In vitro, finasteride is a competitive inhibitor of the human 5αR enzyme, with no affinity for the human androgen receptor or intrinsic androgenic, estrogenic, progestational or other steroidal properties

(Rittmaster 1994; Stoner 1990). Once the existence of two isoenzymes of 5αR was reported, it was determined that finasteride was a potent and selective inhibitor of type 2 5αR, with minimal affinity for the type 1 enzyme (Harris *et al.* 1992). Safety studies conducted across a wide spectrum of biochemical parameters demonstrated excellent safety and tolerability (US Product Circular for Proscar^{R}, 1999). These findings are consistent with those observed in subjects with genetic 5αR deficiency who have been followed for more than 30 years and demonstrate no apparent deleterious effects of inhibition of DHT formation in adulthood (Imperato-McGinley 1997).

18.3 Early studies with finasteride, a type 2 5α-reductase inhibitor

Initial studies with finasteride were conducted in normal volunteers to determine the biochemical efficacy and safety profile of the drug after single doses or with multiple daily dosing.

18.3.1 Effects on serum androgens and gonadotropins

Administration of finasteride markedly reduces circulating DHT levels in adult men (~70% below baseline), and inhibition of DHT formation is maintained with chronic dosing (Gormley 1995; Gormley *et al.* 1990; Stoner *et al.* 1994). Because finasteride is a selective inhibitor of the type 2 5αR enzyme in man, complete inhibition of DHT formation does not occur with treatment. Subsequent studies confirmed that the residual level of circulating DHT (~30% of total circulating DHT) observed with finasteride administration derives from the activity of the type 1 5αR enzyme (see Section 18.8), which is unaffected by finasteride. Because conversion of testosterone to DHT is reduced with finasteride administration, metabolism of testosterone is reduced, thereby leading to a small (~15%) increase in serum testosterone. Since testosterone is the primary substrate for estradiol formation by aromatase in men (Fig. 18.1), there is a similar small (~15%) increase in circulating estradiol levels. Because both testosterone and estradiol increase to a similar extent with finasteride treatment, the ratio of circulating testosterone to estradiol is unaltered. Finasteride treatment for six months produced no clinically significant effects on sex hormone-binding globulin or free hormone (testosterone) levels (Tenover *et al.* 1989).

Despite the marked reduction in circulating levels of DHT with finasteride treatment, circulating levels of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), remain within normal limits in treated men, with mean levels either unchanged or increased slightly. Provocative testing of the hypothalamic-pituitary axis, using gonadotropin-releasing hormone (GnRH),

demonstrates that the response of LH and FSH to GnRH stimulation is not affected by finasteride treatment (Rittmaster *et al.* 1992).

18.3.2 Effects on other hormones

Finasteride administration has no effect on circulating levels of cortisol (basal or adrenocorticotropic hormone [ACTH]-stimulated), prolactin, thyroid-stimulating hormone or thyroxine, and no effect on glucose tolerance is observed.

18.3.3 Effects on hematologic parameters

No effects on hematologic or clinical chemistry safety parameters are observed with finasteride treatment.

18.4 Clinical studies with finasteride in men with benign prostatic hyperplasia

Early studies with finasteride in men with benign prostatic hyperplasia (BPH) were designed to confirm the biochemical efficacy of the drug. Prior studies had identified the predominance of DHT, compared to testosterone, within the prostate due to intraprostatic type 2 5αR activity (Bruchovsky and Wilson 1968). In several studies evaluating the ability of finasteride to reduce DHT formation within the prostate, suppression of intraprostatic DHT levels up to 95%, exceeding the maximal suppression of serum DHT (~70%), were demonstrated in a dose-dependent manner (Geller 1990; McConnell *et al.* 1992; Norman *et al.* 1993).

18.4.1 Efficacy based on prostate volume and symptoms

Several controlled studies have established the utility of finasteride 5 mg in the treatment of men with benign prostatic hyperplasia. Early clinical efficacy studies with finasteride in men with BPH demonstrated that the biochemical efficacy of the drug, defined by reductions in serum and intraprostatic DHT levels, were associated with significant reductions (~20%) in prostate volume (Stoner *et al.* 1994). Dose-ranging studies established the optimal dose for treatment of men with BPH to be between 1 and 5 mg per day, based on prostate volume reduction and improvements in BPH symptoms. Subsequent replicate, definitive, multicenter studies demonstrated the superiority of the higher dose and established 5 mg/day as the optimal dose for the treatment of men with this disease. Efficacy was established based on patient self-reported improvement in the symptoms associated with BPH using a validated symptom score questionnaire, and improvement in maximum urinary flow (increase of ~1.5 mL/sec), with these benefits associated with reductions in prostate volume and circulating DHT (Gormley *et al.* 1992; Finasteride Study Group 1993). Finasteride 5 mg (Proscar™) was first approved for marketing

in 1992 and is currently marketed in over 100 countries for the treatment of men with symptomatic BPH.

18.4.2 Long-term effects on disease progression

Two long-term, multicenter studies demonstrated the sustained effects of finasteride 5 mg on BPH symptoms and, more significantly, on the impact of therapy on disease progression.

The Proscar Long-Term Efficacy and Safety Study (PLESS; Roehrborn *et al.* 1999a), a placebo-controlled multicenter study in which 3040 men with symptomatic BPH were randomized (1:1) to finasteride 5 mg or placebo for four years, demonstrated that treatment with finasteride led to sustained improvements in BPH symptoms and reduced the risks of acute urinary retention and need for BPH-related surgery by approximately 50% within four months of treatment and throughout the four years of controlled clinical observation (McConnell *et al.* 1998). While prostate volume and serum prostate-specific antigen (PSA) level were strong predictors of risk of these sequelae of BPH (Roehrborn *et al.* 1999a), risk reduction due to finasteride treatment was observed irrespective of baseline prostate volume or serum PSA level (Roehrborn *et al.* 1999b) (Fig. 18.4). This study was the first of its kind, establishing the concept that medical intervention could favorably alter the natural course of BPH and confirming the hypothesis that chronic inhibition of DHT formation through type 2 5αR inhibition would reduce the undesirable sequelae of the disease.

The MTOPS (Medical Therapy of Prostatic Symptoms) study (McConnell *et al.* 2003), a multicenter factorial study sponsored by the U.S. National Institutes of Health-National Institute of Diabetes and Digestive and Kidney Diseases (NIH-NIDDK), randomized 3047 patients with symptomatic BPH equally to receive one of four treatments: finasteride 5 mg/day; doxazosin (Cardura^{TM}), an α-adrenergic receptor antagonist used in the treatment of men with BPH, 4 to 8 mg/day; both treatments; or placebo. Patients were followed for four to six years. Results of this study confirmed the findings observed in PLESS: treatment with finasteride 5 mg administered alone, or concomitantly with doxazosin, produced significant reductions in the incidences of acute urinary retention and need for BPH-related surgery compared to placebo. While these reductions were not observed in patients treated with doxazosin alone, treatment with either doxazosin or finasteride 5 mg reduced clinical deterioration in BPH symptoms, defined as a $\geq$ 4-point rise from baseline AUA Symptom Score (baseline symptom score at study entry = 8 to 30). Based on a composite endpoint of clinical progression of BPH, all active treatments were superior to placebo, while concomitant treatment with finasteride 5 mg and doxazosin was found to be superior to either agent alone. The results of the MTOPS study

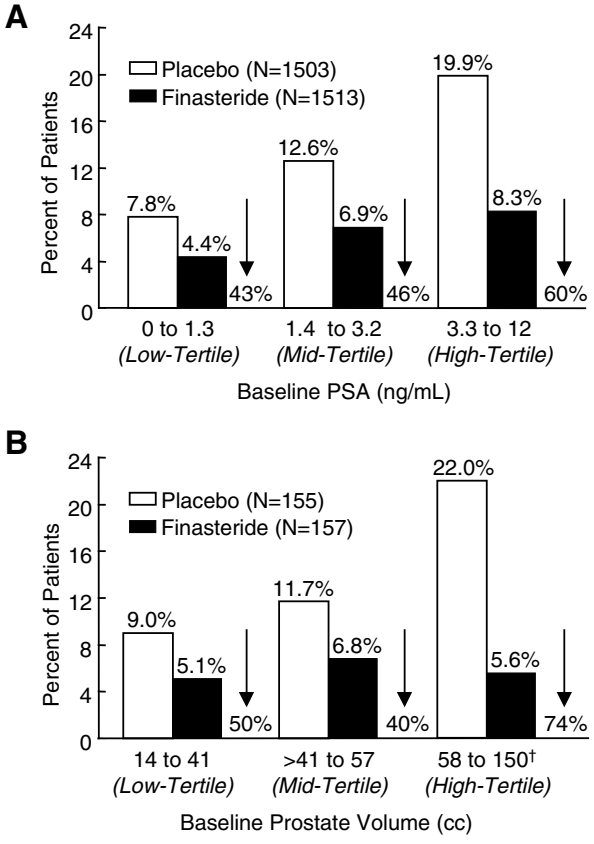


Fig. 18.4 Effects of finasteride 5 mg and placebo on men with BPH: four-year incidences of acute urinary retention or BPH-related surgery over four years in patients grouped by tertiles of: (A) baseline serum prostate-specific antigen (PSA) level, or (B) baseline prostate volume (†one placebo patient had a prostate volume of 222) (adapted from Roehrborn *et al*. 1999b).

confirm the results obtained previously in PLESS and provide new information on the long-term effects of these two agents used in the treatment of men with BPH.

18.4.3 Safety

Based on multiple controlled clinical trials in men with BPH, treatment with finasteride has been shown to be generally well tolerated. Side-effects of treatment are generally transient and usually do not result in discontinuation of drug use. The most frequently reported side-effects include impairment of sexual function (decreased libido, erectile dysfunction, and decreased ejaculate volume). There is no evidence of increased side-effects with increased duration of treatment. Some patients have reported development of breast tenderness or enlargement: in controlled clinical trials, the incidence of breast enlargement reported with finasteride

5 mg was approximately 1% greater than that reported with placebo. Breast neoplasms have also been reported in men receiving finasteride. In the MTOPS study, which randomized 3047 patients to one of four treatment arms (1:1:1:1) over four to six years of observation, breast cancer was reported in four men randomized to the two treatment arms that included finasteride (finasteride alone or finasteride plus doxazosin), with no cases reported in men randomized to the two treatment arms that did not include finasteride (doxazosin alone or placebo). However, in PLESS, which randomized 3040 patients to finasteride 5 mg or placebo (1:1) over four years of observation, two cases of breast cancer were reported in men receiving placebo, with no cases reported in men receiving finasteride, and in the largest and longest study, the Prostate Cancer Prevention Trial (PCPT; see Feigl *et al.* 1995 and Section 18.9.1), which randomized 18,882 men to finasteride 5 mg or placebo (1:1) over seven years of observation, an equal number of cases of breast cancer was reported in men in each treatment group (one in the group receiving finasteride 5 mg and one in the group receiving placebo).

18.5 Clinical studies with finasteride in men with androgenetic alopecia

As in the development program in men with BPH, initial clinical studies with finasteride in men with male pattern hair loss (androgenetic alopecia, AGA) were directed toward demonstration of biochemical efficacy (Kaufman 1996). Androgen receptor number, DHT content and 5αR activity were all reported to be higher in balding than non-balding scalp from subjects with AGA, lending further support to the hypothesis that lowering DHT content in the scalp would be useful in the treatment of patients with AGA (Dallob *et al.* 1994; Price 1975; Randall *et al.* 1991; Sawaya 1991). Subsequent immunolocalization and enzyme inhibitor studies demonstrated that type 2 5αR protein was expressed in structures within the hair follicle (Bayne *et al.* 1999; Hoffmann and Happle 1999). The early studies with finasteride in men with AGA demonstrated that daily oral administration reduced the DHT content of the affected scalp in a dose-dependent manner, based on analysis of scalp biopsies, and suggested that the dose range from 0.2 to 5 mg be evaluated for assessment of clinical benefit (Dallob *et al.* 1994; Drake *et al.* 1999).

18.5.1 Efficacy based on hair count, hair weight, clinical photography, patient assessment

Several controlled clinical studies established the efficacy of finasteride 1 mg in the treatment of men with AGA (Kaufman and Dawber 1999; Shapiro and Kaufman 2003). A placebo-controlled, proof-of-concept pilot study with finasteride 5 mg confirmed the utility of the mechanism of 5αR inhibition and suppression of DHT formation in the treatment of men with AGA (Roberts *et al.* 1999). Subsequent clinical dose-ranging studies established 1 mg as the optimal daily dose for treatment

of this disorder, consistent with the results of earlier studies evaluating the biochemical efficacy of finasteride in this patient population (Roberts *et al.* 1999). Definitive multicenter placebo-controlled studies established the beneficial effect of finasteride 1 mg on scalp hair growth in both the vertex and frontal (anterior mid-scalp) areas of the scalp, using a comprehensive set of endpoints in men with AGA (Kaufman *et al.* 1998; Leyden *et al.* 1999). Summarizing a body of evidence, the benefit of finasteride treatment was consistently demonstrated using a variety of evaluation techniques across multiple studies. These included: (1) macrophotographic hair count, obtained from a defined area of the vertex or frontal scalp (Canfield 1996; Kaufman *et al.* 1998; Leyden *et al.* 1999); (2) phototrichogram analysis, obtained from a defined area of the vertex scalp (Van Neste *et al.* 2000); (3) hair weight, obtained from a defined area of the frontal scalp (Price *et al.* 2002); (4) scalp biopsy, obtained from a defined area of the vertex scalp adjacent to the area used for macrophotographic hair count (Whiting 1990; 1993; Whiting *et al.* 1999); (5) standardized 'global' clinical photography of the vertex or frontal scalp, which also provides a measure of change in the degree of scalp coverage (Canfield 1996) (6) investigator clinical assessment of scalp hair growth; and (7) patient self-assessment of scalp hair growth and satisfaction with scalp hair appearance (Barber *et al.* 1998). Each of these evaluation techniques supports, and the consistency of the results confirms, that, by reducing perifollicular DHT through suppression of DHT formation, finasteride treatment leads to improvement in scalp hair growth in men with AGA. Finasteride 1 mg (Propecia^{TM}) was first approved for marketing in 1997 and is currently approved for the treatment of men with AGA in over 60 countries.

18.5.2 Study in monozygotic twins

Stough *et al.* (2002) reported results from a randomized, placebo-controlled study evaluating the effects of finasteride 1 mg vs. placebo in nine male monozygotic (identical) twin pairs with AGA over one year of observation, with each twin pair randomized to either finasteride 1 mg or matching placebo. While the sample of subjects available for such a study is necessarily limited in size, the results observed in these identical twin pairs were consistent with results from other clinical trials enrolling larger numbers of subjects, supporting the conclusions regarding the benefits of finasteride treatment in this unique and rare patient population studied.

18.5.3 Long-term follow-up

The two definitive, placebo-controlled studies in men with predominantly vertex hair loss were initially conducted over two years, with cohorts of patients randomized to active (finasteride 1 mg) or placebo treatment continuously or switched to

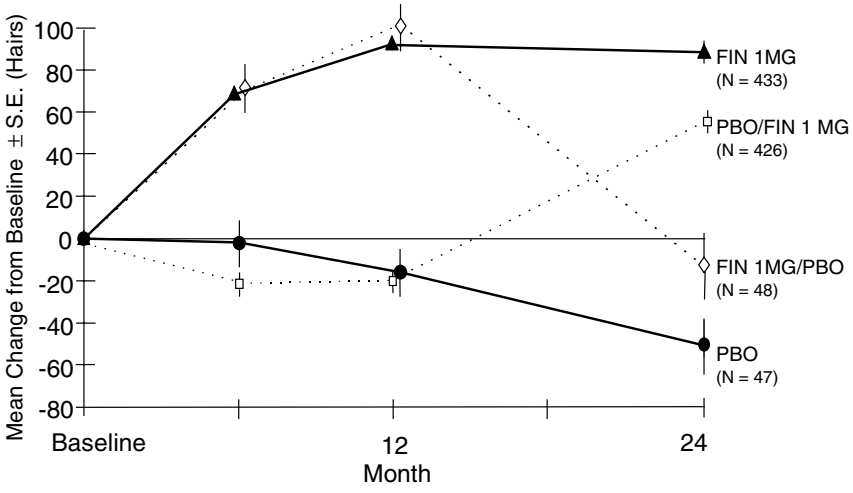


Fig. 18.5 Effects of finasteride 1 mg and placebo on men with AGA: hair count in a defined area of the vertex scalp over two years (Kaufman *et al.* 1998).

the alternate treatment after the first year (Kaufman *et al.* 1998). Fig. 18.5 shows the effect of treatment allocation on the primary efficacy endpoint of these studies, macrophotographic hair count obtained in a defined, 1-inch diameter circular area (5.1 cm^2) of scalp hair at the anterior leading edge of the vertex bald spot (baseline hair count = 876 hairs). These studies were continued as controlled clinical trials over five years for determination of the long-term efficacy and safety profile of finasteride 1 mg in the treatment of men with AGA (Finasteride Male Pattern Hair Loss Study Group 2002; Shapiro and Kaufman 2003). The results of the five-year controlled extensions to the two definitive studies in men with predominantly vertex hair loss demonstrated that treatment with finasteride produced durable improvements in scalp hair growth, with the separation between the treatment groups (finasteride vs. placebo) increasing over time. These long-term studies also demonstrated that the incidences of newly-reported side-effects declined with long-term use.

Most of the reviewed studies enrolled men with mild to moderately severe AGA (Norwood-Hamilton scale II–V hair loss patterns) (Hamilton 1951; Norwood 1975) who were between the ages of 18 and 41 years at the time of initial randomization. A separate study evaluating men with more severe AGA was recently concluded but results have not yet been released. In older men (ages 41 to 60 years) with AGA, Whiting and co-workers reported on the results of a two-year placebo-controlled study with finasteride 1 mg in this patient population (Whiting *et al.* 2003). As in the younger population, results in this population of men with AGA demonstrated improvement in scalp hair growth over the two years of observation, although men

ages 41–50 had numerically greater scores of improvement compared to those aged 51–60 based on the primary efficacy endpoint (standardized clinical photography).

18.5.4 Safety

In studies of finasteride 1 mg in young men with AGA, side-effects related to finasteride treatment were confined to transient impairment of sexual function (decreased libido, erectile dysfunction, and decreased ejaculate volume) in a small number of men. In aggregate, in studies in younger men, 3.8% of patients receiving finasteride compared to 2.1% receiving placebo reported these side-effects, yielding a treatment group difference of < 2% (US Product Circular for Propecia® 2002). In the two-year placebo-controlled study of finasteride 1 mg in older men with AGA, more patients reported side-effects related to sexual function in each treatment group, as expected: 8.7% for finasteride and 5.1% for placebo, yielding a treatment group difference of 3.6% (Whiting *et al.* 2003). Few patients discontinued the studies because of these side-effects, and the incidence of reported side-effects declined with continued treatment.

18.6 Safety studies with finasteride in men

18.6.1 Effects on bone

The effects of finasteride, both at a 5 mg and 1 mg daily dose, on bone have been evaluated in several studies. In men with BPH, finasteride 5 mg was shown to have no deleterious effects on markers of bone formation compared to placebo over one year or on bone mineral density compared to placebo over four years. In young men with AGA, finasteride had no deleterious effects on markers of bone formation or bone mineral density compared to placebo in a 48-week study. Taken together, these data demonstrate that finasteride has no deleterious effects on bone integrity in men. Recent evidence supports the hypothesis that estrogen is the primary mediator of bone integrity in both men and women, based on findings in male subjects with aromatase deficiency.

18.6.2 Effects on semen

Three separate studies evaluated the effects of finasteride 5 mg and 1 mg on semen production in young men. In two placebo-controlled studies evaluating the effects of finasteride at a daily dose of 5 mg, small (25%) reductions in ejaculate volume that were reversible upon discontinuation of drug were observed, while sperm concentration was not altered. This transient effect of finasteride 5 mg on ejaculate volume is believed to be due to reduction in the prostatic contribution to the ejaculate (US Product Circulars for Propecia® 2002 and Proscar® 1999). In a subsequent placebo-controlled study evaluating the effects of finasteride at a daily

dose of 1 mg, no significant differences in ejaculate volume, sperm concentration, total sperm per ejaculate, percent motile sperm or percent sperm with normal morphology were observed (Overstreet *et al.* 1999). These findings support the concept of a dose-dependent effect of finasteride on ejaculate volume through an effect on the prostatic contribution to the ejaculate. This dose-dependence of effect of finasteride on prostatic function is consistent with similar findings of a dose dependence between 1 mg and 5 mg daily doses of finasteride, such as has been reported for serum DHT (Gormley *et al.* 1992), intraprostatic DHT suppression (Norman *et al.* 1993), and clinical benefit in men with BPH (Finasteride Study Group 1993; Gormley *et al.* 1992).

18.6.3 Effects on lipids

Finasteride treatment does not affect the fasting lipid profile (total cholesterol, LDL-cholesterol or triglycerides). In some studies, a small (~10%) increase in plasma HDL-cholesterol level has been observed with finasteride (1 mg or 5 mg per day) compared to placebo, but this effect has not been consistently demonstrated.

18.7 Clinical studies with finasteride in women

Finasteride is not indicated for use in women. Due to its mechanism of action (type 2 5αR inhibition), finasteride use is *contraindicated* in women when they are or may be pregnant because of the risk of undervirilization of a developing male fetus. However, several studies have been published testing finasteride in women with a variety of disorders, including female pattern hair loss and hirsutism.

18.7.1 Study in postmenopausal women with androgenetic alopecia

To determine whether finasteride has utility in the treatment of women with AGA (female pattern hair loss), a randomized, placebo-controlled, one-year study of 137 postmenopausal women with AGA was conducted (Price *et al.* 2000). Women were eligible if they were assessed by the investigator as being Ludwig class I to II (Ludwig 1977) and Savin scale hair density and pattern classification 3 to 5 (Savin 1994). At the end of one year, no benefit of finasteride treatment compared to placebo was demonstrated in any predefined efficacy endpoint, including macrophotographic hair count, global photographic assessment, investigator assessment, patient self-assessment, and histopathologic analysis of scalp biopsies (Whiting *et al.* 1999). Thus, the pathophysiology of AGA in postmenopausal women appears to differ from that of men with AGA. This difference in response between men and postmenopausal women is likely related to the differing hormonal environment of the hair follicle between men and women and may explain the differing phenotypes of male and female pattern hair loss (Olsen 1994; Sawaya and Price 1997). Other

lines of evidence supporting a differing pathophysiology between male and female pattern hair loss have been provided, including differences in the perifollicular hormonal environment and levels of 5αR activity in vitro. Taken together, these reports have prompted scientists to question whether typical female pattern hair loss is, in fact, androgen-dependent (Olsen 2001).

18.7.2 Studies in women with hirsutism

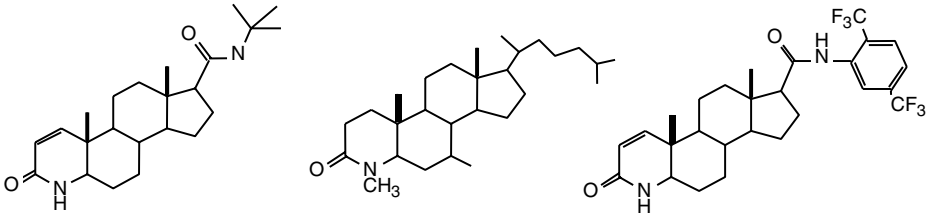
Several clinical trials evaluating the utility of finasteride in the treatment of women with hirsutism have been published (Castello *et al.* 1996; Ciotta *et al.* 1995; Erenus *et al.* 1997; Faloia *et al.* 1998; Falsetti *et al.* 1997; Fruzzetti *et al.* 1994; 1999; Moghetti *et al.* 1994; Sahin *et al.* 1998; Tolino *et al.* 1996; Venturoli *et al.* 1999; Wong *et al.* 1995). While some studies were uncontrolled and thus of limited value, others were conducted as placebo- or active comparator-controlled (with spironolactone, flutamide, ketoconazole or cyproterone acetate as comparator) trials. It should be noted that most of these studies enrolled premenopausal women of childbearing potential who were counseled regarding appropriate contraception requirements to avoid pregnancy; as was noted earlier, finasteride use is contraindicated in women when they are or may be pregnant. Nonetheless, the results of some studies demonstrated that finasteride treatment produced modest benefit in women with hirsutism up to 12 months of observation, based on improvements in the Ferriman-Gallwey score (Ferriman and Gallwey 1961), terminal hair diameter, or other measures, that was superior to placebo and generally comparable to the active comparator used. Few side-effects related to finasteride treatment were reported in this patient population.

18.8 Preliminary studies with MK-386, a type 1 5α-reductase inhibitor

Separate studies were conducted with a type 1-selective 5αR inhibitor to determine utility of this mechanism of action, which differs from that of finasteride in inhibiting the alternate isoenzyme of 5αR, in the treatment of clinical disorders. Based on the known stimulation of acne by androgens (Hamilton 1941) and tissue localization of the type 1 isoenzyme (Harris *et al.* 1992; Thiboutot *et al.* 1995), which is prominent in sebaceous glands of the skin, a potential target for intervention was in the treatment of patients with acne vulgaris.

18.8.1 Effects on serum and sebum DHT

Studies conducted in normal volunteers confirmed that the type 1-selective 5αR inhibitor, MK-386 (Merck & Co., Inc.) (Fig. 18.6), reduced serum DHT concentrations by approximately 30% when administered once daily orally (Ellsworth *et al.* 1996; Schwartz *et al.* 1996). Oral administration of MK-386 also reduced *sebum*



Type 2-selective 5αR inhibitor (finasteride) | Type 1-selective 5αR inhibitor (MK-386) | Non-selective 5αR inhibitor (dutasteride)

Fig. 18.6 Structures of type 2-selective, type 1-selective and non-selective 5α-reductase inhibitors (Harris and Kozarich 1997).

DHT concentrations by approximately 50%, based on a standardized, validated method for measuring sebum output (Schwartz *et al.* 1997). In a separate study, Imperato-McGinley and co-workers demonstrated no difference in sebum output between subjects with type 2 5αR deficiency and normals, and included subjects with androgen insensitivity as a 'positive control', who, as expected, demonstrated no measurable sebum output over the measurement period (Imperato-McGinley *et al.* 1993). These findings led to the hypothesis that treatment with a type 1-selective 5αR inhibitor could have utility in the treatment of patients with acne. However, a placebo- and active-controlled clinical proof of concept study with MK-0219 (Merck & Co, Inc.), another type 1-selective 5αR inhibitor, in patients with acne vulgaris failed to demonstrate utility in this disorder (Leyden *et al.* 2004). To date, no type 1-selective 5αR inhibitors have been brought through clinical development to marketing approval.

18.8.1.1 Effects in combination with finasteride

Studies conducted in normal volunteers evaluated the effects of concomitant administration of finasteride (a type 2-selective 5αR inhibitor) and the type 1-selective 5αR inhibitor, MK-386. Administration of finasteride for seven days produced ~65% reduction in circulating DHT, as expected. Subsequent dosing of MK-386 co-administered with finasteride demonstrated additivity of DHT reduction, producing near complete suppression (~95%) of circulating DHT (Schwartz *et al.* 1996). These data were consistent with the prior observation, obtained from patients with type 2 5αR deficiency, that there are two separate 5αR enzymes contributing to circulating DHT, with the type 2 5αR isoenzyme contributing ~2/3 of circulating DHT while the type 1 isoenzyme contributed ~1/3. Further, the results of this study confirmed that isoenzyme-specific 5αR inhibitors can be used selectively or in combination in vivo. The near-complete suppression of circulating DHT observed with co-administration of type 1- and type 2-selective 5αR inhibitors

argues against the likelihood of a third, as yet undiscovered, 5αR isoenzyme in man.

18.9 Future research

18.9.1 Long-term study of finasteride in chemoprevention of prostate cancer

Preclinical studies in animals and in cell lines in vitro support the concept that DHT is an important promoter of prostatic growth and, potentially, of prostate cancer (Gormley 1991; Gormley *et al.* 1995). At present, no 5αR inhibitor is indicated for use in the prevention or treatment of prostate cancer. Recently, the results of the Prostate Cancer Prevention Trial (PCPT), sponsored by the U.S. National Cancer Institute (NCI) and coordinated by the Southwest Oncology Group (SWOG), were released (Feigl *et al.* 1995; Gormley *et al.* 1995; Thompson *et al.* 1997; 2003). This landmark study randomized (1:1) 18,882 men $\geq$55 years of age with no evidence of prostate cancer (normal prostate exam; serum prostate-specific antigen (PSA) level $\leq$3.0 ng/mL) to treatment with finasteride 5 mg per day or matching placebo. All men were to be evaluated for the presence of prostate cancer by needle biopsy of the prostate, either based on signs or symptoms suggestive of prostate cancer during the study or at the end of the study. The trial was stopped early due to premature attainment of the primary endpoint: a 25% reduction in the period prevalence of prostate cancer was observed in the group treated with finasteride 5 mg compared to the group treated with placebo (prostate cancer diagnosed in 18.4% of finasteride-treated subjects compared to 24.4% of placebo). However, a slightly higher percentage of subjects with Gleason grade 7–10 prostate cancer was reported in the finasteride group compared to the placebo group (6.4% vs. 5.1%). While the trial confirmed its primary hypothesis that chronic suppression of intraprostatic DHT reduces the incidence of prostate cancer, the implications of the secondary finding are less well defined. For example, it is not known whether the increase in the percent of patients with Gleason grade 7–10 prostate cancer represents tumors that are clinically more aggressive. Hypotheses to explain the secondary finding include: (1) selection for development of high-grade tumors due to the effects of finasteride; (2) detection bias, favoring diagnosis of higher grade tumors in the finasteride group (i.e., finasteride treatment suppresses low-grade tumors without suppressing high-grade tumors, combined with enhancement of prostate cancer detection by needle biopsy due to finasteride-induced prostate gland shrinkage); and (3) altered histologic appearance, towards appearance of less differentiation of prostate cancer tissue obtained by needle biopsy due to treatment with finasteride. The latter hypothesis is supported by reports of prostatic tissue atrophy and cell apoptosis associated with finasteride treatment (Rittmaster *et al.* 1996), and is a well-documented phenomenon associated with antiandrogen therapy in patients

with prostate cancer. Further analyses of data from the PCPT may clarify which of these hypotheses, if any, clarify this secondary finding.

18.9.2 Development of other 5α-reductase inhibitors

Since the development of finasteride, only one other 5αR inhibitor has been marketed for clinical use. Dutasteride ((5α,17β)-N-{2,5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide), a non-selective 5αR inhibitor from GlaxoSmithKline with affinity for both the type 1 and type 2 5αR isoenzymes in man (Bramsen *et al.* 1997) (Fig. 18.6), was approved for marketing in 2001 at a 0.5 mg daily dose (Avodart^{TM}) as a treatment for men with benign prostatic hyperplasia. While studies with dutasteride have also been conducted in men with AGA, definitive studies in this population have not been completed and the drug has not been approved for the treatment of men with this disorder. In clinical studies in men with BPH, dutasteride demonstrates an efficacy and safety profile that appears to be generally similar to that of finasteride 5 mg (U.S. Product Circular for Avodart^{R}, 2002). However, long-term studies covering more than two years have not been published, and there is no genetic model of dual (or of type 1) 5αR inhibition from which to obtain information regarding the implications of chronic inhibition of both 5αR isoenzymes in man. Other 5αR inhibitors have been synthesized and subsequently tested in clinical trials (Bakshi *et al.* 1995; Hirsch *et al.* 1993; Jones *et al.* 1993; Kojo *et al.* 1995; Levy *et al.* 1994; Nakayama *et al.* 1997; Ohtawa *et al.* 1991; Van Hecken *et al.* 1994), but to date none has reached marketing approval.

18.10 Key messages

- The development of 5αR inhibitors which followed the identification of a putative role for DHT, a key metabolite of testosterone, in the pathogenesis of androgen-dependent disorders of adult men has significantly expanded our understanding of androgen biology and contributed to novel treatments for patients.
- Finasteride, the first marketed 5αR inhibitor, is selective for the type 2 isoenzyme in man and is marketed for the treatment of men with BPH at a 5 mg daily dose and for the treatment of men with AGA at a 1 mg daily dose.
- Recently, dutasteride, a non-selective 5αR inhibitor, was approved for marketing for the treatment of men with BPH.
- No type 1-selective 5αR inhibitors have been brought through clinical development to marketing approval.
- Currently, there are no approved uses for 5αR inhibitors in women.
- Future developments, such as detailed understanding of the molecular mechanisms underlying the discrete actions of specific androgens, such as testosterone and DHT, offer the promise of greater insight into the pathogenesis of androgen-mediated disorders in affected patients.

18.11 REFERENCES

Anderson KM, Liao S (1968) Selective retention of dihydrotestosterone by prostatic nuclei. Nature 219:277–279

Andersson S, Berman DM, Jenkins EP, Russell DW (1991) Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. Nature 354:159–161

Bakshi RK, Rasmusson GH, Patel GF, Mosley RT, Chang B, Ellsworth K, Harris GS, Tolman RL (1995) 4-Aza-3-oxo-5α-androst-1-ene-17β-N-aryl-carboxamides as dual inhibitors of human type 1 and type 2 steroid 5α-reductases. Dramatic effect of N-aryl substituents on type 1 and type 2 5α-reductase inhibitory potency. J Med Chem 38:3189–3192

Barber BL, Kaufman KD, Kozloff RC, Girman CJ, Guess HA (1998) A hair growth questionnaire for use in the evaluation of therapeutic effects in men. J Dermatol Treat 9:181–186

Bayne EK, Flanagan J, Einstein M, Ayala J, Chang B, Azzolina B, Whiting, DA, Mumford RA, Thiboutot D, Singer II, Harris G (1999) Immunohistochemical localization of types 1 and 2 5α-reductase in human scalp. Brit J Dermatol 141:481–91

Bramson HN, Hermann D, Batchelor KW, Lee FW, James MK, Frye SV (1997) Unique preclinical characteristics of GG745, a potent dual inhibitor of 5AR. J Pharmacol Exp Ther 282:1496–1502

Brooks JR, Berman C, Garnes D, Giltinan D, Gordon LR, Malatesta PF, Primka RL, Reynolds GF, Rasmusson GH (1986) Prostatic effects induced in dogs by chronic or acute oral administration of 5 α-reductase inhibitors. Prostate 9:65–75

Bruchovsky N and Wilson JD (1968) The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243:2012–2021

Canfield D (1996) Photographic documentation of hair growth in androgenetic alopecia. Dermatol Clin 14:713–721

Castello R, Tosi F, Perone G, Negri C, Muggeo M, Moghetti P (1996) Outcome of long-term treatment with the 5α-reductase inhibitor finasteride in idiopathic hirsutism: clinical and hormonal effects during a 1-year course of therapy and 1-year follow-up. Fertil Steril 66: 34–740

Ciotta L, Cianci A, Calogero AE, Palumbo, MA, Marletta E, Sciuto A, Palumbo G (1995) Clinical and endocrine effects of finasteride, a 5α-reductase inhibitor, in women with idiopathic hirsutism. Fertil Steril 64:99–306

Dallob AL, Sadick NS, Unger W, Lipert S, Geissler LA, Gregoire SL, Nguyen HH, Moore EC, Tanaka WK (1994) The effect of finasteride, a 5α-reductase inhibitor, on scalp skin testosterone and dihydrotestosterone concentrations in patients with male pattern baldness. J Clin Endocrinol Metab 79:703–706

Drake L, Hordinsky M, Fiedler V, Swinehart J, Unger WP, Cotterill PC, Thiboutot DM, Lowe N, Jacobson C, Whiting D, Stieglitz S, Kraus SJ, Griffin EI, Weiss D, Carrington P, Gencheff C, Cole GW, Pariser DM, Epstein ES, Tanaka W, Dallob A, Vandormael K, Geissler L, Waldstreicher J. (1999) The effects of finasteride on scalp skin and serum androgen levels in men with androgenetic alopecia. J Am Acad Dermatol 41:550–554

Ellsworth K, Azzolina B, Baginsky W, Bull H, Chang B, Cimis G, Mitra S, Toney J, Bakshi RK, Rasmusson GR, Tolman RL, Harris GS (1996) MK386: a potent, selective inhibitor of the human type 1 5α-reductase. J Steroid Biochem Mol Biol 58:377–384

Erenus M, Yucelten D, Durmusoglu F, Gurbuz O (1997) Comparison of finasteride versus spironolactone in the treatment of idiopathic hirsutism. Fertil Steril 68:1000–1003

Faloia E, Filipponi S, Mancini V, Di-Marco S, Mantero F (1998) Effect of finasteride in idiopathic hirsutism. J Endocrinol Invest 21:694–698

Falsetti L, De-Fusco D, Eleftheriou G, Rosina B (1997) Treatment of hirsutism by finasteride and flutamide in women with polycystic ovary syndrome. Gynecol Endocrinol 11:251–257

Fang S, Anderson KM, Liao S (1969) Receptor proteins for androgens. On the role of specific proteins in selective retention of 17β-hydroxy-5α-androstan-3-one by rat ventral prostate in vivo and in vitro. J Biol Chem 244:6584–6595

Feigl P, Blumenstein B, Thompson I, Crowley J, Wolf M, Kramer BS, Coltman CA, Brawley OW, Ford GF (1995) Design of the Prostate Cancer Prevention Trial (PCPT) Cont Clin Trials 16:150–163

Ferriman D, Gallwey JD (1961) Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 21:1440–1447

Finasteride Male Pattern Hair Loss Study Group (2002) Long-term (5-year) multinational experience with finasteride 1 mg in the treatment of men with androgenetic alopecia. Eur J Dermatol 12:38–49

Finasteride Study Group (1993) Finasteride (MK-906) in the treatment of benign prostatic hyperplasia. Prostate 22:291–299

Fruzzetti F, de Lorenzo D, Parrini D, Ricci C (1994) Effects of finasteride, a 5α -reductase inhibitor, on circulating androgens and gonadotropin secretion in hirsute women. J Clin Endocrinol Metab 79:831–835

Fruzzetti F, Bersi C, Parrini D, Ricci C, Genazzani AR (1999) Treatment of hirsutism: comparisons between different antiandrogens with central and peripheral effects. Fertil Steril 71:445–451

Geller J (1990) Effect of finasteride, a 5α-reductase inhibitor on prostatic tissue androgens and prostate-specific antigen. J Clin Endocrinol Metab 71:1552–1555

Gormley GJ (1991) The role of 5α-reductase inhibitors in the treatment of prostate cancer. Urol Clin North Am 18:93–98

Gormley GJ (1995) Finasteride: a clinical review. Biomed and Pharmacother 49:319–324

Gormley GJ, Stoner E, Rittmaster RS, Gregg H, Thompson DL, Lasseter KC, Vlasses PH, Stein EA (1990) Effects of finasteride (MK-906), a 5 alpha-reductase inhibitor, on circulating androgens in male volunteers. J Clin Endocrinol Metab 70:1136–1141

Gormley GJ, Stoner E, Bruskewitz RC, Imperato-McGinley J, Walsh PC, McConnell JD, Andriole GL, Geller J, Bracken BR, Tenover JS, Vaughn ED, Pappas F, Taylor A, Binkowitz B, Ng J (1992) The effect of finasteride in men with benign prostatic hyperplasia. N Engl J Med 327:1185–1191

Gormley GJ, Brawley O, Thompson I (1995) The potential application of finasteride for chemoprevention of prostate cancer. Ann NY Acad Sci 768:163–169

Hamilton JB (1941) Male hormone substance: a prime factor in acne. J Clin Endocrinol Metab 1:570–592

Hamilton JB (1942) Male hormone stimulation is a prerequisite and an incitant in common baldness. Am J Anat 71:451–480

Hamilton JB (1951) Patterned loss of hair in man: types and incidence. Ann NY Acad Sci 53: 708–728

Harris GS, Kozarich JW (1997) Steroid 5α-reductase inhibitors in androgen-dependent disorders. Curr Opin Chem Biol 1:254–259

Harris G, Azzolina B, Baginsky W, Cimis G, Rasmusson GH, Tolman RL, Raetz CR, Ellsworth K (1992) Identification and selective inhibition of an isozyme of steroid 5 alpha-reductase in human scalp. Proc Natl Acad Sci USA 89:10787–10791

Hirsch KS, Jones CD, Audia JE, Andersson S, McQuaid L, Stamm NB, Neubauer BL, Pennington P, Toomey RE, Russell DW (1993) LY191704: a selective, nonsteroidal inhibitor of human steroid 5α-reductase type 1. Proc Natl Acad Sci USA 90:5277–5281

Hoffmann R, Happle R (1999) Finasteride is the main inhibitor of 5α-reductase activity in microdissected dermal papillae of human hair follicles. Arch Dermatol Res 291: 100–103

Imperato-McGinley J (1997) 5α-reductase-2 deficiency. Curr Ther Endocrinol Metab 6:384–387

Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE (1974) Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science 186:1213–1215

Imperato-McGinley J, Peterson RE, Gautier T, Sturla A (1979) Male pseudohermaphroditism secondary to 5α-reductase deficiency – a model for the role of androgens in both the development of the male phenotype and the evolution of a male gender identity. J Steroid Biochem 11:637–645

Imperato-McGinley J, Gautier T, Cai LQ, Yee B, Epstein J, Pochi P (1993) The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. J Clin Endocrinol Metab 76:524–528

Jones CD, Audia JE, Lawhorn DE, McQuaid LA, Neubauer BL, Pike AJ, Pennington PA, Stamm NB, Toomey RE, Hirsch KS (1993) Nonsteroidal inhibitors of human type I steroid 5α-reductase. J Med Chem 36:421–423

Katz MD, Cai L-Q, Zhu Y-S, Herrera C, DeFillo-Ricart M, Shackelton CHL, Imperato-McGinley J (1995) The biochemical and phenotypic characterization of females homozygous for 5 alpha-reductase-2 deficiency. J Clin Endocrinol Metab 80:3160–3167

Kaufman KD (1996) Androgen metabolism as it affects hair growth in androgenetic alopecia. Dermatol Clin 14:697–711

Kaufman KD, Dawber RP (1999) Finasteride, a type 2 5α-reductase inhibitor, in the treatment of men with androgenetic alopecia. Exp Opin Invest Drugs 8:403–415

Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, Price VH, Van-Neste D, Roberts JL, Hordinsky M, Shapiro J, Binkowitz B, Gormley GJ (1998) Finasteride in the treatment of men with androgenetic alopecia. J Am Acad Dermatol 39:578–589

Kojo H, Nakayama O, Hirosumi J, Chida N, Notsu Y, Okuhara M (1995) Novel steroid 5α-reductase inhibitor FK143: its dual inhibition against the two isozymes and its effect on transcription of the isozyme genes. Mol Pharmacol 48:410–406

Levy MA, Brandt M, Sheedy KM, Dinh JT, Holt DA, Garrison LM, Bergsma DJ, Metcalf BW (1994) Epristeride is a selective and specific uncompetitive inhibitor of human steroid 5 alpha-reductase isoform 2. J Steroid Biochem Molec Biol 48:197–206

Leyden J, Dunlap F, Miller B, Winters P, Lebwohl M, Hecker D, Kraus S, Baldwin H, Shalita A, Draelos Z, Markou M, Thiboutot D, Rapaport M, Kang S, Kelly T, Pariser D, Webster G, Hordinsky M, Rietschel R, Katz I, Terranella L, Best S, Round E, Waldstreicher J (1999) Finasteride in the treatment of men with frontal male pattern hair loss. J Am Acad Dermatol 40:930–937

Leyden J, Bergfeld W, Drake L, Dunlap F, Goldman MP, Gottlieb AB, Heffernan MP, Hickman JG, Hordinsky M, Jarrett M, Kang S, Lucky A, Peck G, Phillips T, Rapaport M, Roberts J, Savin R, Sawaya ME, Shalita A, Shavin J, Shaw JC, Stein L, Stewart D, Strauss J, Swinehart J, Swinyer L, Thiboutot D, Washenik K, Weinstein G, Whiting D, Pappas F, Sanchez M, Terranella L, Waldstreicher J (2004) A systemic type I 5α-reductase inhibitor is ineffective in the treatment of acne vulgaris. J Am Acad Dermatol (in press)

Liang T, Rasmusson GH, Brooks JR (1983) Biochemical and biological studies with 4-aza-steroidal 5α-reductase inhibitors. J Steroidal Biochem 19:385–390

Liang T, Heiss CE, Cheung AH, Reynolds GF, Rasmusson GH (1984) 4-azasteroidal 5α-reductase inhibitors without affinity for the androgen receptor. J Biol Chem 259:734–739

Ludwig E (1977) Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Dermatol 97:247–254

Mainwaring WIP (1969) A soluble androgen receptor in the cytoplasm of rat prostate. J Endocrinol 45:531–541

Mainwaring WIP (1977) The mechanism of action of androgens. Monographs in Endocrinology. New York, Springer-Verlag

McConnell JD, Wilson JD, George FW, Geller J, Pappas F, Stoner E (1992) Finasteride, an inhibitor of 5α-reductase, suppresses prostatic dihydrotestosterone in men with benign prostatic hyperplasia. J Clin Endocrinol Metab 74:505–508

McConnell JD, Bruskewitz R, Walsh P, Andriole G, Lieber M, Holtgrewe HL, Albertsen P, Roehrborn CG, Nickel JC, Wang DZ, Taylor AM, Waldstreicher J (1998) The effect of finasteride on the risk of acute urinary retention and the need for surgical treatment among men with benign prostatic hyperplasia. N Engl J Med 338:557–563

McConnell JD, Roehrborn CG, Bautista OM, Andriole GL, Dixon CM, Kusek JW, Lepor H, McVary KT, Nyberg LM, Clarke HS, Crawford ED, Diokno A, Foley JP, Foster HE, Jacobs SC, Kaplan SA, Kreder KJ, Lieber MM, Lucia MS, Miller GJ, Menon M, Milam DF, Ramsdell JW, Schenkman NS, Slawin KM, Smith JA, for the Medical Therapy of Prostatic Symptoms (MTOPS) Research Group (2003) The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. N Engl J Med 349:2387–2398

Moghetti P, Castello R, Magnani CM, Tosi F, Negri C, Armanini D, Belloti G, Muggeo M (1994) J Endocrinol Metab 79:1115–1121

Morris JM, Mahesh VB (1963) Further observations on the syndrome, "testicular feminization." Am J Obstet Gynecol 87:731–734

Nakayama O, Hirosumi J, Chida N, Takahashi S, Sawada K, Kojo H, Notsu Y (1997) FR146687, a novel steroid 5 alpha-reductase inhibitor: in vitro and in vivo effects on prostates. Prostate 31:241–249

Norman RW, Coakes KE, Wright AS, Rittmaster RD (1993) Androgen metabolism in men receiving finasteride before prostatectomy J Urol 150:1736–1739

Norwood OT (1975) Male pattern baldness: classification and incidence. South Med J 68:1359–1365

Ohtawa M, Morikawa H, Shimazaki J (1991) Pharmacokinetics and biochemical efficacy after single and multiple oral administration of N-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, a new type of specific competitive inhibitor of testosterone 5α-reductase, in volunteers. Eur J Drug Metab Pharmacokin 16:15–21

Olsen EA (1994) Androgenetic alopecia. In: Olsen EA (ed) Disorders of hair growth: diagnosis and treatment. McGraw-Hill, Inc, New York, pp 257–283

Olsen EA (2001) Female pattern hair loss. J Am Acad Dermatol 45:S70–S80

Overstreet JW, Fuh, VL, Gould J, Howards SS, Lieber MM, Hellstrom W, Shapiro S, Carroll P, Corfman RS, Petrou S, Lewis R, Toth P, Shown T, Roy J, Jarow J, Bonillak J, Jacobsen C, Wang DZ, Kaufman KD (1999) Chronic treatment with finasteride does not affect spermatogenesis or semen production in young men. J Urol 162:1295–1300

Price VH (1975) Testosterone metabolism in the skin. A review of its function in androgenetic alopecia, acne vulgaris, and idiopathic hirsutism including recent studies with antiandrogens. Arch Dermatol 111:1496–1502

Price VH, Roberts JL, Hordinsky M, Olsen EA, Savin R, Bergfeld W, Fiedler V, Lucky A, Whiting DA, Pappas F, Culbertson J, Kotey P, Meehan A, Waldstreicher J (2000) Lack of efficacy of finasteride in postmenopausal women with androgenetic alopecia. J Am Acad Dermatol 43:768–776

Price V, Menefee E, Sanchez M, Ruane P, Kaufman KD (2002) Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride 1 mg daily. J Am Acad Dermatol 46:517–523

Randall VA, Thornton JM, Hamada K, Redfern CP, Nutbrown M, Ebling FJ, Messenger AG (1991) Androgens and the hair follicle. Cultured human dermal papilla cells as a model system. Ann NY Acad Sci 642:355–375

Rasmusson GH, Liang T, Brooks JR (1983) A new class of 5α-reductase inhibitors. In: Roy AK, Clark JH (eds), Gene regulation by steroid hormones II. Springer-Verlag, New York, pp 311–333

Rittmaster RS (1994) Finasteride. N Engl J Med 330:120–125

Rittmaster RS, Lemay A, Zwicker H, Capizzi TP, Winch S, Moore E, Gormley GJ (1992) Effect of finasteride, a 5α-reductase inhibitor, on serum gonadotropins in normal men. J Clin Endocrinol Metab 75:484–488

Rittmaster RS, Norman RW, Thomas LN, Rowden G (1996) Evidence for atrophy and apoptosis in the prostates of men given finasteride. J Clin Endocrinol Metab 81:814–819

Roberts JL, Fiedler V, Imperato-McGinley J, Whiting D, Olsen E, Shupack J, Stough D, DeVillez R, Rietschel R, Savin R, Bergfeld W, Swinehart J, Funicella T, Hordinsky M, Lowe N, Katz I, Lucky A, Drake L, Price VH, Weiss D, Whitmore E, Millikan L, Muller S, Gencheff C, Carrington P, Binkowitz B, Kotey P, He W, Bruno K, Jacobsen C, Terranella L, Gormley GJ, Kaufman KD (1999) Clinical dose ranging studies with finasteride, a type 2

5α-reductase inhibitor, in men with male pattern hair loss. J Am Acad Dermatol 41:555–563

Roehrborn, CG, Boyle P, Bergner D, Gray T, Gittleman M, Shown T, Melman A, Bracken B, deVereWhite, R Taylor, A Wang, D, Waldstreicher W, for the PLESS Study Group. (1999a) Serum prostate-specific antigen and prostate volume predict long-term changes in symptoms and flow rate: results of a four-year, randomized trial comparing finasteride versus placebo. Urology 54:662–669

Roehrborn CG, McConnell JD, Lieber M, Kaplan S, Geller J, Gholem M. H, Castellanos R, Coffield S, Saltzman B, Resnick M, Cook TJ, Waldstreicher J. (1999b) Serum prostate-specific antigen concentration is a powerful predictor of acute urinary retention and need for surgery in men with clinical benign prostatic hyperplasia. Urology 53:473–480

Russell DW, Wilson JD (1994) Steroid 5α-reductase: two genes/two enzymes. Ann Rev Biochem 63:25–61

Sahin Y, Bayram F, Kelestimur F, Muderris I (1998) Comparison of cyproterone acetate plus ethinyl estradiol and finasteride in the treatment of hirsutism. J Endocrinol Invest 2:348–352

Saunders FJ (1963) In: Vollmer EP (ed) Biology of the prostate and related tissue. U.S. Govt. Printing Office, Washington DC, pp 139–159

Savin RC (1994) Upjohn Dermatology Division, Upjohn Company

Sawaya ME (1991) Steroid chemistry and hormone controls during the hair follicle cycle. Ann NY Acad Sci 642:376–385

Sawaya ME, Price VH (1997) Different levels of 5 alpha-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. J Invest Dermatol 109:296–300

Schwartz JI, Van Hecken A, De Schepper PJ, De-Lepeleire I, Lasseter KC, Shamblen EC, Winchell GA, Constanzer ML, Chavez CM, Wang DZ, Ebel DL, Justice SJ, Gertz BJ (1996) Effect of MK-386, a novel inhibitor of type 1 5α-reductase, alone and in combination with finasteride, on serum dihydrotestosterone concentrations in men. J Clin Endocrinol Metab 81:2942–2947

Schwartz JI, Tanaka WK, Wang DZ, Ebel DL, Geissler LA, Dallob A, Hafkin B, Gertz BJ (1997) MK-386, an inhibitor of 5α-reductase type 1, reduces dihydrotestosterone concentrations in serum and sebum without affecting dihydrotestosterone concentrations in semen. J Clin Endocrinol Metab 82:1373–1377

Shapiro J, Kaufman KD (2003) Use of finasteride in the treatment of men with androgenetic alopecia (male pattern hair loss) J Invest Dermatol Symp Proc 8:20–23

Stoner E (1990) The clinical development of a 5α-reductase inhibitor, finasteride. J Steroid Biochem Mol Biol 37:375–378

Stoner E and the Finasteride Study Group (1994) The clinical effects of a 5 alpha-reductase inhibitor, finasteride, on benign prostatic hyperplasia. Urology 43:284–294

Stough DB, Rao NA, Kaufman KD, Mitchell C (2002) Finasteride improves male pattern hair loss in a randomized study in identical twins. Eur J Dermatol 12:32–37

Tenover JS, Zeitner ME, Plymate SR (1989) Effects of 24-week administration of a 5α-reductase inhibitor (MK-906) on serum levels of testosterone (T), free T, and gonadotropins in men. 71st Annual Meeting of the Endocrine Society (Seattle, Washington), Abstract 583

Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S (1995) Activity of the type 1 5α-reductase exhibits regional differences in isolated sebaceous glands and whole skin. J Invest Dermatol 105:209–214

Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW (1993) Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression. J Clin Invest 92: 903–910

Thompson IM, Coltman CA, Crowley J (1997) Chemoprevention of prostate cancer: the Prostate Cancer Prevention Trial. Prostate 33:217–222

Thompson IM, Goodman PJ, Tangen, CM, Lucia MS, Miller G, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA (2003) The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224

Tolino A, Petrone A, Sarnacchiaro F, Cirillo D, Ronsini S, Lombardi G, Nappi C (1996) Finasteride in the treatment of hirsutism: new therapeutic perspectives. Fertil Steril 66:61–65

US Product Circular for PROPECIA® (finasteride 1 mg tablets) (April 2002) In: PDR Electronic Library™, Thomson Micromedex

US Product Circular for PROSCAR® (finasteride 5 mg tablets) (August 1999) In: PDR Electronic Library™, Thomson Micromedex

US Product Circular for AVODART® (dutasteride 0.5 mg capsules) (October 2002), In: PDR Electronic Library™, Thomson Micromedex

Van Hecken A, Depre M, Schwartz JI, Tjandramaga TB, Winchell GA, De-Lepeleire I, Ng J, De-Schepper PJ (1994) Plasma concentrations and effect on testosterone metabolism after single doses of MK-0434, a steroid 5α™-reductase inhibitor, in healthy subjects. Eur J Clin Pharmacol 46:123–126

Van Neste D, Fuh V, Sanchez-Pedreno P, Lopez-Bran E, Wolff H, Whiting D, Roberts J, Kopera D, Stene J, Calvieri S, Tosti A, Prens E, Guarrera M, Kanojia P, He W, Kaufman K. D (2000) Finasteride increases anagen hair in men with androgenetic alopecia. Br J Dermatol 143:804–810

Venturoli S, Marescalchi O, Colombo FM, Macrelli S, Ravaioli B, Bagnoli A, Paradisi R, Flamigni C (1999) A prospective randomized trial comparing low dose flutamide, finasteride, ketoconazole, and cyproterone acetate-estrogen regimens in the treatment of hirsutism. J Clin Endocrinol Metab 84:1304–1310

Walsh PC, Madden JD, Harrod MJ, Goldstein JL, Macdonald PC, Wilson JD (1974) Familial incomplete male pseudohermaphroditism, type 2: decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N Engl J Med 291:944–949

Whiting DA (1990) The value of horizontal sections of scalp biopsies. J Cutan Aging Cosmetic Dermatol 1:165–173

Whiting DA (1993) Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. J Am Acad Dermatol 28:755–763

Whiting DA, Waldstreicher J, Sanchez M, Kaufman KD (1999) Measuring reversal of hair miniaturization in androgenetic alopecia by follicular counts in horizontal sections of serial scalp biopsies: Results of finasteride 1 mg treatment of men and postmenopausal women. J Invest Dermatol Symp Proc 4:282–284

Whiting DA, Olsen EA, Savin R, Halper L, Rodgers A, Wang L, Hustad C, Palmisano J for the Finasteride Male Pattern Hair Loss Study Group (2003) Efficacy and tolerability of finasteride 1 mg in men aged 41 to 60 years old with male pattern hair loss. Eur J Dermatol 13:150–160

Wilson JD (1972) Recent studies on the mechanism of action of testosterone. N Engl J Med 287:1284–1291

Wilson JD, Gloyna RE (1970) The intranuclear metabolism of testosterone in the accessory organs of reproduction. Rec Prog Horm Res 26:309–336

Wilson JD, Lasnitzki I (1971) Dihydrotestosterone formation in fetal tissues of the rabbit and rat. Endocrinology 89:659–668

Wong IL, Morris RS, Chang L, Spahn MA, Stanczyk FZ, Lobo RA (1995) A prospective randomized trial comparing finasteride to spironolactone in the treatment of hirsute women. J Clin Endocrinol Metab 80:233–238

19

Dehydroepiandrosterone (DHEA) and androstenedione

B. Allolio and W. Arlt

Contents

19.1 Introduction

Man together with higher primates have adrenals secreting large amounts of dehydroepiandrosterone (DHEA) and its sulfate ester, DHEAS. The physiological role of these steroid hormones has long been elusive. However, a growing number of

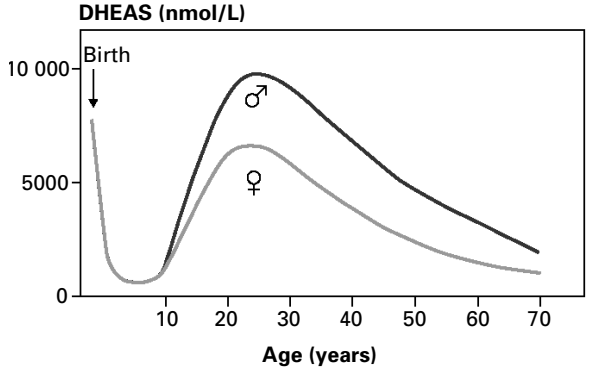


Fig. 19.1 Serum DHEAS concentrations during the human life cycle.

well-designed studies has helped to shed light on the role of DHEA in human health. Nevertheless, many aspects remain to be elucidated.

DHEA is distinct from other major adrenocortical steroids – cortisol and aldosterone – in declining with advancing age. Moreover, administration of DHEA to experimental animals has demonstrated a multitude of beneficial effects on the prevention of cancer, heart disease, diabetes and obesity (Svec and Porter 1998). This has led to the assumption that the age-related decline of DHEA may play a role in the degenerative changes observed in human aging and that administration of DHEA may reverse some of these changes. Moreover, the availability of DHEA as a food supplement in the USA resulted in aggressive marketing of DHEA as an anti-aging drug and in largescale self-administration without medical supervision.

However, in rodents circulating levels of DHEA and DHEAS are several orders of magnitude lower than in humans and no age-related decline in DHEA concentrations has been documented. This indicates that experimental studies in laboratory animals receiving high doses of DHEA have little bearing for human physiology.

This chapter, therefore, will focus mainly on data generated in humans. DHEA(S) will refer to both DHEA and DHEAS. In addition, clinical studies concerning androstenedione, another steroid hormone precursor, will also be covered.

19.2 DHEA secretion and age

In humans and in some non-human primates the secretion of DHEA(S) shows a characteristic pattern throughout the life cycle (Orentreich *et al.* 1984; Palmert *et al.* 2001; Reiter *et al.* 1977) (Fig. 19.1). DHEA(S) is secreted in high quantities by the fetal zone of the adrenal cortex, leading to high circulating DHEAS levels at birth. As the fetal zone involutes, a sharp fall in serum DHEA(S) concentrations is observed post partum to almost undetectable levels after the first months of life.

Levels remain very low until they gradually increase between the sixth and tenth years of age owing to increasing DHEA(S) production by the zona reticularis, a phenomenon termed adrenarche (Reiter *et al.* 1977; Sklar *et al.* 1980). DHEA(S) concentrations peak during the third decade, followed by a steady decline with advancing age so that levels during the eighth and ninth decade are only 10–20% of those in young adults (Orentreich *et al.* 1992). This decline has been termed "adrenopause" in spite of unchanged or even increased cortisol secretion (Laughlin and Barrett-Connor 2000). The age-related decline in DHEA(S) levels shows high interindividual variability and is associated with a reduction in size of the zona reticularis (Parker Jr. *et al.* 1997). DHEA secretion follows a diurnal rhythm similar to that of cortisol while DHEA(S) does not vary throughout the day. Liu *et al.* (1990) observed an age-associated attenuation of the diurnal rhythm and the pulse amplitude of DHEA secretion. Moreover, the ACTH-induced increase in DHEA secretion is reduced in elderly subjects (Parker Jr. *et al.* 2000), whereas the cortisol response to an ACTH challenge is constant or even increased. There is a clear gender difference in DHEA(S) concentrations with lower DHEAS concentrations in adult women compared to men (Orentreich *et al.* 1984). The physiological basis for this gender difference is not fully clear. However, while some of the circulating DHEA in males is contributed by the testes (Nieschlag *et al.* 1973; Zappulla *et al.* 1981) no such contribution from the ovaries is found, although they may indirectly affect DHEA(S) levels (Cumming *et al.* 1982).

There is also a clear genetic component to circulating DHEA(S) levels which vary significantly in different populations (Khaw 1996). Moreover, the high interindividual variability in any group of similar age is apparently in part inherited and serum DHEAS has thus been reported to be a specific individual marker (Thomas *et al.* 1994).

19.3 Epidemiology

There is some gender effect of high DHEAS levels in epidemiologic studies: Barrett-Connor *et al.* (1986) reported an inverse correlation between DHEAS levels and death from any cause for men (>50 years of age) but not for women (Barrett-Connor and Khaw 1987; Barrett-Connor and Goodman-Gruen 1995). In a prospective cohort study in 622 subjects of 65 years and older mortality at two and four years was associated with low serum DHEAS at baseline in men but not in women (Berr *et al.* 1996). Similarly in a recent report including men ($n = 963$) and women ($n = 1171$) >65 years of age, all cause and cardiovascular disease mortality were highest in the lowest DHEAS quartile for men. Again no significant association of circulating DHEAS and mortality was found for women (Trivedi and Khaw 2001). In addition, Mazat *et al.* (2001) found no association between

mortality and DHEAS levels in women, whereas in men the relative risk of death was 1.9 ($p < 0.01$) for those with the lowest concentrations of DHEAS. Accordingly, in a study of healthy very old men (90–106 years) low DHEAS concentrations were associated with poor functional status (Ravaglia *et al.* 1996). This sex difference observed in many (but not all) studies could be explained, in part, by sex-specific differences in bioconversion of DHEA(S) (Arlt *et al.* 1998; 1999b) (see below).

In addition, low DHEAS levels may be a non-specific marker of poor health status and thereby associated with an increased risk of severe illness and death. Low DHEA(S) concentrations have been found in systemic lupus erythematosus, dementia, breast cancer and rheumatoid arthritis and there is an inverse relationship between serum DHEAS levels and severity of disease (Deighton *et al.* 1992). Chronic disease often leads to a shift of intra-adrenal biosynthesis away from DHEA(S) production favoring cortisol secretion (Parker *et al.* 1985). Thus, low DHEAS levels may indicate the presence of a not yet apparent disease, which determines a future risk of morbidity or even mortality.

19.4 Mechanisms of action

19.4.1 DHEA

Three mechanisms of action have been described for DHEA: as precursor for active sex steroids, as a neurosteroid interacting with neurotransmitter receptors and as a ligand for a specific DHEA receptor (for review see Allolio and Arlt 2002).

As the human steroidogenic enzyme P450c17 converts almost no 17α-hydroxyprogesterone to androstenedione, the biosynthesis of virtually all sex steroids begins after the conversion of 17-hydroxypregnenolone to DHEA. Thus, only DHEA is converted to androstenedione by the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) and then further converted to testosterone and estradiol by isoenzymes of 17β-hydroxysteroid dehydrogenase (17β-HSD) and P450 aromatase, respectively (Fig. 19.2). Only lipophilic DHEA can be converted intracellularly to androgens and estrogens. Thus the local availability and activity of DHEA sulfotransferase and steroid sulfatase determines the ratio of DHEA activation (via conversion to sex steroids) to inactivation (via secretion as DHEAS back into the circulation). Analysis of the pharmacokinetics of DHEA and DHEAS following oral administration of DHEA suggests that DHEA and DHEAS undergo continuous interconversion (Arlt *et al.* 1998; 1999b). Measured with a constant infusion technique the conversion ratios for the conversion of DHEAS to DHEA were 0.006 for men and 0.004 for women, indicating that a significant amount of DHEA arises from DHEAS (Bird *et al.* 1984). However, the precise contribution of DHEA generation from DHEAS remains to be established.

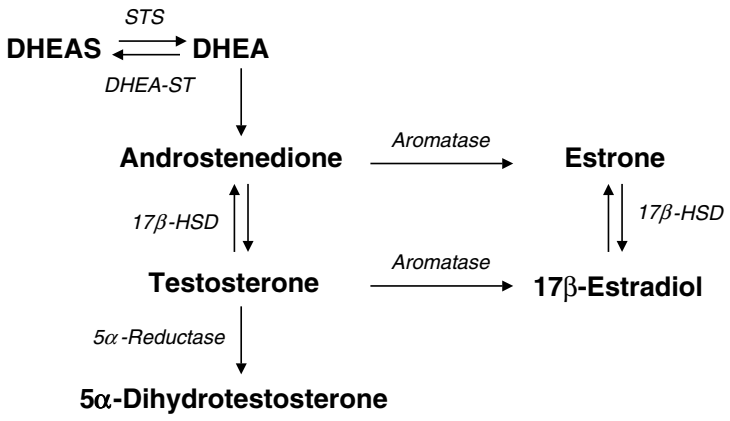


Fig. 19.2 Bioconversion of DHEA and androstenedione to sex steroids.

The widespread presence of 3β-HSD, 17β-HSD, 5α-reductase and P450 aromatase results in almost ubiquitous peripheral generation of sex steroids from DHEA (English *et al.* 2000; Jakob *et al.* 1997; Martel *et al.* 1994). Tissues involved include liver, skin, prostate, bone, breast, and brain. It has been estimated that 30–50% of androgen synthesis in men and 50–100% of estrogen synthesis in pre- and postmenopausal women occurs from adrenal steroids in peripheral tissues (Labrie 1991).

The concept of peripheral synthesis, action, and metabolism of steroid hormones from inactive precursors within the same target cell has been coined "intracrinology". Such intracrine processes are difficult to study, as serum parameters may only partially reflect target cell physiology. Nonetheless there is evidence that the bioconversion of DHEA(S) follows a sexually dimorphic pattern with preferential increases in androgenic activity in women and increases in circulating estrogens in men (Arlt *et al.* 1998; 1999b). However, in men with combined adrenal insufficiency and hypogonadism due to hypopituitarism oral DHEA administration induces significant increases in both estrogens and androgens (Young *et al.* 1997). Moreover, after oral administration of DHEA to men a pronounced increase in circulating 5α-androstane-3α,17β-diolglucoronide (ADG) is found, indicating increased peripheral androgen synthesis not reflected by changes in circulating testosterone levels (Arlt *et al.* 1999b). As ADG is the major metabolite of dihydrotestosterone (DHT) (Giagulli *et al.* 1989), it reflects increased DHT generation in peripheral androgen target tissues.

In addition, DHEA is considered a neurosteroid. There is compelling evidence for DHEA synthesis and action in the central nervous system (Corpechot *et al.* 1981). Several studies have demonstrated the synthesis of P450c17 and other key steroidogenic enzymes in the brain (Compagnone *et al.* 1995; Zwain and Yen 1999),

thereby providing the tools to generate DHEA in the absence of adrenal and gonadal function. DHEA influences neuronal activity via interaction with various receptors (N-methyl-D-aspartate [NMDA] receptor, sigma receptor, γ-aminobutyric acid [$GABA_A$] receptor) (Bergeron *et al.* 1996; Demirgoren *et al.* 1991; Majewska *et al.* 1990). Animal and in vitro studies have shown that DHEA(S) affect neuronal growth and development, improve glial survival, learning and memory (Compagnone and Mellon 1998; Svec and Porter 1998).

Thirdly, there is growing evidence for DHEA action via specific receptors, although such a DHEA receptor has not yet been fully characterized or even cloned. High affinity binding sites for DHEA have been described in murine and human T cells (Meikle *et al.* 1992; Okabe *et al.* 1995) but their specificity for DHEA remained questionable. More recently high affinity binding sites for DHEA were identified in bovine endothelial cells (Liu and Dillon 2002). In these cells DHEA activates endothelial nitric oxide synthase (eNOS) via a G-protein coupled plasma membrane receptor (Liu and Dillon 2002). Similarly DHEA affects extracellular-signal-regulated kinase 1 (ERK-1) phosphorylation in human vascular smooth muscle cells independently of androgen and estrogen receptors (Williams *et al.* 2002). These observations strengthen the concept of direct and specific hormonal activity of DHEA independent of its potential bioconversion to other steroids.

Taken together the available evidence clearly indicates that DHEA has a complex and specific activity profile, which is gender specific due to its sex-related differential pattern of downstream bioconversion to potent sex steroids. The gender specificity of the DHEA activity profile thus provides an elegant explanation for the profound gender effect observed in epidemiological studies on the association of serum DHEA(S) levels and mortality/morbidity (see 19.3).

19.4.2 Androstenedione

Androstenedione is not only a product of DHEA metabolism, but may be regarded as a prohormone itself. It can be converted to testosterone by 17βHSDs or to estrone by the aromatase enzyme complex (Leder *et al.* 2000). Accordingly, administration of androstenedione may alter circulating steroid hormone concentrations. In women pronounced increases not only in circulating androstenedione but also in testosterone have been described following administration of 100 mg androstenedione (Kicman *et al.* 2003) (Fig. 19.3). In contrast, in men the effects of oral androstenedione have been variable: in some trials serum total testosterone concentrations were not affected by 100 mg androstenedione (Brown *et al.* 2000; King *et al.* 1999). However, 300 mg androstenedione induced increases in testosterone levels (Leder *et al.* 2000). Importantly, clear increases in estrogens were observed after oral ingestion of androstenedione in young and elderly men (Brown *et al.* 2000; King *et al.* 1999; Leder *et al.* 2000), an effect quite similar to oral DHEA administration.

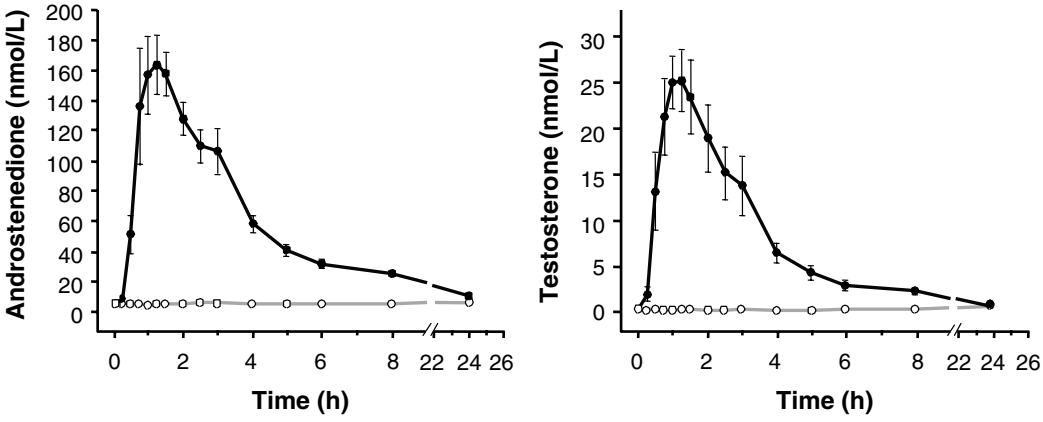


Fig. 19.3 Increase of testosterone in healthy young women after oral administration of 100 mg androstenedione (Kicman *et al.* 2003).

The intracrine activation of androstenedione – similar to DHEA – was highlighted by a detailed analysis of the metabolism of orally administered androstenedione in young men (Leder *et al.* 2001) observing increases in the excretion rates of conjugated testosterone, androsterone, etiocholanolone and dihydrotestosterone. It was concluded that orally administered androstenedione is largely metabolized to androgen metabolites before release into the general circulation. Thus again the biological activity of androstenedione is incompletely reflected by circulating active sex steroids. Similar hormone profiles were obtained using sublingual androstenediol in young men (Brown *et al.* 2002). Clear increases in serum testosterone were found by bypassing firstpass hepatic metabolism using this sublingual administration.

At present there is no evidence that androstenedione has biological activity independent of its downstream conversion to sex steroids.

19.5 Treatment with DHEA – clinical studies

19.5.1 Patients with adrenal insufficiency

The classical approach to study the physiological role of a hormone in humans is to analyze the effect of a hormonal deficit and the changes induced by replacement of the missing hormone. Thus adrenal insufficiency is the most useful model disease to understand the clinical activity of DHEA. As in adrenal insufficiency (AI) not only DHEA but also cortisol and (in primary AI) aldosterone is lacking, one might speculate that replacement of cortisol and aldosterone alone is not sufficient to fully restore wellbeing in AI. Intriguingly, it has only recently been clearly demonstrated that replacement of glucocorticoids (GC) and mineralocorticoids (MC) alone is indeed not sufficient to fully compensate the impairment of health induced by AI.

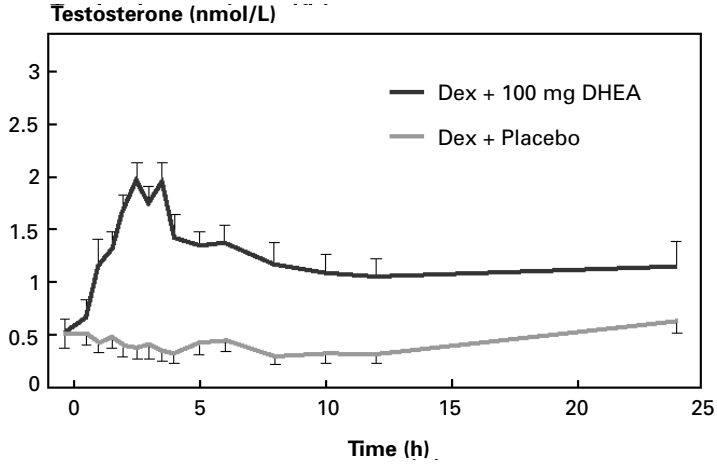


Fig. 19.4 Lasting increases of serum testosterone in healthy dexamethasone-suppressed women after oral administration of 100 mg DHEA versus placebo (Arlt *et al.* 1998).

Lovas *et al.* (2002) have demonstrated in 88 patients with primary AI receiving GC and MC impaired self-perception of general health and vitality. In addition, the overall scores for fatigue clearly indicated more fatigue in this patient population. These findings were confirmed by Gurnell *et al.* (2002) in a population of patients with primary AI demonstrating similar changes in health-related quality of life, indicating that conventionally treated AI is associated with a specific pattern of chronic disability. Jakobi *et al.* (2001) have provided some more insight into the mechanism of increased fatigue in conventionally treated patients with AI. Muscle function (twitch tension, central activation) was reduced and patients self-terminated a submaximal fatigue protocol significantly earlier than controls (5 ± 1 vs 10 ± 1 min, $p = 0.006$) (Jakobi *et al.* 2001).

For replacement of DHEA(S) in AI an oral dose of 25–50 mg DHEA per day has consistently been found to restore circulating DHEA(S) into the normal range of young adults (Arlt *et al.* 1999a; Hunt *et al.* 2000; Young *et al.* 1997). Owing to the long half-life of DHEAS and the interconversion of DHEA and DHEAS a single dose is sufficient to maintain normal DHEA(S) levels over 24 hours. Due to the downstream bioconversion lasting increases in circulating androgens have been demonstrated in women (Arlt *et al.* 1998; 1999a). In fact, the superior pharmacokinetics of oral DHEA make it a promising tool for androgen replacement in women (Fig. 19.4) with the additional potential advantage of a DHEA-specific neurosteroidal effect (see also Chapter 17).

To date there are four published randomized double blind trials on DHEA treatment in patients with adrenal insufficiency (Arlt *et al.* 1999a; Hunt *et al.* 2000; Johannsson *et al.* 2002; Lovas *et al.* 2003). A fifth large trial has recently been

completed in England; however, up to now the results have only been presented in abstract form (Gurnell *et al.* 2002). Three of the trials studied women only (Arlt *et al.* 1999a; Johannsson *et al.* 2002; Lovas *et al.* 2003), whereas the other two trials included both men and women (Hunt *et al.* 2000; Gurnell *et al.* 2002). In the first double-blind study (Arlt *et al.* 1999a) 24 women with adrenal insufficiency received in random order 50 mg of DHEA orally each morning for four months and placebo for four months with a one-month washout period. Treatment with DHEA raised the initially low concentrations of DHEA(S), androstenedione, and testosterone into the normal range. Serum concentrations of sex hormone-binding globulin (SHBG), total cholesterol and high-density lipoprotein (HDL) cholesterol decreased significantly. DHEA improved wellbeing and sexuality: compared to placebo DHEA resulted in a decrease in the scores for depression ($p = 0.02$), anxiety ($p = 0.01$) as well as for a global severity index ($p = 0.02$). Scores on all three subscales of the Multidimensional Mood Questionnaire also significantly improved after treatment with DHEA. Beneficial effects of DHEA treatment on anxiety ($p = 0.04$) and depression ($p = 0.01$) were also observed for the Hospital Anxiety and Depression Scale. A reduction in fatigue was evident from the Giessen Complaint List ($p = 0.03$). Treatment with DHEA resulted in significant increases in the initially low scores of all four visual-analogue scales for sexuality. DHEA did not affect fasting serum glucose, insulin and parameters of body composition (Callies *et al.* 2001). Using an incremental cycling test maximum workload was 95.8 ± 20.4 W after DHEA compared to 91.7 ± 24.1 W after placebo ($p = 0.057$). DHEA induced a significant decrease in serum leptin ($p = 0.01$) and an increase in serum osteocalcin ($p = 0.02$) compared to placebo. Androgenic skin effects of DHEA treatment were reported in 19 out of the 24 women but were mostly mild and transient (Arlt *et al.* 1999a).

Improvement in mood and fatigue was also observed after DHEA replacement in Addison's disease in the trial reported by Hunt *et al.* (2000). In this double blind trial 39 patients (24 women, 15 men) received either 50 mg oral DHEA for 12 weeks followed by a four–week washout period, then 12 weeks of placebo or vice versa. The hormonal changes induced by DHEA in females were virtually identical to those reported by Arlt *et al.* (1999a) with increases in serum DHEA(S), androstenedione, and testosterone into normal range for women. In males, serum testosterone and SHBG did not change. Hunt *et al.* (2000) found a significant increase in self-esteem after DHEA substitution ($p < 0.001$). Using a Profile of Mood State Questionnaire it was demonstrated that evening mood ($p = 0.018$) and evening fatigue ($p = 0.002$) was improved by DHEA. No changes in BMD, body mass index, serum cholesterol or insulin sensitivity were observed after DHEA treatment. Adverse events of DHEA replacement were few and mild (facial acne in nine patients vs. five patients receiving placebo). As the beneficial effects in this study were also observed in male

patients who exhibited no change in testosterone, it was concluded that DHEA acts directly at the central nervous system rather than via peripheral conversion to androgens (Hunt *et al.* 2000). The same group followed up on these results and performed a study in patients with primary adrenal insufficiency ($n = 106$) who received 12 months of DHEA replacement (50 mg/day) or placebo in a parallel group study design (Gurnell *et al.* 2002). Preliminary results of this phase III trial demonstrated significant improvement in health-related quality of life during DHEA replacement at three and six months of treatment. An attenuation of this effect after 12 months was observed. However, after withdrawal of DHEA, well-being scores significantly worsened. In addition they found significant beneficial effects of DHEA replacement on femoral neck bone mineral density as assessed by dual energy x-ray absorptiometry (DXA). Body composition analysis by DXA also revealed a significant increase in lean body mass while fat mass remained the same (Gurnell *et al.* 2002).

In a recent study DHEA (20–30 mg/day) was used in 38 women with secondary AI due to hypopituitarism (Johannsson *et al.* 2002). DHEA or placebo was given for six months in a randomized, placebo-controlled double blind study, followed by a six-months open treatment period. DHEA(S) increased into the normal range during DHEA administration, whereas androstenedione and testosterone rose only to subnormal levels. The percentage of partners of the patients who reported improved alertness, stamina, and initiative by their spouses were 70%, 64%, and 55%, respectively, in the DHEA group and 11%, 6%, and 11%, respectively, in the placebo group ($p < 0.05$) Sexual relations tended to improve ($p = 0.06$). An increase in or the reappearance of axillary and/or pubic hair was seen in all women given 30 mg DHEA and in 69% of women receiving 20 mg DHEA but was not found in women receiving placebo. Glucose metabolism and lipoproteins remained unaffected by DHEA with the exception of transient decrease in HDL cholesterol. Interestingly, based on age-adjusted reference values the study group had $79 \pm 20\%$ and $67 \pm 23\%$ of predicted values for peak and mean handgrip strength over 10 seconds. These values had significantly increased at 12 months ($p < 0.05$). Bone markers and BMD remained unchanged. Androgenic skin effects were again more often seen during DHEA treatment.

In contrast, the most recent study using 25 mg DHEA in 39 patients with primary AI in a parallel group design failed to detect a benefit for subjective health status and sexuality (Lovas *et al.* 2003). The reason for the negative result is most likely the fact that the study was grossly underpowered to detect significant changes. This trial, therefore, is a good example that inadequately designed studies can cause more harm than provide benefits in the development of new treatment strategies (Arlt and Allolio 2003b).

Besides the results of these randomized trials, evidence from case reports (Kim and Brody 2001; Wit *et al.* 2001) in AI is available. Kim and Brody (2001) have

described a 24 year-old female with Addison's disease and the complaint of neither axillary nor pubic hair growth. DHEA was added to the conventional replacement therapy. Serum DHEAS and testosterone levels increased. Pubic hair growth changed from Tanner stage I to Tanner stage III within two years of receiving DHEA at a final dose of 25 mg daily. Similarly, Wit *et al.* (2001) used oral DHEAS (15 mg/m^2) for atrichia pubis in four female adolescents with panhypopituitarism (n = 2) or 17-hydroxylase deficiency (n = 2). They found DHEAS an efficacious treatment leading from atrichia pubis to Tanner stage 4–5 pubic hair.

19.5.2 Elderly subjects

The age-related decline in circulating DHEA(S) has led to a number of randomized trials to assess the effect of oral DHEA in otherwise healthy elderly subjects. In a first double blind placebo-controlled trial using a cross-over design 13 men and 17 women aged 40–70 years received either 50 mg DHEA or placebo for three months (and vice versa) (Morales *et al.* 1994). The subjects reported an improvement in wellbeing using a non-validated questionnaire for self-assessment of wellbeing. No change in insulin sensitivity and body composition was found. Bioavailable IGF-1 increased slightly during DHEA, whereas HDL-cholesterol decreased in women. Short-term (2 weeks) randomized double-blind studies by Wolf *et al.* (1997; 1998) failed to demonstrate any benefit of DHEA on wellbeing, mood and cognition. Similarly, in a double-blind placebo-controlled cross-over trial Arlt *et al.* (2001) found no effect of DHEA (50 mg/day) on mood, wellbeing and sexuality in 20 men aged 50–69 years after four months of therapy. In another placebo-controlled randomized crossover trial by van Niekerk *et al.* (2001) no effect of 50 mg/day DHEA for 13 weeks on wellbeing and cognition was found using a wide range of validated self-assessment questionnaires and standardized test batteries, respectively. No effect of DHEA on activities of daily living was found after three months of 100 mg DHEA/d in 39 men aged 60–84 years in another placebo-controlled crossover trial (Flynn *et al.* 1999).

In the largest study to date, Baulieu *et al.* (2000) studied the effects of 50 mg DHEA/day vs. placebo in a double-blind randomized parallel study including 140 men and 140 women aged 60–79 years. In general the results were disappointing. Neither wellbeing nor cognition was improved by DHEA using a wide range of validated tools. In women >70 years libido was increased and slight but significant gains in bone mineral density were observed in women but not in men.

Taking all studies on DHEA supplementation in elderly subjects together, the results show only very limited effects of DHEA compared to placebo. An important explanation for this lack of efficacy may be related to a selection bias. In almost all studies, only healthy subjects with excellent performance status at baseline were included, thereby leaving limited space for further improvement. However, from these studies it can be concluded that age-related low DHEA concentrations do

not necessarily lead to impaired wellbeing, cognition and sexuality per se (Allolio and Arlt 2002). Thus an aging-associated decline in serum DHEA(S) differs by orders of magnitude from the very low DHEAS concentrations observed in adrenal insufficiency.

19.5.3 Patients with impaired mood and wellbeing

Consistent with the effects of DHEA on mood and wellbeing in patients with adrenal insufficiency beneficial effects were also observed in randomized double-blind studies in patients with major depression (Wolkowitz *et al.* 1999) and midlife dysthymia (Bloch *et al.* 1999). DHEA also improved scores on an ADL scale in patients with myotonic dystrophy (Sugino *et al.* 1998). Reiter *et al.* (1999) have reported an improvement in erectile function, sexual satisfaction and orgasmic function in 40–60 year old men suffering from erectile dysfunction and receiving 50 mg DHEA/day for six months in a randomized double-blind fashion. To compare the efficacy of DHEA vs. placebo in Alzheimer disease 58 patients were randomized to six months of treatment with DHEA (100 mg/day) or placebo. A transient effect on cognitive performance narrowly missed significance (Wolkowitz *et al.* 2003), possibly because of the small patient sample. Recently Strous *et al.* (2003) have studied the efficacy of DHEA (100 mg/day) in schizophrenic patients with prominent negative symptoms. In a double-blind trial a significant improvement in negative symptoms ($p < 0.001$), as well as in depressive ($p < 0.05$) and anxiety ($p < 0.001$) symptoms was seen in individuals receiving DHEA.

It seems noteworthy that the pattern of improvement observed in these trials closely resembled the changes observed in patients with adrenal insufficiency.

19.5.4 Patients with immunological disorders

In a number of studies DHEA supplementation has been used to modify immune functions and alter the course of immunopathies. Most studies have been performed in patients with systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disease of unknown etiology (Chang *et al.* 2002; Petri *et al.* 2002; Van Vollenhoven *et al.* 1995). The concept to use DHEA in the treatment of SLE was based on the observation that women are more often affected and that androgens and DHEA concentrations are low in patients with SLE (Lahita *et al.* 1987). Moreover, androgen treatment can modify the disease progression in an animal model of SLE (Melez *et al.* 1980). After preliminary evidence of a glucocorticoid-sparing effect of DHEA in patients with mild SLE (Van Vollenhoven *et al.* 1994) a randomized double-blind placebo-controlled trial was performed (200 mg DHEA orally for three months) (Van Vollenhoven *et al.* 1995). It demonstrated beneficial effects of DHEA on patient and physician overall assessment, SLE disease activity index (SLEDAI) and glucocorticoid requirements. This was

confirmed in recent double-blind randomized, placebo-controlled trials demonstrating that DHEA (200 mg/day) was well tolerated, reduced the number of SLE flares, reduced disease activity and allowed reducing the dosage of glucocorticoids (Chang *et al.* 2002; Petri *et al.* 2002). It is important to note that these studies included women only and that it remains unclear whether similar results can be obtained in men. In a phase II uncontrolled pilot trial DHEA (200 mg/day) was effective and safe in patients with refractory Crohn's disease and ulcerative colitis (Andus *et al.* 2003). However, to date no placebo-controlled trials have been performed in inflammatory bowel disease. In all these trials side-effects were mild with acne being the most frequently seen adverse event despite the use of undoubtedly supraphysiological DHEA doses (200 mg/d).

DHEA supplementation has also been used to enhance the antibody response to tetanus and influenza vaccines (Danenberg *et al.* 1997; Degelau *et al.* 1997; Evans *et al.* 1996). However, in these randomized placebo-controlled trials no consistent effect of DHEA on protective antibody titers was found.

19.6 Androstenedione administration in clinical studies

Effects of oral androstenedione have not been studied in women and have been largely disappointing in men. Short-term (5 days) androstenedione (100 mg/day) had no anabolic effect on muscle protein metabolism in eugonadal young men (Rasmussen *et al.* 2000). In 30–56 year-old men androstenedione (3×100 mg/day) for 28 days slightly reduced HDL-cholesterol without affecting prostate specific antigen (PSA), suggesting some androgenic activity (Brown *et al.* 2000). Serum HDL-cholesterol was also reduced in an eight-week randomized trial in 20 young men receiving oral androstenedione (300 mg/day) (King *et al.* 1999). Androstenedione failed to enhance muscle adaptation to resistance training in this population (King *et al.* 1999).

At present both treatment duration and sample sizes have been too limited to draw any firm conclusions on the clinical efficacy of androstenedione. However, the profound increases in circulating testosterone observed in women after oral androstenedione deserve attention and should preclude its use as food supplement (Kicman *et al.* 2003).

19.7 The emerging therapeutic profile of DHEA

19.7.1 Effects on the central nervous system

Improvement in mood and wellbeing have consistently been observed in patients with adrenal insufficiency (Arlt *et al.* 1999a; Hunt *et al.* 2000; Johannsson *et al.* 2002) and in patients with depressive disorders (Bloch *et al.* 1999; Wolkowitz *et al.*

1999) and schizophrenia (Strous *et al.* 2003), particularly improving symptoms of anxiety and depression and their physical correlates. It is important to note that improvements have only been observed in subjects with impaired mood and wellbeing at baseline and that DHEA-induced improvements led to scores in the range of normal healthy subjects. This indicates that DHEA may normalize impaired wellbeing but will not lead to "supranormal" wellbeing in otherwise healthy subjects (irrespective of the presence of low endogenous DHEAS concentrations).

Several cases of mania have been reported with DHEA treatment (Kline and Jaggers 1999; Markowitz *et al.* 1999) and we also have observed a similar case in a woman with adrenal insufficiency receiving a daily dose of 25 mg DHEA, although a direct causal role for DHEA is difficult to establish.

The basis for the anxiolytic and antidrepressive activity of DHEA remains to be elucidated but may be related to both androgenic effects and neurosteroidal actions of DHEA.

In contrast, there is little evidence that DHEA affects memory or cognition. Negative results have been found not only in healthy elderly subjects (Baulieu *et al.* 2000) but also in adrenal insufficiency (Arlt *et al.* 2000). Moreover, in Addison disease cognition is not impaired despite severe endogenous DHEA deficiency (Arlt *et al.* 2000). Thus it is unlikely that cognition is a major target of DHEA action.

Libido and sexual satisfaction are influenced by DHEA in women with AI (Arlt *et al.* 1999a) and in elderly women with age-related low DHEAS (Baulieu *et al.* 2000). Also in men, only impaired sexuality benefits from DHEA administration (Reiter *et al.* 1999) while normal baseline performance cannot be enhanced (Arlt *et al.* 2001). The effect of DHEA on libido and sexuality is most likely a consequence of increased androgenic activity derived from DHEA by peripheral bioconversion. In recent years it has become increasingly clear that androgens play a keyrole for female sexuality (Arlt 2003; Shifren *et al.* 2000). In fact, the adrenals are a major source of female androgens (Labrie *et al.* 2003) and their fundamental role for female sexuality (Waxenberg *et al.* 1959) has been rediscovered by studies on the therapeutic potential of DHEA. The available evidence and the superior pharmacokinetic properties make DHEA a highly attractive tool for treatment of impaired sexuality in women. However, firm conclusions must await the results of further trials.

19.7.2 Metabolism and body composition

The effects of DHEA on metabolic parameters (e.g. lipids, insulin sensitivity) and body composition are mostly not consistent and largely unimpressive. Insulin sensitivity was unaffected in women with adrenal insufficiency and also in healthy elderlies receiving replacement doses of DHEA (Callies *et al.* 2001; Casson *et al.*

1995; Morales *et al.* 1994; Morales *et al.* 1998; Yen *et al.* 1995), whereas Diamond *et al.* (1996) observed decreased fasting glucose and insulin in 15 women receiving DHEA cream (10%) but no effect on total areas under the curve during oral glucose tolerance test (OGTT). However, a recent study in 24 men with hypercholesterolemia demonstrated improved endothelial function and insulin sensitivity following three months of DHEA 25 mg/d (Kawano *et al.* 2003).

Most studies were possibly of too short duration to reliably detect changes in body composition. Body composition remained either unaffected (Arlt *et al.* 2001; Casson *et al.* 1995; Diamond *et al.* 1996; Morales *et al.* 1994) or showed variable and gender-specific changes with reduction in fat mass in men only (Morales *et al.* 1998; Yen *et al.* 1995) and an increase in total body mass in women (Morales *et al.* 1998). Of note, in the only long-term study (12 months) in patients with adrenal insufficiency preliminary results suggest a DHEA-induced increase in lean body mass without effects on fat mass. Thus it may be possible that the androgenic activity of DHEA favors a shift in the ratio of lean mass to fat mass favoring muscle mass. This view is supported by the documentation of impaired muscle function in patients with adrenal insufficiency receiving glucocorticoid and mineralocorticoid replacement, but not DHEA (Jakobi *et al.* 2001; Johannsson *et al.* 2002). Accordingly, increased muscle strength after DHEA administration (100 mg/day) was reported by Yen *et al.* (1995) and Morales *et al.* (1998). However, in the largest study to date in elderly subjects DHEA (50 mg/day) failed to affect muscle area or strength (Percheron *et al.* 2003). Thus at present a significant effect of DHEA on muscle remains uncertain.

In studies administering DHEA in physiological (25–50 mg/day) or near physiological doses (100 mg/day) a significant decrease in apolipoprotein A1 and HDL-cholesterol was seen in women but not in men (Diamond *et al.* 1996; Morales *et al.* 1994; 1998). This corresponded to an increase in circulating androgen concentrations in women but not in men. In one study employing a dose of 100 mg/day DHEA, a slight, but significant, HDL-cholesterol reduction was also seen in men (Flynn *et al.* 1999) who concurrently showed an increase in both free testosterone and 17β-estradiol serum concentrations.

A slight but significant increase in serum IGF-I in response to oral DHEA treatment has been reported in some studies (Morales *et al.* 1994; 1998; Villareal *et al.* 2000). However, others found no significant changes in parameters of the somatotropic axis (Baulieu *et al.* 2000; Casson *et al.* 1998; Diamond *et al.* 1996). Thus, the significance of these findings remains questionable.

19.7.3 Skeletal system

Possible effects of DHEA on bone mineral density and bone markers have been of considerable interest, as sex steroids have been demonstrated to influence bone

remodeling and to prevent osteoporosis. However, small sample size and short duration of treatment precluded clear conclusions in many trials. Moreover, only randomized placebo-controlled trials allow a robust assessment of the effects of DHEA on bone. Some open label studies have reported increases in bone mineral density (BMD) (Labrie *et al.* 1997; Villareal *et al.* 2000) whereas in placebo-controlled trials DHEA failed to affect BMD or bone markers (Kahn and Halloran 2002; Morales *et al.* 1998; Yen *et al.* 1995). In the DHEAge study (Baulieu *et al.* 2000) some increases in BMD were found in women (<70 years of age) at the femoral neck. However, no effects were observed in men. This finding is in keeping with preliminary results from patients with primary adrenal insufficiency (Gurnell *et al.* 2002) reporting also an increase in femoral neck BMD as assessed by DXA. DHEA effects on bone markers were missing in men (Arlt *et al.* 2001; Baulieu *et al.* 2000; Kahn and Halloran 2002) and variable in women with either increases, decreases or no change in bone resorption markers (Baulieu *et al.* 2000; Callies *et al.* 2001; Villareal *et al.* 2000) and increases or no change in osteocalcin (Baulieu *et al.* 2000; Callies *et al.* 2001).

At present it seems likely that beneficial effects of DHEA on BMD are small and restricted to women, possibly due to androgenic biotransformation of DHEA. However, only large prospective controlled trials will settle this issue.

19.7.4 Skin

Skin is an important target of DHEA action: DHEA increases sebum secretion and skin hydration (Baulieu *et al.* 2000; Labrie *et al.* 1997) and has been reported to reduce facial skin pigmentation (yellowness) in elderlies (Baulieu *et al.* 2000). Androgenic changes such as acne and hirsutism including facial hair growth have been reported as possible side effects in numerous controlled trials (Arlt *et al.* 1999a; Van Vollenhoven *et al.* 1995).

19.7.5 Immune system

Based on data from animal experiments (Svec and Porter 1998) and from in vitro studies (Meikle *et al.* 1992; Okabe *et al.* 1995) DHEA has been suggested as a steroid with immune-regulatory activity. This view is supported by the clinical studies in patients with SLE demonstrating glucocorticoid-sparing activity of DHEA and clinical improvement (Chang *et al.* 2002; Petri *et al.* 2002; Van Vollenhoven *et al.* 1995). However, in these studies DHEA was given at a clearly supraphysiological dose (200 mg/day) and physiological replacement doses (50 mg/d) given to healthy elderlies in the DHEAge study did not have any effect on B- and T-cell populations, cytokine production or natural killer cell cytotoxicity (unpublished observations). In vitro studies with human cells also show DHEA-induced increases in IL-2 secretion (Suzuki *et al.* 1991) and NK cell activity (Solerte *et al.* 1999) and inhibition

of IL-6 release (Gordon *et al.* 2001; Straub *et al.* 1998). IL-2 secretion in SLE correlates with circulating DHEAS and in vitro DHEA restores IL-2 secretion from T lymphocytes of SLE patients (Suzuki *et al.* 1995). No consistent in vivo data on immune effects of DHEA in humans are reported. Again it is likely that beneficial effects of DHEA are more easily detectable in patients with immunopathies and an altered immune system at baseline.

19.8 Practical approach to the patient with DHEA deficiency

At present there is no established indication and no generally accepted pharmacological preparation of DHEA for treatment. However, there is growing acceptance (Achermann and Silverman 2001; Arlt and Allolio 2003a; Oelkers 1999) of the view that DHEA replacement in patients with adrenal insufficiency may be beneficial in a substantial percentage of cases. In these patients not only very low or absent circulating DHEA(S) is demonstrated, but there is evidence of impaired wellbeing, reduced vitality and increased fatigue (Lovas *et al.* 2002), symptoms that are likely to respond to DHEA replacement (25–50 mg/day). Treatment usually starts with 25 mg/day. Serum DHEAS concentrations can easily be monitored and should be in the respective sex- and age-adjusted reference range (Orentreich *et al.* 1984). It is important to know that significant improvement may occur only after two to four months of treatment.

Treatment of elderlies with age-related low endogenous DHEA(S) is per se not justified. All available evidence indicates that an age-related decline in DHEA(S) concentration is not necessarily associated with impairment in wellbeing and mood or with increased fatigue. Accordingly, DHEA supplementation offers no apparent benefit for such a population. This is a situation very similar to postmenopausal hormone replacement: despite very low estradiol concentrations estrogen replacement may be more often detrimental than beneficial. This does not exclude the possibility that certain subgroups of elderly subjects may benefit from DHEA supplementation, but these subgroups need to be defined. In particular, to date there is little evidence that DHEA supplementation reverses relevant aspects of aging.

Much more plausible is an approach that focuses on specific complaints like anxiety, depression, increased fatigue or impaired female sexuality which may be amenable to DHEA treatment. In particular such complaints in patients receiving chronic glucocorticoid therapy with concomitant suppression of adrenal androgen secretion may occasionally justify an individual trial of DHEA therapy (25 mg/day). However, patients need to be informed about the experimental nature of such treatment. In particular, the possible risks of androgenic side-effects and the potential promotion of sex steroid-dependent tumor growth need to be addressed.

Table 19.1 Action profile of DHEA

Central Nervous System:
- mood ↑, well-being ↑, anxiety ↓, depression ↓, fatigue ↓
- libido → (↑ in women), sexual satisfaction → (↑)

Metabolism:
- insulin sensitivity →, fasting glucose →
- HDL-cholesterol ↓ (→), total cholesterol →
- IGF-1 → (↑)

Muscle:
- strength → (↑), muscle area →
- lean body mass → (↑)

Bone:
- bone mineral density → (↑ in women)
- bone maker →(↑↓)

Skin:
- sebum production ↑
- skin hydration ↑
- acne ↑

Immune System:
- glucocorticoid demand in immunopathies (↓)
- immune cell distribution → (B-cells, T-cells, natural killer cells)

19.9 Future perspectives

In less than a decade tremendous progress has been made in the field of DHEA research. The therapeutic potential of DHEA is now more clearly visible (see Table 19.1) and it is predicted that DHEA will become part of routine replacement for the majority of patients with adrenal insufficiency, although large phase III trials will be necessary to firmly establish its role in the treatment of adrenal failure. While hopes of using DHEA as an anti-aging remedy have not been fulfilled, there is growing evidence that DHEA may have therapeutic potential for other patient groups. These include patients with psychiatric illnesses (depression, schizophrenia, dysthymia), immunopathies (systemic lupus erythematosus) and women with androgen deficiency related complaints (e.g. loss of libido).

In these patient groups administration of DHEA must not be regarded as substitution therapy but rather as pharmacotherapy. Accordingly, only large prospective randomized double-blind trials will allow us to define the benefits and also the risks of such DHEA pharmacotherapy.

An important contribution to the development of treatment strategies with DHEA will come from a better understanding of the mechanisms of action of

DHEA. It is predicted that the next decade of DHEA research will be successful in identifying more specifically the multiple mechanisms of action of DHEA, most likely including the identification and characterization of a membrane bound G-protein coupled DHEA receptor. Of particular interest will be the investigation of specific DHEA actions on the immune and central nervous systems.

In conclusion, DHEA has emerged as a fascinating adrenal steroid but its physiology and therapeutic potential are still waiting to be fully revealed.

19.10 Key messages

- DHEA(S) secretion shows a characteristic pattern during the human life cycle with a prepubertal rise (adrenarche) and a continuous decline (adrenopause) after a peak in early adulthood. There is high interindividual variability and a sex difference in circulating DHEA(S) levels with higher concentrations in males.
- Low DHEA(S) concentrations predict imminent mortality in men but not in women. Low DHEAS levels may be a non-specific marker of poor health and rather an epiphenomenon but not a cause of disease.
- DHEA exerts its biological activity via its downstream conversion to potent sex steroids, as a neurosteroid interacting with neurotransmitter receptors, and most likely also via a membrane-bound specific G-protein coupled DHEA receptor.
- Like DHEA, androstenedione acts as a prohormone and is converted into androgens and estrogens after oral administration.
- Treatment of patients with adrenal insufficiency (50 mg DHEA/day) improves wellbeing, mood and fatigue and may also improve sexuality in female patients. By contrast, in healthy elderly subjects with age-related low endogenous DHEA(S) beneficial effects of DHEA supplementation remain doubtful.
- DHEA administration has been found to improve anxiety and depression in midlife dysthymia, patients with depression and schizophrenia. In systemic lupus erythematosus DHEA reduces disease activity, flares and allows reduction of the glucocorticoid dose.
- In short-term studies, androstenedione and DHEA did not improve muscle function or muscle strength.
- The available clinical evidence suggests that the main target tissues for DHEA are the central nervous system, the skin and possibly the immune system. Beneficial effects on muscle function and bone remain to be established.

19.11 REFERENCES

Achermann JC, Silverman BL (2001) Dehydroepiandrosterone replacement for patients with adrenal insufficiency. Lancet 357:1381–1382

Allolio B, Arlt W (2002) DHEA treatment: myth or reality? Trends Endocrinol Metab 13:288–294

Andus T, Klebl F, Rogler G, Bregenzer N, Scholmerich J, Straub RH (2003) Patients with refractory Crohn's disease or ulcerative colitis respond to dehydroepiandrosterone: a pilot study. Aliment Pharmacol Ther 17:409–414

Arlt W (2003) Management of the androgen-deficient woman. Growth HormIGFRes 13: Suppl, S85–S89

Arlt W, Allolio B (2003a) Adrenal insufficiency. Lancet 361:1881–1893

Arlt W, Allolio B (2003b) DHEA replacement in adrenal insufficiency. J Clin Endocrinol Metab 88:4001–4002

Arlt W, Justl HG, Callies F, Reincke M, Hubler D, Oettel M, Ernst M, Schulte HM, Allolio B (1998) Oral dehydroepiandrosterone for adrenal androgen replacement: pharmacokinetics and peripheral conversion to androgens and estrogens in young healthy females after dexamethasone suppression. J Clin Endocrinol Metab 83:1928–1934

Arlt W, Callies F, Van Vlijmen JC, Koehler I, Reincke M, Bidlingmaier M, Huebler D, Oettel M, Ernst M, Schulte HM, Allolio B (1999a) Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 341:1013–1020

Arlt W, Haas J, Callies F, Reincke M, Hubler D, Oettel M, Ernst M, Schulte HM, Allolio B (1999b) Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. J Clin Endocrinol Metab 84:2170–2176

Arlt W, Callies F, Allolio B (2000) DHEA replacement in women with adrenal insufficiency–pharmacokinetics, bioconversion and clinical effects on wellbeing, sexuality and cognition. Endocr Res 26:505–511

Arlt W, Callies F, Koehler I, Van Vlijmen JC, Fassnacht M, Strasburger CJ, Seibel MJ, Huebler D, Ernst M, Oettel M, Reincke M, Schulte HM, Allolio B (2001) Dehydroepiandrosterone supplementation in healthy men with an age-related decline of dehydroepiandrosterone secretion. J Clin Endocrinol Metab 86:4686–4692

Barrett-Connor E, Goodman-Gruen D (1995) Dehydroepiandrosterone sulfate does not predict cardiovascular death in postmenopausal women. The Rancho Bernardo Study Circulation 91:1757–1760

Barrett-Connor E, Khaw KT (1987) Absence of an inverse relation of dehydroepiandrosterone sulfate with cardiovascular mortality in postmenopausal women. N Engl J Med 317:711

Barrett-Connor E, Khaw KT, Yen SS (1986) A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. N Engl J Med 315:1519–1524

Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B, Faucounau V, Girard L, Hervy MP, Latour F, Leaud MC, Mokrane A, Pitti-Ferrandi H, Trivalle C, De Lacharriere O, Nouveau S, Rakoto-Arison B, Souberbielle JC, Raison J, Le Bouc Y, Raynaud A, Girerd X, Forette F (2000) Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. Proc Natl Acad Sci USA 97:4279–4284

Bergeron R, De Montigny C, Debonnel G (1996) Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. J Neurosci 16:1193–1202

Berr C, Lafont S, Debuire B, Dartigues JF, Baulieu EE (1996) Relationships of dehydroepiandrosterone sulfate in the elderly with functional, psychological, and mental status, and short-term mortality: a French community-based study. Proc Natl Acad Sci USA 93:13410–13415

Bird CE, Masters V, Clark AF (1984) Dehydroepiandrosterone sulfate: kinetics of metabolism in normal young men and women. Clin Invest Med 7:119–122

Bloch M, Schmidt PJ, Danaceau MA, Adams LF, Rubinow DR (1999) Dehydroepiandrosterone treatment of midlife dysthymia. Biol Psychiatry 45:1533–1541

Brown GA, Vukovich MD, Martini ER, Kohut ML, Franke WD, Jackson DA, King DS (2000) Endocrine responses to chronic androstenedione intake in 30- to 56-year-old men. J Clin Endocrinol Metab 85:4074–4080

Brown GA, Martini ER, Roberts BS, Vukovich MD, King DS (2002) Acute hormonal response to sublingual androstenediol intake in young men. J Appl Physiol 92:142–146

Callies F, Fassnacht M, Van Vlijmen JC, Koehler I, Huebler D, Seibel MJ, Arlt W, Allolio B (2001) Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. J Clin Endocrinol Metab 86:1968–1972

Casson PR, Faquin LC, Stentz FB, Straughn AB, Andersen RN, Abraham GE, Buster JE (1995) Replacement of dehydroepiandrosterone enhances T-lymphocyte insulin binding in postmenopausal women. Fertil Steril 63:1027–1031

Casson PR, Santoro N, Elkind-Hirsch K, Carson SA, Hornsby PJ, Abraham G, Buster JE (1998) Postmenopausal dehydroepiandrosterone administration increases free insulin-like growth factor-I and decreases high-density lipoprotein: a six-month trial. Fertil Steril 70:107–110

Chang DM, Lan JL, Lin HY, Luo SF (2002) Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. Arthritis Rheum 46:2924–2927

Compagnone NA, Mellon SH (1998) Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. Proc Natl Acad Sci USA 95:4678–4683

Compagnone NA, Bulfone A, Rubenstein JL, Mellon SH (1995) Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. Endocrinology 136:5212–5223

Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu EE (1981) Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci USA 78:4704–4707

Cumming DC, Rebar RW, Hopper BR, Yen SS (1982) Evidence for an influence of the ovary on circulating dehydroepiandrosterone sulfate levels. J Clin Endocrinol Metab 54:1069–1071

Danenberg HD, Ben Yehuda A, Zakay-Rones Z, Gross DJ, Friedman G (1997) Dehydroepiandrosterone treatment is not beneficial to the immune response to influenza in elderly subjects. J Clin Endocrinol Metab 82:2911–2914

Degelau J, Guay D, Hallgren H (1997) The effect of DHEAS on influenza vaccination in aging adults. J Am Geriatr Soc 45:747–751

Deighton CM, Watson MJ, Walker DJ (1992) Sex hormones in postmenopausal HLA-identical rheumatoid arthritis discordant sibling pairs. J Rheumatol 19:1663–1667

Demirgoren S, Majewska MD, Spivak CE, London ED (1991) Receptor binding and electrophysiological effects of dehydroepiandrosterone sulfate, an antagonist of the GABAA receptor. Neuroscience 45:127–135

Diamond P, Cusan L, Gomez JL, Belanger A, Labrie F (1996) Metabolic effects of 12-month percutaneous dehydroepiandrosterone replacement therapy in postmenopausal women. J Endocrinol 150 Suppl, S43–S50

English MA, Hughes SV, Kane KF, Langman MJ, Stewart PM, Hewison M (2000) Oestrogen inactivation in the colon: analysis of the expression and regulation of 17 beta-hydroxysteroid dehydrogenase isozymes in normal colon and colonic cancer. Br J Cancer 83:550–558

Evans TG, Judd ME, Dowell T, Poe S, Daynes RA, Araneo BA (1996) The use of oral dehydroepiandrosterone sulfate as an adjuvant in tetanus and influenza vaccination of the elderly. Vaccine 14:1531–1537

Flynn MA, Weaver-Osterholtz D, Sharpe-Timms KL, Allen S, Krause G (1999) Dehydroepiandrosterone replacement in aging humans. J Clin Endocrinol Metab 84:1527–1533

Giagulli VA, Verdonck L, Giorgino R, Vermeulen A (1989) Precursors of plasma androstanediol- and androgen-glucuronides in women. J Steroid Biochem 33:935–940

Gordon CM, Leboff MS, Glowacki J (2001) Adrenal and gonadal steroids inhibit IL-6 secretion by human marrow cells. Cytokine 16:178–186

Gurnell EM, Hunt PJ, Curran SE, Conway CL, Huppert FA, Herbert J, Chatterjee VK (2002) A longer term trial of DHEA replacement in Addison's disease. Endocrine Society's 84th Annual Meeting, San Francisco, Abstract S19-2

Hunt PJ, Gurnell EM, Huppert FA, Richards C, Prevost AT, Wass JA, Herbert J, Chatterjee VK (2000) Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. J Clin Endocrinol Metab 85:4650–4656

Jakob F, Siggelkow H, Homann D, Kohrle J, Adamski J, Schutze N (1997) Local estradiol metabolism in osteoblast- and osteoclast-like cells. J Steroid Biochem Mol Biol 61:167–174

Jakobi JM, Killinger DW, Wolfe BM, Mahon JL, Rice CL (2001) Quadriceps muscle function and fatigue in women with Addison's disease. Muscle Nerve 24:1040–1049

Johannsson G, Burman P, Wiren L, Engstrom BE, Nilsson AG, Ottosson M, Jonsson B, Bengtsson BA, Karlsson FA (2002) Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. J Clin Endocrinol Metab 87:2046–2052

Kahn AJ, Halloran B (2002) Dehydroepiandrosterone supplementation and bone turnover in middle-aged to elderly men. J Clin Endocrinol Metab 87:1544–1549

Kawano H, Yasue H, Kitagawa A, Hirai N, Yoshida T, Soejima H, Miyamoto S, Nakano M, Ogawa H (2003) Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. J Clin Endocrinol Metab 88:3190–3195

Khaw KT (1996) Dehydroepiandrosterone, dehydroepiandrosterone sulphate and cardiovascular disease. J Endocrinol 150: Suppl, S149–S153

Kicman AT, Bassindale T, Cowan DA, Dale S, Hutt AJ, Leeds AR (2003) Effect of androstenedione ingestion on plasma testosterone in young women; a dietary supplement with potential health risks. Clin Chem 49:167–169

Kim SS, Brody KH (2001) Dehydroepiandrosterone replacement in addison's disease. Eur J Obstet Gynecol Reprod Biol 97:96–97

King DS, Sharp RL, Vukovich MD, Brown GA, Reifenrath TA, Uhl NL, Parsons KA (1999) Effect of oral androstenedione on serum testosterone and adaptations to resistance training in young men: a randomized controlled trial. JAMA 281:2020–2028

Kline MD, Jaggers ED (1999) Mania onset while using dehydroepiandrosterone. Am J Psychiatry 156:971

Labrie F (1991) Intracrinology. Mol Cell Endocrinol 78:C113–C118

Labrie F, Diamond P, Cusan L, Gomez JL, Belanger A, Candas B (1997) Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women. J Clin Endocrinol Metab 82:3498–3505

Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX, Pelletier G (2003) Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev 24:152–182

Lahita RG, Bradlow HL, Ginzler E, Pang S, New M (1987) Low plasma androgens in women with systemic lupus erythematosus. Arthritis Rheum 30:241–248

Laughlin GA, Barrett-Connor E (2000) Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo Study. J Clin Endocrinol Metab 85:3561–3568

Leder BZ, Longcope C, Catlin DH, Ahrens B, Schoenfeld DA, Finkelstein JS (2000) Oral androstenedione administration and serum testosterone concentrations in young men. JAMA 283:779–782

Leder BZ, Catlin DH, Longcope C, Ahrens B, Schoenfeld DA, Finkelstein JS (2001) Metabolism of orally administered androstenedione in young men. J Clin Endocrinol Metab 86:3654–3658

Liu CH, Laughlin GA, Fischer UG, Yen SS (1990) Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for a reduced 17, 20-desmolase enzymatic activity. J Clin Endocrinol Metab 71:900–906

Liu D, Dillon JS (2002) Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Galpha(i2,3). J Biol Chem 277:21379–21388

Lovas K, Loge JH, Husebye ES (2002) Subjective health status in Norwegian patients with Addison's disease. Clin Endocrinol (Oxf) 56:581–588

Lovas K, Gebre-Medhin G, Trovik TS, Fougner KJ, Uhlving S, Nedrebo BG, Myking OL, Kampe O, Husebye ES (2003) Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. J Clin Endocrinol Metab 88:1112–1118

Majewska MD, Demirgoren S, Spivak CE, London ED (1990) The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABAA receptor. Brain Res 526:143–146

Markowitz JS, Carson WH, Jackson CW (1999) Possible dihydroepiandrosterone-induced mania. Biol Psychiatry 45:241–242

Martel C, Melner MH, Gagne D, Simard J, Labrie F (1994) Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey. Mol Cell Endocrinol 104:103–111

Mazat L, Lafont S, Berr C, Debuire B, Tessier JF, Dartigues JF, Baulieu EE (2001) Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits, and 10-year mortality. Proc Natl Acad Sci USA 98:8145–8150

Meikle AW, Dorchuck RW, Araneo BA, Stringham JD, Evans TG, Spruance SL, Daynes RA (1992) The presence of a dehydroepiandrosterone-specific receptor binding complex in murine T cells. J Steroid Biochem Mol Biol 42:293–304

Melez KA, Boegel WA, Steinberg AD (1980) Therapeutic studies in New Zealand mice VII Successful androgen treatment of NZB/NZW F1 females of different ages. Arthritis Rheum 23:41–47

Morales AJ, Nolan JJ, Nelson JC, Yen SS (1994) Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. J Clin Endocrinol Metab 78:1360–1367

Morales AJ, Haubrich RH, Hwang JY, Asakura H, Yen SS (1998) The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. Clin Endocrinol (Oxf) 49:421–432

Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB (1973) The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. J Endocrinol 57: 123–134

Oelkers W (1999) Dehydroepiandrosterone for adrenal insufficiency. N Engl J Med 341: 1073–1074

Okabe T, Haji M, Takayanagi R, Adachi M, Imasaki K, Kurimoto F, Watanabe T, Nawata H (1995) Up-regulation of high-affinity dehydroepiandrosterone binding activity by dehydroepiandrosterone in activated human T lymphocytes. J Clin Endocrinol Metab 80:2993–2996

Orentreich N, Brind JL, Rizer RL, Vogelman JH (1984) Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551–555

Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H (1992) Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab 75:1002–1004

Palmert MR, Hayden DL, Mansfield MJ, Crigler JF, Jr, Crowley WF, Jr, Chandler DW, Boepple PA (2001) The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. J Clin Endocrinol Metab 86:4536–4542

Parker CR Jr, Mixon RL, Brissie RM, Grizzle WE (1997) Aging alters zonation in the adrenal cortex of men. J Clin Endocrinol Metab 82:3898–3901

Parker LN, Levin ER, Lifrak ET (1985) Evidence for adrenocortical adaptation to severe illness. J Clin Endocrinol Metab 60:947–952

Parker CR, Jr, Slayden SM, Azziz R, Crabbe SL, Hines GA, Boots LR, Bae S (2000) Effects of aging on adrenal function in the human: responsiveness and sensitivity of adrenal androgens and cortisol to adrenocorticotropin in premenopausal and postmenopausal women. J Clin Endocrinol Metab 85:48–54

Percheron G, Hogrel JY, Denot-Ledunois S, Fayet G, Forette F, Baulieu EE, Fardeau M, Marini JF (2003) Effect of 1-year oral administration of dehydroepiandrosterone to 60- to 80-year-old individuals on muscle function and cross-sectional area: a double-blind placebo-controlled trial. Arch Intern Med 163:720–727

Petri MA, Lahita RG, Van Vollenhoven RF, Merrill JT, Schiff M, Ginzler EM, Strand V, Kunz A, Gorelick KJ, Schwartz KE (2002) Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum 46:1820–1829

Rasmussen BB, Volpi E, Gore DC, Wolfe RR (2000) Androstenedione does not stimulate muscle protein anabolism in young healthy men. J Clin Endocrinol Metab 85:55–59

Ravaglia G, Forti P, Maioli F, Boschi F, Bernardi M, Pratelli L, Pizzoferrato A, Gasbarrini G (1996) The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds. J Clin Endocrinol Metab 81:1173–1178

Reiter EO, Fuldauer VG, Root AW (1977) Secretion of the adrenal androgen, dehydroepiandrosterone sulfate, during normal infancy, childhood, and adolescence, in sick infants, and in children with endocrinologic abnormalities. J Pediatr 90:766–770

Reiter WJ, Pycha A, Schatzl G, Pokorny A, Gruber DM, Huber JC, Marberger M (1999) Dehydroepiandrosterone in the treatment of erectile dysfunction: a prospective, double-blind, randomized, placebo-controlled study. Urology 53:590–594

Shifren JL, Braunstein GG, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA (2000) Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688

Sklar CA, Kaplan SL, Grumbach MM (1980) Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. J Clin Endocrinol Metab 51:548–556

Solerte SB, Fioravanti M, Vignati G, Giustina A, Cravello L, Ferrari E (1999) Dehydroepiandrosterone sulfate enhances natural killer cell cytotoxicity in humans via locally generated immunoreactive insulin-like growth factor I. J Clin Endocrinol Metab 84:3260–3267

Straub RH, Konecna L, Hrach S, Rothe G, Kreutz M, Scholmerich J, Falk W, Lang B (1998) Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence. J Clin Endocrinol Metab 83:2012–2017

Strous RD, Maayan R, Lapidus R, Stryjer R, Lustig M, Kotler M, Weizman A (2003) Dehydroepiandrosterone augmentation in the management of negative, depressive, and anxiety symptoms in schizophrenia. Arch Gen Psychiatry 60:133–141

Sugino M, Ohsawa N, Ito T, Ishida S, Yamasaki H, Kimura F, Shinoda K (1998) A pilot study of dehydroepiandrosterone sulfate in myotonic dystrophy. Neurology 51:586–589

Suzuki T, Suzuki N, Daynes RA, Engleman EG (1991) Dehydroepiandrosterone enhances IL2 production and cytotoxic effector function of human T cells. Clin Immunol Immunopathol 61:202–211

Suzuki T, Suzuki N, Engleman EG, Mizushima Y, Sakane T (1995) Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 99:251–255

Svec F, Porter JR (1998) The actions of exogenous dehydroepiandrosterone in experimental animals and humans. Proc Soc Exp Biol Med 218:174–191

Thomas G, Frenoy N, Legrain S, Sebag-Lanoe R, Baulieu EE, Debuire B (1994) Serum dehydroepiandrosterone sulfate levels as an individual marker. J Clin Endocrinol Metab 79:1273–1276

Trivedi DP, Khaw KT (2001) Dehydroepiandrosterone sulfate and mortality in elderly men and women. J Clin Endocrinol Metab 86:4171–4177

Van Niekerk JK, Huppert FA, Herbert J (2001) Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. Psychoneuroendocrinology 26:591–612

Van Vollenhoven RF, Engleman EG, Mcguire JL (1994) An open study of dehydroepiandrosterone in systemic lupus erythematosus. Arthritis Rheum 37:1305–1310

Van Vollenhoven RF, Engleman EG, Mcguire JL (1995) Dehydroepiandrosterone in systemic lupus erythematosus: Results of a double-blind, placebo-controlled, randomized clinical trial. Arthritis Rheum 38:1826–1831

Villareal DT, Holloszy JO, Kohrt WM (2000) Effects of DHEA replacement on bone mineral density and body composition in elderly women and men. Clin Endocrinol (Oxf) 53:561–568

Waxenberg SE, Drellich MG, Sutherland AM (1959) The role of hormones in human behavior I: Changes in female sexuality after adrenalectomy. J Clin Endocrinol Metab 19:193–202

Williams MR, Ling S, Dawood T, Hashimura K, Dai A, Li H, Liu JP, Funder JW, Sudhir K, Komesaroff PA (2002) Dehydroepiandrosterone inhibits human vascular smooth muscle cell proliferation independent of ARs and ERs. J Clin Endocrinol Metab 87:176–181

Wit JM, Langenhorst VJ, Jansen M, Oostdijk WA, Van Doorn J (2001) Dehydroepiandrosterone sulfate treatment for atrichia pubis. Horm Res 56:134–139

Wolf OT, Neumann O, Hellhammer DH, Geiben AC, Strasburger CJ, Dressendorfer RA, Pirke KM, Kirschbaum C (1997) Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and wellbeing in healthy elderly women and men. J Clin Endocrinol Metab 82:2363–2367

Wolf OT, Naumann E, Hellhammer DH, Kirschbaum C (1998) Effects of dehydroepiandrosterone replacement in elderly men on event-related potentials, memory, and wellbeing. J Gerontol A Biol Sci Med Sci 53:M385–M390

Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, Roberts E (1999) Double-blind treatment of major depression with dehydroepiandrosterone. Am J Psychiatry 156:646–649

Wolkowitz OM, Kramer JH, Reus VI, Costa MM, Yaffe K, Walton P, Raskind M, Peskind E, Newhouse P, Sack D, De Souza E, Sadowsky C, Roberts E (2003) DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. Neurology 60:1071–1076

Yen SS, Morales AJ, Khorram O (1995) Replacement of DHEA in aging men and women: Potential remedial effects. Ann NY Acad Sci 774:128–142

Young J, Couzinet B, Nahoul K, Brailly S, Chanson P, Baulieu EE, Schaison G (1997) Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. J Clin Endocrinol Metab 82:2578–2585

Zappulla F, Ventura D, Capelli M, Cassio A, Balsamo A, Frejaville E, Bolelli G, Cacciari E (1981) Gonadal and adrenal secretion of dehydroepiandrosterone sulfate in prepubertal and pubertal subjects. J Endocrinol Invest 4:197–202

Zwain IH, Yen SS (1999) Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. Endocrinology 140:3843–3852

20

Selective androgen receptor modulators (SARMs)

S.S. Wolf and M. Obendorf

Contents

20.1 Introduction

Nuclear hormone receptors (NHRs) as members of the nuclear receptor (NR) gene family that regulate a wide range of physiological and pathophysiological effects, are activated through binding of small ligands (hormones), and acting as transcription factors by interaction with distinct DNA motifs on gene promoter to control target gene transcription. Because this mechanism is similar in all tissues it was highly interesting to discover that specific ligands have selective effects on target genes and target tissues, opening up the concept of creating selective NHR modulators for particular therapeutic endpoints. A group of compounds were described

that function as estrogen receptor (ER) agonists in some tissues (e.g. bone) but have opposite estrogen action in others (e.g. breast), establishing the concept of selective estrogen receptor modulators (SERMs) and are already available as drugs (Cosman and Lindsay 1999). From the molecular mode of action on the nuclear receptor, SERMs are characterised as mixed agonists/antagonists as measurable in cell culture using reporter gene assays in the absence or presence of the ligand. This mixed behaviour in cell culture is reflected by their in vivo action, and both, agonistic and antagonistic properties may be combined in selected target organs (Katzenellenbogen and Katzenellenbogen 2002). In the agonistic conformation as shown in LBD crystal structure of ERα in complex with estradiol (E2), the helix-12 is in the ligand binding pocket in complex with the agonist, but displaced in the antagonist complex with raloxifen (Brzozowski *et al.* 1997). It seems reasonable that similar mechanisms are employed involving other NHRs such as progesterone receptor (PR) or androgen receptor (AR) with the corresponding hormones leading to selective progesterone receptor modulators (SPRMs) or selective androgen receptor modulators (SARMs) respectively.

In analogy to the definition of SERMs, SARMs would be characterised as androgen receptor specific ligands which reveal a mixed agonist/antagonistic behaviour in cell culture in vitro. However the term SARM was introduced four years ago for molecules that target the AR in a distinct way with tissue-specific desired biological effects (Negro-Vilar 1999). In this definition based on in vivo action SARMs are described as compounds which elicits androgen agonism in one or more target tissues (e.g. muscle and bone) and antagonism and/or minimal agonism in other tissues (e.g. prostate and skin) without further questioning the molecular mode of action. Here, in this chapter, SARMs are defined as mixed receptor agonists/antagonists in vitro elucidated with transactivation assay systems. Such mixed agonist/antagonist activity on transactivation can lead to selective action in vivo targeting AR in different tissues. In a way the tissue specific action of SERMs on ER are considered useful for treatment of menopausal women as well as for breast cancer. However if such tissue-selective action of SARMs have similar desirable in vivo action, such as agonistic activity in brain, bone, and muscle, but not in the prostate, remains to be elucidated. We will describe further possibilities to determine the selective effects of androgens in different tissues and explain possible mechanisms beside the mixed agonist/antagonist activity on receptor mediated transactivation. The idea is that the different mechanism mentioned here will help to develop organ- or tissue-selective AR agonists in some tissues (e.g. brain) with low or antagonistic activity in other tissues (e.g. prostate).

To achieve different effects in tissues and organs for hormonal action several possibilities for the ligand as well as the receptor do exist (Fig. 20.1). After passive diffusion of an androgen into the cell it will either act directly or be converted, as the

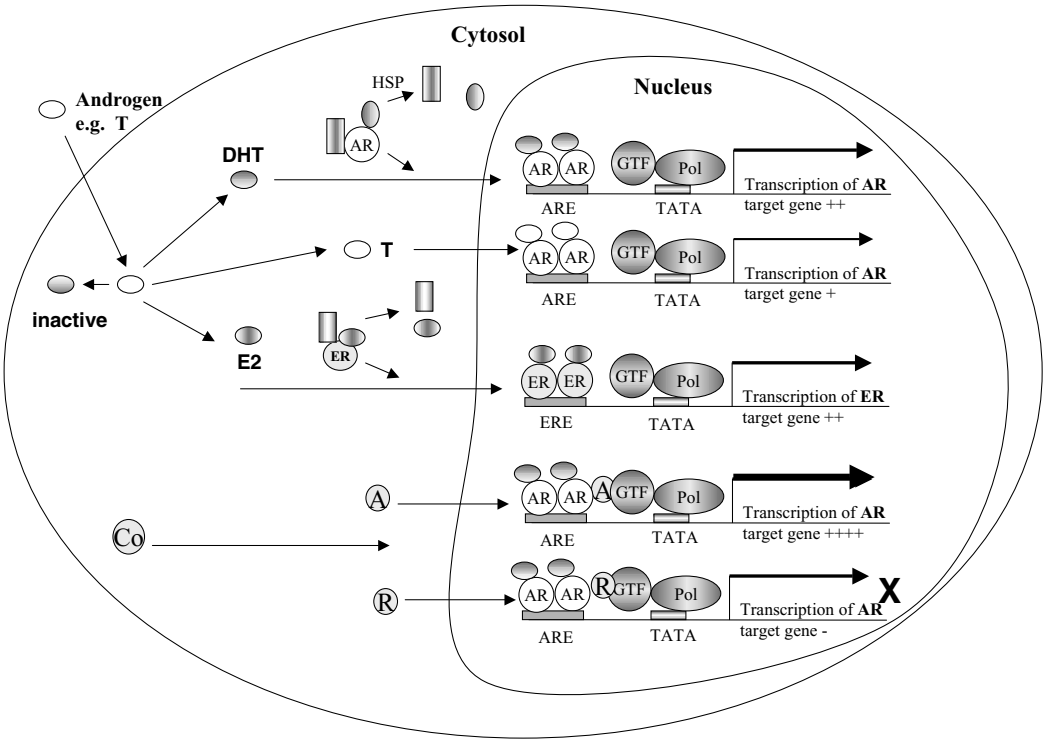


Fig. 20.1 Possible action of androgens
An androgen (e.g. T) diffuses into the cell and may either be inactive, bind to AR directly to release chaperone proteins (e.g. HSP90), or be converted to a stronger androgen (e.g. DHT) or even to another class of hormones (e.g. E2) to activate the corresponding target genes. Comodulators may either enhance (coactivator) or repress (corepressor) the nuclear receptor mediated transactivation (T testosterone; DHT dihydrotestosterone; E2 estradiol; AR androgen receptor; ER estrogen receptor; HSP heat shock protein; ARE androgen response element; ERE estrogen response element; TATA TATA-box; GTF general transcription factors; Pol RNA polymerase II; Co comodulator; A coactivator; R corepressor).

metabolism of the hormone is important for the function which finally determines the receptor mediated activity. The androgen can either bind to the receptor as it enters the cell controlling target gene activation, or the androgen may be transformed into an inactive form resulting in low or no receptor mediated action, or finally the androgen is metabolised through cellular enzymes (e.g. 5α-reductase or aromatase) leading to compounds which either have an increased affinity for the AR, or interact with a different nuclear receptor. Also intermolecular contacts between the AR itself (N/C-terminal interaction) as well as interaction of the receptor with comodulators influence androgen-mediated transcriptional transactivation. The comodulators are either able to enhance (coactivator) or repress (corepressor) target

gene transcription. Last but not least, the distribution of AR and AR comodulators in specific tissues are important for the selective action of androgens.

Testosterone is a ligand for the AR and essential for development and maintenance of the male reproductive system and secondary male sex characteristics. Testosterone is an example for an agonistic and non tissue-selective androgen as it shares the same activities in anabolic as well as in reproductive target tissues. Anabolic steroids are known to partially separate between anabolic activities on muscle, hematopoesis and androgenic activities on the prostate. There is growing interest in finding selective male hormones which fulfil unmet medical needs to act as tissue-selective androgens with agonist activity in e.g. muscle, brain and bone but the prostate and antagonist activity only in the prostate. Furthermore, tissue-selective androgens may also useful for treatment of some conditions in menopausal women, especially for those complaining about reduced libido. The development of tissue-selective androgens are just at the beginning with few in clinical phase evaluation but will increase substantively in the near future. This chapter will summarise the knowledge about the mechanism of tissue-selectivity of androgens with emphasis on the development of an ideal tissue-selective androgen.

20.2 Mechanism of tissue selectivity

20.2.1 Metabolism of androgens in different tissues

In adult men testosterone itself is the hormone governing libido, gonadotropin feedback regulation, and growth and function of extragenital tissues, such as muscle, kidney, liver, and bone. When testosterone diffuses from the outside into the cytosol of cells, it binds either to AR directly, or is converted into DHT with higher affinity to the receptor, or, after it is aromatised to estradiol (E2) it can interact with ERα or ERβ. In addition, it is feasible that the androgen is metabolised to an inactive compound which has no effect on NR target genes or on cellular function.

Testosterone is metabolised to the more active androgen dihydrotestosterone (DHT) by 5α-reductase, which has much greater affinity to the AR than T and enhances AR-mediated transcription of target genes amplifying therefore the action of testosterone. The reductase is expressed especially in organs like skin, hair follicle and prostate and thereby contribute to undesired side-effects of androgens on baldness, acne, hirsutism (in women) and on the prostate.

Testosterone is also metabolised in vivo to E2 by aromatase which is in male predominantly expressed in brain, liver and adipose tissues exerting estrogenic activity and leading to the activation of estrogen-responsive target genes (Harada *et al.* 1993; McEwen 1980; Nimrod and Ryan 1975; Simpson *et al.* 1994). Therefore, the androgens have the potential to activate a different set of target genes after tissue-specific aromatisation to estrogens. Indeed several beneficial effects on the

brain, as well as bone protection, libido and reproduction, are most likely mediated not by the androgen itself, but by its aromatised product. The additional effects of aromatization were described for the preservation of osteopenia (for overview Riggs *et al.* 2002) and neuroprotection (Azcoitia *et al.* 2001). A similar role for estrogens in enhancement of libido in human is controversially discussed in the literature (e.g. Gooren 1985; Roselli and Resko 2001). Most data clearly supporting the hypothesis were from studies in birds or rats. However, the few data available from human and other primates stressed the importance of not only systemical estrogens (Carani *et al.* 1999), but also locally synthesised estrogens (Zumpe *et al.* 1993).

20.2.2 AR interaction with DNA motifs in promoter of target genes

When the androgen bound AR translocates from the cytosol to the nucleus, the interaction with the androgen response elements (AREs) on the promoter of androgen target genes will lead to activation of transcription. Structural studies showed that not only the steroid hormone is able to alter nuclear receptor conformation, but also the contact of the receptor with distinct responsive elements on the DNA of gene promoter may alter its form as it was shown for ERs and retinoid X receptor (RXR) (Loven *et al.* 2001; Yi *et al.* 2002; Zhao *et al.* 2000). The interaction between NR and the DNA influences the ability of comodulator recruitment and finally NR signalling. It can be speculated that the conformation mediated by ligands and DNA-binding synergistically influence the interaction with particular comodulators which determine the effect on gene transactivation. The overall receptor conformation influenced by ligand and ARE may produce a unique scaffold surface allowing interaction with distinct proteins modulating gene transcription. Further evidence for this so far highly speculative theory is the observation of promoter selective agonistic action of tamoxifen on ER (Berry *et al.* 1990; Shull *et al.* 1992; Tzukerman *et al.* 1994).

20.2.3 Protein/protein interaction of the AR

The AR is able to perform interactions with proteins. The AR monomer can build an intermolecular interaction bridge and makes contact with a variety of other proteins. These interactions are vital for the AR-mediated transcriptional transactivation of target genes as they modulate the activity of the receptor.

Direct contact between the amino-terminal and the carboxyl-terminal regions (N/C-interaction) of the AR was found using a two-hybrid system and glutathione-S-transferase fusion protein studies. Hereby, distinct amino-terminal sequence motifs in the receptor mediates interaction with the AF2 region of the LBD (He *et al.* 2000). Because androgens are able to bind the LBD located at the C-terminus of the AR, the intermolecular interaction between the receptor sides is controlled by the

hormone itself but independent of the binding to DNA (Langley *et al.* 1995). The N/C-interaction of AR is required for potent agonists to be effective at low concentrations but is not required for weak agonist activity at higher ligand concentrations. Therefore, the formation of the N/C-interaction contributes to the stability of AR at low ligand concentrations (Kemppainen *et al.* 1999). As found recently, there is also strong correlation between the strength of the N/C-terminal interactions and the corresponding clinical phenotype in androgen insensitive syndrome (AIS), indicating the importance of the structural conformation of the receptor (Ghali *et al.* 2003). Furthermore, comodulators such as TIF2 can augment the N/C-terminal interaction of the AR and the nuclear orphan receptor DAX-1, which has low expression level in benign prostate hyperplasia (BPH) tissue, is able to disrupt the N/C-terminal interaction of the AR but does not influence the interaction between AR and SRC-1 (Agoulnik *et al.* 2003; Ghali *et al.* 2003). Therefore some orphan receptors and comodulators are important mediators of hormonal signals in human diseases.

As it became clear that the transcriptional activities of AR and other members of the NR superfamily are modulated by coregulatory proteins, many groups tried to identify interacting partners. To search for possible binding proteins for the AR the yeast two-hybrid system, established over ten years ago (Fields and Song 1989), and direct cDNA expression library screening using affinity chromatography, were performed (e.g. Hsiao and Chang 1999). Mostly, the cDNA libraries used to identify AR-interacting proteins are deriving from prostate and testis and only rarely from other tissues, such as brain or hepatic cell lines. Up to now, over 70 proteins described as able to interact with the AR, were compiled in different reviews (Heinlein and Chang 2002; Hermanson *et al.* 2002; McKenna *et al.* 1999; Robyr *et al.* 2000) and also elsewhere (ww2.mcgill.ca/androgendb). When the properties of these proteins were analysed in detail, it becomes clear that most were not exclusive for the AR as they interact with other NRs as well. In addition, some putative AR interacting proteins may be located either in a tissue where the AR is not present, or even localised differently inside a single cell. This promiscuity makes the search for AR-specific comodulators difficult especially when it is not clear what defines comodulator selectivity. Nevertheless, some comodulators specific for the AR were described (e.g. FHL2). The transcriptional effect of FHL2 was measured using transient transfection experiments in vitro that examine the ability of the comodulator to alter the AR-mediated transcriptional activity on an artificial reporter construct whereas the transactivation of other nuclear receptors (GR, PR, and MR) were not mediated by FHL2 (Müller *et al.* 2000).

Coregulatory proteins are able to interact with the hormone receptor either direct or indirect via secondary proteins to enhance (coactivator) or reduce (corepressor) the receptor mediated transactivation of target genes. The modulators may stabilise

the receptor/ligand binding and influence the translocation of the receptor into the nucleus. ARA70, one of the first comodulator for AR described, enhances AR-dependent transcription in prostate cells. Additionally, the interaction of ARA70 with hormone bound AR enhances stability of the receptor (Yeh and Chang 1996). E2 usually binds AR with a 100-fold lower affinity than DHT and does not mediate AR-dependent transactivation. However in the presence of ARA70, E2 activates AR-mediated transcription in PC3 (Han *et al.* 2001) and DU145 (Yeh *et al.* 1998). It may be possible that AR-dependent genes are induced by E2 in tissues where ARA70 expression is elevated causing androgen effects mediated by E2. Additionally, it was shown that ARA70 enables the AR antagonists hydroxyflutamide and casodex to behave as agonists (Miyamoto *et al.* 1998; Yeh *et al.* 1997). This is especially relevant for prostate cancer patients treated with androgen antagonists as part of androgen ablation therapy. When these compounds were used in the clinic, it must be considered if comodulators are present in the tissue, as they lead to enhancement of gene activation.

Comodulators determine the tissue specificity of SERMs with respect to agonistic and antagonistic activities (Shang and Brown 2002). Recently, it was shown that the coactivator/corepressor ratio also modulate PR-mediated transcription by a selective receptor modulator (RU38486) acting as an agonist in T47D cells and as an antagonist in HeLa cells (Liu *et al.* 2002). It may be possible that similar mechanisms are valid for AR-mediated transactivation in different cells where the relative amount of comodulators play a vital role.

The mechanism how comodulators act on transcription in detail is only partially understood. They may work as bridging factors between the DNA-bound nuclear receptor and the basal transcriptional machinery, as chromatin modelling factors, as protein modifying enzymes or even as DNA binding factors to regulate their corresponding genes. Importantly, the tissue distribution of comodulators differs in the organism and so far not much information is available concerning the regulation and activation of AR interacting proteins.

20.2.4 Tissue distribution of AR and AR-specific comodulators

When hormones are effective acting on cells specifically, it is obvious that the corresponding receptor needs to be present to mediate transcriptional activity and induce expression of target genes. Therefore, the availability of AR is a prerequisite for the induction of androgen target genes. AR is widely distributed in mammalian reproductive and non-reproductive tissues but is predominantly expressed in testis, heart, prostate, muscle and ovary as determined by Northern blot analysis (Fig. 20.2).

While the abundance of comodulators is reflected by tissue-specific expression fingerprints, the coexpression of multiple comodulators in a single tissue appears to

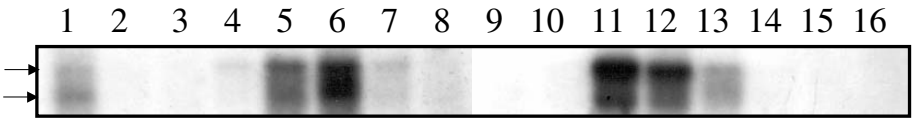
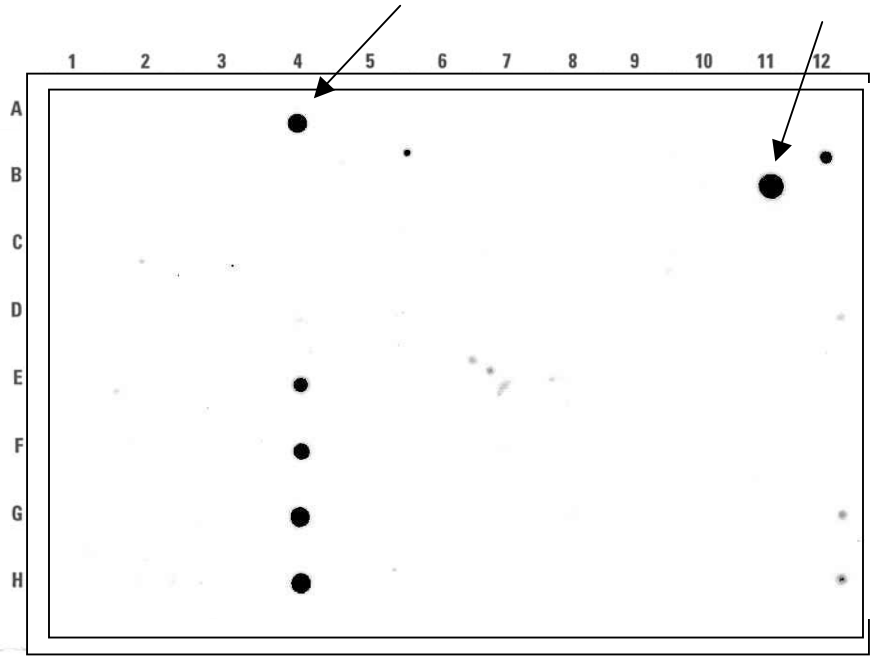


Fig. 20.2 Tissue distribution of the AR by Northern blotting
AR (arrows) is expressed in human androgen target tissues. PolyA+ RNAs prepared from human tissues were separated, blotted and hybridised with a labelled fragment for the human AR following autoradiography. (1 heart; 2 brain; 3 placenta; 4 lung; 5 liver; 6 muscle; 7 kidney; 8 pancreas; 9 spleen; 10 thymus; 11 prostate; 12 testis; 13, ovary; 14 intestine; 15 colon; 16 lymphocytes).

be a general rule (McKenna and O'Malley 2000). The distribution of comodulators must overlap with the expression of the receptor, only then will it be possible to influence NR-mediated transcriptional activation of target genes. Many comodulators (e.g. ARA70, ARA54) are expressed in the same tissues as the AR (Kang *et al.* 1999; Yeh and Chang 1996). Interestingly, the AR-specific coactivator, FHL2, is very highly expressed in the human fetal and adult heart and may influence the androgen action in this organ (Müller *et al.* 2000). Furthermore, the expression of FHL2 is dominant in the ventricle, the septum and the apex of the heart, as determined with an expression array including more then 70 human tissue and cell line samples (Fig. 20.3). Overall it is necessary for comodulators to act in concert with the receptor so that both are expressed at the same time and are able to mediate the NR-mediated transcriptional activation.

20.2.5 Other approaches for selective actions

Modifications of the AR such as splice variants, isoforms or postranslational modifications may effect the interaction of the androgen with the receptor and determine tissue selectivity, but not much is known yet. Recent publications (Kousteni *et al.* 2001; Migliaccio *et al.* 2000) stress the importance of transcription-independent, nongenomic actions of steroids, reflecting the observation of rapid effects, mediated by hormones and their hormone receptors within minutes, thereby excluding transcription-dependent activity (genomic action). It was demonstrated that kinases such as PI3K/AKT (Castoria *et al.* 2003) on one side or Src/Ras/MEK (Kousteni *et al.* 2003) on the other side are downstream targets of the activated receptors. It is very likely that the necessary interaction with proteins for nongenomic action also depends on the ability of the ligand bound hormone receptor to adopt specific conformations which may differ from the conformation necessary for DNA-binding and transactivation. However this is highly speculative and it is unknown at this time if a preferential transcription-independent action could



	1	2	3	4	5	6	7	8	9	10	11	12
A	whole brain	cerebellum, left	substantia nigra	heart	esophagus	colon, transverse	kidney	lung	liver	leukemia, HL-60	fetal brain	yeast total RNA
B	cerebral cortex	cerebellum, right	accumbens nucleus	aorta	stomach	colon, desending	skeletal muscle	placenta	pancreas	HeLa S3	fetal heart	yeast tRNA
C	frontal lobe	corpus callosum	thalamus	atrium, left	duodenum	rectum	spleen	bladder	adrenal gland	leukemia, K-562	fetal kidney	*E. coli* rRNA
D	parietal lobe	amygdala	pituitary gland	atrium, right	jejunum		thymus	uterus	thyroid gland	leukemia, MOLT-4	fetal liver	*E. coli* DNA
E	occipital lobe	caudate nucleus	spinal cord	ventricle, left	ileum		peripheral blood leukocyte	prostate	salivary gland	Burkitt's lymphoma, Raji	fetal spleen	Poly r(A)
F	temporal lobe	hippo-campus		ventricle, right	ilocecum		lymph node	testis	mammary gland	Burkitt's lymphoma, Daudi	fetal thymus	human C_0t -1 DNA
G	p. g.* of cerebral cortex	medulla oblongata		inter-ventricular septum	appendix		bone morrow	ovary		colorectal adeno-carcinoma, SW480	fetal lung	human DNA 100 ng
H	pons	putamen		apex of the heart	colon, ascending		trachea			lung carcinoma, A549		human DNA 500 ng

* paracentral gyrus

Fig. 20.3 The expression of the AR-specific coactivator FHL2 is restricted to distinct tissue

FHL2 is expressed in the heart only (arrows). PolyA+ RNAs from human tissues and cell lines were prepared, transferred onto a membrane and probed with the labelled cDNA for FHL2 followed by autoradiography. Major expression (arrows) of FHL2 was in the fetal (B11) and adult (A4) heart, left and right ventricle (E4, F4), the interventricular septum (G4) and the apex (H4) of the heart.

be beneficial for hypogonadism or PCa treatment. Effects which are suggested for nongenomic actions of steroid receptors includes beneficial and undesired effects on proliferation, protection of neurons and osteoblasts, and vasorelaxation (Cato *et al.* 2002; Sci STKE).

The identification of hormones which selectively alter genomic vs. nongenomic effects and signalling may lead to the development of novel compounds that specifically modulate the signals in vivo. First examples for androgens which were able to modulate genomic and nongenomic responses differentially were described (Lutz *et al.* 2003). However, it is not clear if such approaches will lead to tissue-selective hormones.

20.3 Search for tissue-specific androgens

20.3.1 Transcriptional reporter assays

For the investigation of androgen effects on target genes cellular in vitro assay systems are implemented. This includes a suitable cell line either expressing the AR or, if the AR is not present, its expression is accomplished by cell transfection techniques. In addition, reporter genes (e.g. luciferase or ß-galactosidase) under the control of an androgen responsive promoter such as MMTV (mouse mammary tumour virus), PSA (prostate specific antigen) or probasin employing AREs are transfected into the cells. After androgen treatment of the cells, the measurement of the reporter gene is corresponded with the activity of the AR-dependent transactivation and the potency of the hormone. If in addition comodulators are transfected into the cells, the activity of the AR-dependent reporter signal should either be enhanced or decreased. Importantly, when internal comodulators are expressed in the cells already that activate the AR-dependent reporter signal, an overexpression of these proteins may not modulate the reporter signal. It is therefore essential to know the expression level of the corresponding comodulators in the cells investigated.

A simple however straightforward tool to identify tissue-selective androgens is the comparison of androgen action as measured by reporter gene expression under the control of the same androgen responsible promoter employing different cells, representing the corresponding organs. In this system, the involved cell- or tissue-specific comodulators need not be known in detail. However a possible pitfall may be the alteration of comodulator gene expression in cell lines in comparison to tissue cells in vivo due to changes in the cellular properties, gene regulation and ultimately also AR signalling.

20.3.2 Promoter specific regulation

The transcriptional activation method with different androgen-dependent promoters may be implemented to find dissociated androgens. The structure of

promoters of AR target genes were described including one or more binding sites for the receptor. Subtle differences in the sequence of response elements can dictate androgen specific responses and creates new possibilities in the research on hormone-selective action and provides a new angle in the search for selective ligands that might influence AR mediated action via the type of DNA motif (Verrijdt *et al.* 2003). The rat probasin promoter, the human secretory component upstream enhancer and the mouse sex-limited protein enhancer are examples of elements specifically regulated by androgens involving androgen-specific DNA regions. As ligand bound AR interacts with the DNA, the conformation of the receptor will be altered. This was shown for the ER interaction with the DNA (see 20.2.3), but no data are available for AR. However, it is very likely that also for the AR individual AREs cause distinct conformation changes of the receptor. Particular ligands may alter the AR conformation synergistically with the individual ARE. Use and comparison of distinct androgen-dependent gene promoters might be a rationale for the identification of androgens with selective action.

20.3.3 In vivo test systems

The rodent prostate hypertrophy test was developed half a century ago (Hershberger and Shipley 1953) and is still used successfully to determine androgen effects in vivo (e.g. Yin *et al.* 2003b). Chemical substances are administered to immature male rodents (rats) or those from which the testes has been removed. Whether these compounds show the androgen-like effects or not is evaluated by factors such as changes in organ weights as well as by histological inspection. The agonist and antagonist effects of androgens are measured in this model in which after castration and hormone treatment surrogate endpoints are measured in tissues including prostate, seminal vesicle, and levator ani muscle. In addition, to characterise in vivo properties of compounds in more detail, measurement of biochemical markers for anabolic effects (e.g. IGF and GH) or markers demonstrating action on the brain which are involved in feedback-regulation of steroid synthesis (e.g. plasma hormone levels such as LH) can be used.

Furthermore, animal models including a hormone-dependent tumour are available for assessing the antagonist activity of a potential SARM. Using a rat bearing an androgen-dependent prostate tumour (e.g. Dunning rat) the effect of the SARM on the androgen-dependent prostate tumour can be compared to the effect of the SARM on non-tumour tissues (Zaccheo *et al.* 2000).

20.3.4 The ideal tissue-selective androgen

The definition of "ideal" depends strongly on the clinical situation. The treatment of male hypogonadism has to reveal agonistic activities in muscle, bone and brain and no activity in e.g. the prostate. As indicated the dissociation of an androgen

is implemented in vitro by recruitment of comodulators from which are known, they are expressed in the desired target tissues. For example FHL2 may coactivate androgen mediated action in the heart specifically and may be useful for heart muscle specific androgen benefits. In addition, recruitment of corepressors to the AR in prostate cells is beneficial to inhibit the androgen-dependent transactivation of target genes. For reasons to avoid undesired side effects, also androgens which are not 5α-reducible and therefore have no increased activity in organs with 5α-reductase (e.g. prostate, skin and hair follicle) are preferably used (see 20.2.1). Some beneficial androgen action especially in bone, brain and cardiovascular are not mediated by the androgen itself, but the aromatised product (see 20.2.1). The ideal tissue-selective androgen for hormone replacement should have the liability for aromatisation.

In male hypogonadism the internal testosterone secretion is already declined. A further decrease in testosterone production is not desired. Testosterone synthesis is positively controlled by gonadotrophins, especially LH. For that reason an ideal tissue-selective androgen for treatment of hypogonadism should not decrease LH secretion.

In comparison to hypogondism, the definition for an ideal dissociation is different in male contraception or PCa. For male contraception a reduction in gonadotrophins is necessary, for PCa protection it is desired. For PCa, an ideal dissociated androgen should have antagonistic action on the prostate, but maintaining agonistic effects on brain, muscle and bone, to avoid side effects, like hot flushes, loss of libido, mood disturbance, muscle wasting and osteopenia.

The requirements for female androgen substitution differ from males. Anabolic action on muscle and bone as well as libido are the positive effects of androgen action in women. Hirsutism, acne, male pattern baldness and voice change are severe side effects. In general a weak, but safe androgen is required, in order to avoid these side effects. Again an androgen which is not 5 α-reducible would be useful, as 5 α-reductase mainly enhances androgen action in skin and hair follicles (see 20.2.1). Alternatively, a SARM with antagonistic effects in these organs, but agonistic response in muscle, bone and brain would be ideal.

20.4 Examples of tissue-specific androgens

20.4.1 General

Currently the prime use of androgens are in the treatment of reproductive disorders, male hypogonadism and anabolic effects on non-gonadal disorders such as erythropoiesis, osteopenia, and wasting disease. The main problem for the indications is that the natural androgen testosterone has anabolic as well as androgenic effects acting equally on different tissues. But the discovery and development of tissue-selective androgens offers a huge opportunity to differentially regulate the

androgen effects in various target tissues, thus minimising the interference to normal physiological processes while targeting desirable therapeutic goals.

Chemicals that modulate the transcriptional activity of the AR can be divided in two structural (steroidal and non-steroidal) and two functional (androgenic and antiandrogenic) classes. Androgens such as testosterone and related compounds are used clinically to treat androgen-deficiency. Steroidal anti-androgens like cyproterone acetate (CPA) as well as non steroidal antagonists as bicalutamide or flutamide are used to counteract the undesirable effects of androgens as for treatment of PCa. A recent paper reviews the patent publications on tissue-selective androgens with different effects on non-reproductive target tissues (Chengalvala *et al.* 2003).

Non-steroidal synthetic compounds (e.g. tricyclic pyridinodihydroquinoline derivates) show promising anabolic effects without significant action on the prostate and seminal vesicle.

20.4.2 Role of 5α-reduction

Androgens, which are not reducible by the tissue-specific enzyme 5α-reductase are likely to be dissociated (see 20.2.1). Several steroidal compounds on the market with enhanced anabolic effects as well as recently described non-steroidal compounds are not 5α-reducible (e.g. anadrol, oxandrolone) (for overview Chengalvala *et al.* 2003).

A steroidal compound, 7α-methyl-19-nortestosterone (MENT), with selective properties was described for the development for male contraception. MENT which acts as an agonist only, differs from testosterone as it does not undergo 5α-reduction in the prostate as does testosterone (Anderson *et al.* 2003; Cummings *et al.* 1998; Sundaram *et al.* 1993). Therefore a dose of MENT sufficient to maintain normal androgen function in most organs will not hyper-stimulate the prostate because its action is not amplified as is that of testosterone. This compound as well as anabolic compounds described since the 50s were the first hints that androgens with different tissue specific action are possible showing tissue-specific effects in vivo. Contrary to non-steroidal compounds as well as to some steroidal compounds, MENT is aromatisable (LaMorte *et al.* 1994) and may address beneficial estrogenic effects of in vivo aromatized MENT.

Tissue-selective non-steroidal compounds were identified with selected anabolic effects (e.g. Hamann 1999; Yin *et al.* 2003a). In preclinical studies the compounds were less potent and efficacious than testosterone propionate in androgenic organs, but their anabolic activity was similar to that of testosterone propionate. It is possible to achieve with the non-steroidal compounds tissue-selective actions and generate agents with activity profiles meeting specific therapeutic needs. The compounds are of course not aromatisable to an estrogen, only the internal testosterone and androstendione serve as substrates for the aromatase enzyme. Therefore, further

reduction in the amount of the internal aromatisable androgens are not desired. Unfortunately data about effects on gonadotropin synthesis are rare or have limited prediction for the situation in human.

20.4.3 Mixed agonists/antagonists

The development of therapeutically useful mixed AR agonists/antagonists, like the clinically available SERMs for ER, may offer unique therapeutic advantages over their only agonistic counterparts. The steroidal compound mifepristone (RU38486) has partial agonistic and antagonistic actions (Berrevoets *et al.* 2002). Recently non-steroidal ligands known for better receptor selectivity than steroidal ligands were developed for estrogen, gestagen and androgen receptors.

It was shown previously that hydroxyflutamide, which is a known antiandrogen in most tissues, may function as a SARM showing effects on IL-6 production by osteoblastic cells, and that its potency depends on their number of functional AR expressed (Hofbauer *et al.* 1999). The search and validation of mixed agonists/antagonists is still ongoing. The near future will reveal if these molecules are useful for either tissue specific agonistic activity in human disorders like hypogonadism or for tissue-specific antagonistic activities for e.g. treatment of PCa in men or hirsutism in women.

20.5 Key messages

- Selective androgen receptor modulators (SARMs) are ligands for the AR which have mixed agonistic and antagonistic activities.
- Different mechanism are feasible to achieve tissue- and organ-selective androgen action.
- SARMs may be useful to achieve tissue-selective agonistic/antagonistic properties.
- The transformation of the androgen to its metabolites is specific for tissues.
- The AR interacts with DNA as well as with proteins, resulting in altered receptor conformation.
- Comodulators either enhance (coactivator) or repress (corepressor) transcription of target genes.
- The expression of AR and comodulators is specific for tissues and organs.
- Transcriptional reporter assays as well as in vivo test systems are used to discover SARMs and tissue-selective androgens.
- The definition for an ideal tissue-selective androgen depends on the indication.
- Dissociated androgens belong to the classes of steroidal as well as non-steroidal compounds.

20.6 REFERENCES

Agoulnik IU, Krause WC, Bingman WE, III, Rahman HT, Amrikachi M, Ayala GE, Weigel NL (2003) Repressors of androgen and progesterone receptor action. J Biol Chem 278:31136–31148

Anderson RA, Wallace AM, Sattar N, Kumar N, Sundaram K (2003) Evidence for tissue selectivity of the sysnthestic androgen 7alpha-methyl-19-nortestosterone in hypogonadal men. J Clin Endocrinol Metab 88:2784–2793

Azcoitia I, Sierra A, Veiga S, Honda S, Harada N, Garcia-Segura LM (2001) Brain aromatase is neuroprotective. J Neurobiol 47:318–329

Berrevoets CA, Umar A, Brinkmann AO (2002) Antiandrogens: selective androgen receptor modulators. Mol Cell Endocrinol 189: 97–103

Berry M, Metzger D, Chambon P (1990) Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO JY 9:2811–2818

Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753–758

Carani C, Rochira V, Faustini-Fustini M, Balestrieri A, Granata AR (1999) Role of oestrogen in male sexual behaviour: insights from the natural model of aromatase deficiency. Clin Endocrinol 51:517–524

Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domenico M, Bottero D, Vitale F, Migliaccio A, Auricchio F (2003) Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a nontranscriptional receptor action. J Cell Biol 161:547–556

Cato AC, Nestl A, Mink S (2002) Rapid actions of steroid receptors in cellular signaling pathways. Sci STKE 138:RE9

Chengalvala M, Oh T, Roy AK (2003) Selective androgen receptor modulators. Expert Opin Ther Patents 13:59–66

Cosman F and Lindsay R (1999) Selective estrogen receptor modulators: clinical spectrum. Endocr Rev 20:418–434

Cummings DE, Kumar N, Bardin CW, Sundaram K, Bremner WJ (1998) Prostate-sparing effects in primates of the potent androgen 7alpha-methyl-19-nortestosterone: a potential alternative testosterone for androgen replacement and male contraception. J Clin Endocrinol Metab 83:4212–4219

Fields S and Song O (1989) A novel genetic system to detect protein-protein interactions. Nature 340:245–246

Ghali SA, Gottlieb B, Lumbroso R, Beitel LK, Elhaji Y, Wu J, Pinsky L, Trifiro MA (2003) The use of androgen receptor amino/carboxyl-terminal interaction assays to investigate androgen receptor gene mutations in subjects with varying degrees of androgen insensitivity. J Clin Endocrinol Metab 88:2185–2193

Gooren LJ (1985) Human male sexual functions do not require aromatization of testosterone: a study using tamoxifen, testolactone, and dihydrotestosterone. Arch Sex Behav 14:539–548

Hamann LG, Mani NS, Davis RL, Wang XN, Marschke KB, Jones TK (1999) Discovery of a potent, orally active, nonsteroidal androgen receptor agonist: 4-ethyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-γ]- quinoline (LG121071). J Med Chem 42:210–212

Han G, Foster BA, Mistry S, Buchanan G, Harris JM, Tilley WD, Greenberg NM (2001) Hormone status selects for spontaneous somatic androgen receptor variants that demonstrate specific ligand and cofactor dependent activities in autochthonous prostate cancer. J Biol Chem 276:11204–11213

Harada N, Utsumi T, Takagi Y (1993) Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. Proc. Natl Acad Sci USA 90:11312–11316

He B, Kemppainen JA, Wilson EM (2000) FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 275:22986–22994

Heinlein CA and Chang C (2002) Androgen receptor (AR) coregulators: an overview. Endoc Rev 23:175–200

Hermanson O, Glass CK, Rosenfeld MG (2002) Nuclear receptor coregulators: multiple modes of modification. Trends Endocrinol Metab 13:55–60

Hershberger L and Shipley EMR (1953) Myotropic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. Proc Soc Exp Biol Med 83:175–180

Hofbauer LC, Ten RM, Khosla S (1999) The anti-androgen hydroxyflutamide and androgens inhibit interleukin-6 production by an androgen-responsive human osteoblastic cell line. J Bone Miner Res 14:1330–1337

Hsiao PW and Chang C (1999) Isolation and characterization of ARA160 as the first androgen receptor N-terminal-associated coactivator in human prostate cells. J Biol Chem 274:22373–22379

Kang HY, Yeh S, Fujimoto N, Chang C (1999) Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 274:8570–8576

Katzenellenbogen BS and Katzenellenbogen JA (2002) Biomedicine. Defining the "S" in SERMs. Science 295:2380–2381

Kemppainen JA, Langley E, Wong CI, Bobseine K, Kelce WR, Wilson EM (1999) Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone. Mol Endocrinol 13:440–454

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC (2001) Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104:719–730

Kousteni S, Han L, Chen JR, Almeida M, Plotkin LI, Bellido T, Manolagas SC (2003) Kinase-mediated regulation of common transcription factors accounts for the bone-protective effects of sex steroids. J Clin Invest 111:1651–1664

LaMorte A, Kumar N, Bardin CW, Sundaram K (1994) Aromatization of 7 alpha-methyl-19-nortestosterone by human placental microsomes in vitro. J Steroid Biochem Mol Biol 48: 297–304

Langley E, Zhou ZX, Wilson EM (1995) Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 270:29983–29990

Liu Z, Auboeuf D, Wong J, Chen JD, Tsai SY, Tsai MJ, O'Malley BW (2002) Coactivator/corepressor ratios modulate PR-mediated transcription by the selective receptor modulator RU486. Proc Natl Acad Sci USA 99:7940–7944

Loven MA, Likhite VS, Choi I, Nardulli AM (2001) Estrogen response elements alter coactivator recruitment through allosteric modulation of estrogen receptor beta conformation. J Biol Chem 276:45282–45288

Lutz LB, Jamnongjit M, Yang WH, Jahani D, Gill A, Hammes SR (2003) Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. Mol Endocrinol 17:1106–1116

McEwen BS (1980) Binding and metabolism of sex steroids by the hypothalamic-pituitary unit: physiological implications. Annu Rev Physiol 42:97–110

McKenna NJ, Lanz RB, O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344

McKenna NJ and O'Malley BW (2000) From ligand to response: generating diversity in nuclear receptor coregulator function. J Steroid Biochem Mol Biol 74:351–356

Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zannini MS, Abbondanza C, Auricchio F (2000) Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation. *EMBO J.* 19:5406–5417

Miyamoto H, Yeh S, Wilding G, Chang C (1998) Promotion of agonist activity of antiandrogens by the androgen receptor coactivator, ARA70, in human prostate cancer DU145 cells. Proc Natl Acad Sci USA 95:7379–7384

Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R (2000) FHL2, a novel tissue-specific coactivator of the androgen receptor. *EMBO J.* 19:359–369

Negro-Vilar A (1999) Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. J Clin Endocrinol Metab 84:3459–3462

Nimrod A and Ryan KJ (1975) Aromatization of androgens by human abdominal and breast fat tissue. J Clin Endocrinol Metab 40:367–372

Riggs BL, Khosla S, Melton LJ, III (2002) Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302

Robyr D, Wolffe AP, Wahli W (2000) Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 14:329–347

Roselli CE and Resko JA (2001) Cytochrome P450 aromatase (CYP19) in the non-human primate brain: distribution, regulation, and functional significance. J Steroid Biochem Mol Biol 79: 247–253

Shang Y and Brown M (2002) Molecular determinants for the tissue specificity of SERMs. Science 295:2465–2468

Shull JD, Beams FE, Baldwin TM, Gilchrist CA, Hrbek MJ (1992) The estrogenic and antiestrogenic properties of tamoxifen in GH4C1 pituitary tumor cells are gene specific. Mol Endocrinol, 6:529–535

Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD (1994) Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev 15:342–355

Sundaram K, Kumar N, Bardin CW (1993) 7 alpha-methyl-nortestosterone (MENT): the optimal androgen for male contraception. Ann Med 25:199–205

Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW, McDonnell DP (1994) Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8:21–30

Verrijdt G, Haelens A, Claessens F (2003) Selective DNA recognition by the androgen receptor as a mechanism for hormone-specific regulation of gene expression. Mol Genet Metab 78:175–185

Yeh S and Chang C (1996) Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93:5517–5521

Yeh S, Miyamoto H, Chang C (1997) Hydroxyflutamide may not always be a pure antiandrogen. Lancet 349:852–853

Yeh S, Miyamoto H, Shima H, Chang C (1998) From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proc Natl Acad Sci USA 95:5527–5532

Yi P, Driscoll MD, Huang J, Bhagat S, Hilf R, Bambara RA, Muyan M (2002) The effects of estrogen-responsive element- and ligand-induced structural changes on the recruitment of cofactors and transcriptional responses by ER alpha and ER beta. Mol Endocrinol 16:674–693

Yin D, Gao W, Kearbey JD, Chung K, He Y, Marhefka CA, Veverka KA, Miller DD, Daltom JT (2003a) Pharmacodynamics of selective androgen receptor modulators. J Pharmacol Exp Ther 304:1334–1340

Yin D, Xu H, He Y, Kirkovsky LI, Miller DD, Dalton JT (2003b) Pharmacology, pharmacokinetics, and metabolism of acetothiolutamide, a novel nonsteroidal agonist for the androgen receptor. J Pharmacol Exp Ther 304:1323–1333

Zaccheo T, Giudici D, Panzeri A, di Salle E (2000) Combined treatment of Dunning R3327 rat prostatic tumor with the 5alpha-reductase inhibitor PNU 157706 and the antiandrogen bicalutamide. Cancer Chemother Pharmacol 45:31–37

Zhao Q, Chasse SA, Devarakonda S, Sierk ML, Ahvazi B, Rastinejad F (2000) Structural basis of RXR-DNA interactions. J Mol Biol 296:509–520

Zumpe D, Bonsall RW, Michael RP (1993) Effects of the nonsteroidal aromatase inhibitor, fadrozole, on the sexual behavior of male cynomolgus monkeys (Macaca fascicularis). Horm Behav 27:200–215

21

Methodology for measuring testosterone, DHT and SHBG in a clinical setting

M. Simoni

Contents

21.1 Introduction

During the era of automated hormone measurements a chapter about testosterone assay may seem obsolete. Yet, various methods of measuring testosterone continue to be challenged in the endocrinological literature by studies comparing the (poor) performance of commercial kits (Boots *et al.* 1998; Taieb *et al.* 2003; Wang *et al.* 2004). This contribution aims to help the reader choose the testosterone detection

Table 21.1 Transport of endogenous testosterone and DHT in male and female serum

	Serum concentration (nM)	Unbound (%)	SHBG (%)	CBG (%)	Albumin (%)
Testosterone					
Adult men	23.0	2.23	44.3	3.56	49.9
Adult women					
Follicular phase	1.3	1.36	66.0	2.26	30.4
Luteal phase	1.3	1.37	65.7	2.20	30.7
Pregnancy	4.7	0.23	95.4	0.82	3.60
DHT					
Adult men	1.70	0.88	49.7	0.22	39.2
Adult women					
Follicular phase	0.65	0.47	78.4	0.12	21.0
Luteal phase	0.65	0.48	78.1	0.12	21.3
Pregnancy	0.93	0.07	97.8	0.04	2.15

From Dunn *et al.*, 1981

system most suitable for his/her needs and to be aware of its analytical performance and limitations. In addition, because of their relevance for testosterone physiology and substitution, methods for assessing of sex hormone binding globulin (SHBG), free testosterone and dihydrotestosterone (DHT) will be considered as well.

21.2 Testosterone in blood

Testosterone circulates in serum largely bound to transport proteins. Like other steroids and thyroid hormones, both albumin and specific binding globulins are involved in testosterone binding. Testosterone binds to albumin with low affinity but, due to its high concentration, albumin displays a very high binding capacity. The specific transport protein for testosterone, some other androgens and estradiol is SHBG. A systematic analysis of serum transport of steroid hormones and their interaction with binding proteins revealed an association constant of SHBG of $1.6 \times 10^9\ M^{-1}$ for testosterone and of $5.5 \times 10^9\ M^{-1}$ for DHT at 37°C (Dunn *et al.* 1981). By comparison the association constant of albumin for testosterone is five orders of magnitude lower ($6 \times 10^4\ M^{-1}$) (Anderson 1974). The relative amounts of protein binding of circulating testosterone in men and women is shown in Table 21.1.

About 1.5–2% of serum testosterone is free and is believed to represent bioactive testosterone. According to the free hormone hypothesis, it is only the free hormone fraction that is accessible to all body compartments and can enter the cells, exerting its action where androgen receptors are available. The free diffusion of unbound

testosterone in all cells and organs is demonstrated by the same free testosterone concentration in all body fluids, e.g. in blood and in saliva. Free and protein-bound testosterone and DHT are in equilibrium, so that when free hormone is subtracted from circulation because of entry into tissue, new testosterone dissociates from albumin and SHBG, a new equilibrium is promptly reached and the free hormone concentration in serum remains constant. Conversely, pathophysiological conditions causing changes in binding protein concentration (e.g. pregnancy, hypo- or hyperthyroidism, growth hormone excess, treatment with antiepileptic drugs) or displacement of testosterone from SHBG by drugs (e.g. danazol) results in changes in total testosterone concentration in order to maintain constant free testosterone levels (Pugeat *et al.* 1981).

As indicated below in this chapter, measurement of SHBG is valuable for assessment of androgenization and of free testosterone. In earlier times SHBG was measured indirectly, by estimating its binding capacity. The classic method used tritiated DHT as ligand because of its higher affinity to SHBG and lack of binding to cortisol binding globulin (CBG). Saturating amounts of labeled DHT were added to the samples and SHBG was then precipitated by ammonium sulfate. The amount of labeled DHT precipitated provided a direct measurement of SHBG binding capacity. This method did not allow absolute changes in SHBG protein concentrations to be measured, which can now be assessed by modern immunoradiometric assays. Modern assays have demonstrated that, in general, SHBG binding capacity (expressed in terms of DHT binding) corresponds acceptably to the molar SHBG concentration.

The free hormone hypothesis has been repeatedly challenged in the scientific literature, mainly due to the difficulty of reconciling the existing experimental evidence with appropriate mathematical models of hormone transport (Ekins 1990; Mendel 1992). For instance, the low affinity of testosterone for albumin binding and some experimental data led to the idea that albumin-bound testosterone is readily available for delivery to the tissues (i.e. bioavailable) while only SHBG-bound testosterone is not biologically active (Manni *et al.* 1985). This view has now been corroborated by serum androgen bioassays (see below). In contrast, SHBG itself has been proposed to interact with cell surface receptors, thereby contributing to the biological activity of androgens (Rosner *et al.* 1999). This novel, putative function of SHBG is of particular interest in the light of the essential lack of any physiological explanation why primates, unlike all other species, possess such a protein. SHBG seems to "buffer" serum testosterone levels, which, beside the physiological circadian rhythm, show only minor circhoral variations despite highly pulsatile LH secretion (Simoni *et al.* 1988 and 1992). On the contrary, serum testosterone levels oscillate widely in rodents, which do not have SHBG. In addition SHBG reduces the rate of hepatic testosterone degradation. There are no known cases of congenital

absence of SHBG in humans but an analbuminemic strain of rats, a species which does not have circulating SHBG, is normally fertile and shows normal free testosterone levels, arguing for a dispensable role of serum testosterone binding proteins (Mendel *et al.* 1989). Similarly, the congenital absence of thyroxin binding globulin (TBG) in humans is compatible with normal thyroid function (Dussault *et al.* 1977).

Since SHBG concentrations influence total and free testosterone levels, it is important to know which factors influence SHBG production. Of the hormones, estrogens stimulate and androgens inhibit SHBG secretion. Administration of 20 μg daily of ethinyl estradiol to men for 5 weeks resulted in a 150% increase in SHBG and, as a consequence of the reduced free testosterone levels, in a 50% increase in total serum testosterone (Anderson 1974). The estrogen effect is evident in women, who have SHBG serum levels double of those in men, and during pregnancy, when SHBG rises to levels 5–10 times higher than in non-pregnant women. In addition, SHBG levels are stimulated by thyroid hormones, resulting in high levels in thyrotoxycosis and low levels in hypothyroidism, and are reduced by growth hormone and cortisol, resulting in low levels in acromegaly and in Cushing syndrome. Finally, SHBG levels are higher in children than in adults and increase in men after the age of 50, contributing to the possible decline of free testosterone levels observed in aging men.

The most important bioactive metabolite of testosterone is DHT. The reduction of testosterone to DHT occurs in those tissues expressing 5α-reductase (see Chap. 1 and Chap. 18) and DHT is well measurable in circulation. In eugonadal, adult men serum DHT concentrations are about 10–12 times lower than testosterone and DHT is mainly bound to SHBG. Given the role of DHT in prostate growth, the measurement of serum DHT is of relevance during testosterone treatment, especially when testosterone is administered via the trans-cutaneous route, (e.g. testosterone gel or patches) since the skin is the primary organ for 5α-reduction.

21.3 Principles of immunological testosterone assays

The principles of hormone measurement in general also apply to testosterone and good chapters on hormone assays are available in various textbooks of endocrinology (e.g. Segre and Brown 1998). As for all other hormones, the accurate measurement of testosterone in blood was made possible by the advent of radioimmunoassays (RIA). In immunological assays the hormone being measured (i.e. the antigen) competes with the labeled hormone (i.e. the tracer) for binding to an antiserum (the antibody). Since the amount of antibody available for reaction is limited, the higher the concentration of the hormone in blood, the lower the amount of tracer bound

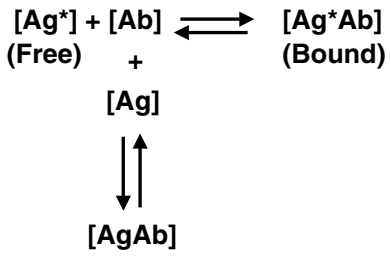


Fig. 21.1 Principle of immunological testosterone assay. Testosterone in the serum sample (Ag) and labeled testosterone (Ag*) competes for binding to a limited number of binding sites (Ab). The reaction is governed by the law of mass action. The asterisk indicates any type of label (e.g. an isotope, a non-radioactive label, an enzyme, etc.). At the end of the reaction the Free is removed and the Bound is counted.

by the antibody. At the end of the reaction, the antibody bound to the hormone (B) is separated from the free fraction (F) and the radioactivity or the signal emitted by a non-radioactive tracer is measured (Fig. 21.1).

In case of testosterone RIA, the tracer can be tritiated or iodinated. ^{3}H-testosterone can be tritiated in two or four positions (Fig. 21.2). Iodination, which can be achieved by oxidation (e.g. by reaction with chloramine-T) of a thyrosine or another amino acid residue in peptidic hormones, requires conjugation with a histamine residue in the case of a steroid molecule (Fig. 21.2). Both tritiated and iodinated testosterone tracers are commercially available. The half-life of the tracers depends on the isotope. Tritiated tracers can be stored and used for years but, since the slow but progressive decay results in impurities which reduce the assays' performance, they should be purified by chromatography at 6–12 months intervals. Iodinated tracers, e.g. testosterone-3-(O-carboxymethyl)oximino-(2-[^{125}I] iodohistamine, show a much shorter half-life and can be used only for about one month, but they have a much higher specific activity than the tritiated tracers allowing the use of lower antiserum concentrations and improved assay sensitivity. Iodinated testosterone is usually purified by high performance liquid chromatography (HPLC) by the manufacturer and does not require further cleaning before use. If the tracer is produced in-house, it should be purified by HPLC or other chromatographic technique (e.g. gel filtration on Sephadex) before use. In recent years non-radioactive testosterone tracers have been produced and are widely used in clinical routine measurements (see below). They offer the advantage of a lower environmental impact, but the assays employing such tracer function according to the same principles of RIAs.

Beside the specific activity of the tracer, the assays' sensitivity depends on the affinity of the antiserum, which should, if possible, be identical for both the antigen and the tracer. Since testosterone is not antigenic when injected in animals, a testosterone

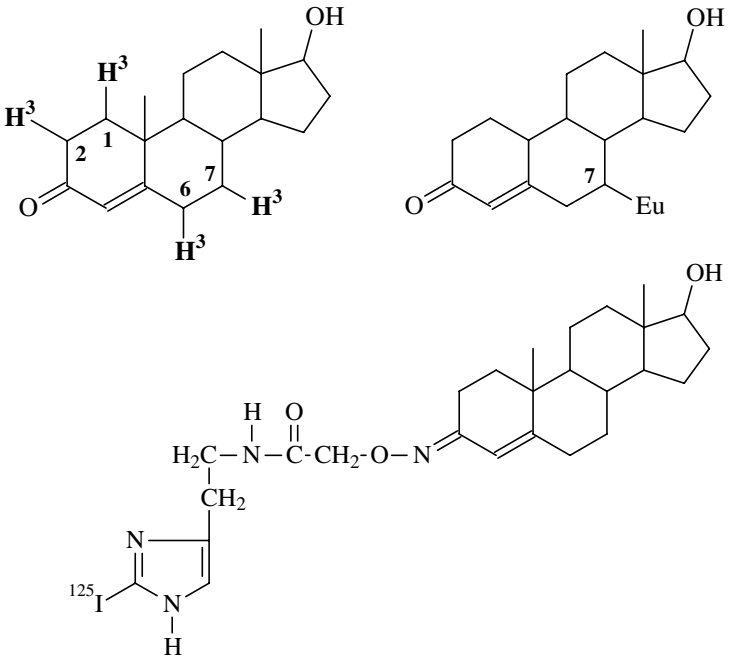


Fig. 21.2 Tracers used in testosterone immunoassays. RIAs are based on tritiated or iodinated (testosterone-3-(*O*-carboxymethyl) oximino-(2-[^{125}I]iodohistamine) tracers. The Europium-labeled testosterone is an example of non-radioactive tracer used in fluoroimmuno assays (FIA). Other non-radioactive immunoassays are based on testosterone molecules coupled with enzymes or luminescent substances.

conjugate conferring aptene properties to the steroid must be used to obtain antisera. As for iodination, position 3 in the A ring of testosterone is usually exploited for conjugation with the CMO (carboxymethyloximino) group, a spacer necessary for coupling the antigen to BSA which renders the conjugate antigenic. The antibodies for testosterone immunoassays are usually polyclonal antisera obtained in rabbits, but monoclonal antibodies are used in some kits. Polyclonal antisera have the advantage of high affinity, but good monoclonal antibodies might have better specificity, obviating, at least in part, the problem of cross-reactivity of most polyclonal antisera with DHT.

After the antigen-antibody reaction has reached equilibrium, separation of the antibody-bound (B) from the free tracer (F) can be accomplished specifically by adding an antiserum directed against the immunoglobulins of the species from which the first antibody was obtained (e.g. goat antirabbit antiserum), together with preimmune serum (e.g. normal rabbit serum) to achieve complete precipitation of the immune complexes. The reaction tubes are then centrifuged and the radioactivity or the signal emitted by the non-radioactively-labeled tracer is counted.

Alternatively, non-specific precipitating agents (e.g. ammonium sulphate, polyethylene glycol, [PEG]) or subtances which absorb the free antigen (e.g. dextran-coated charcoal) can be used. In practice, in a testosterone RIA based on rabbit antiserum, B/F separation is performed very efficiently by addition of rabbit immunoglobulins, antirabbit antiserum and PEG.

After counting the results can be calculated in several ways. The signal emitted by the unknown samples is compared to that of the samples with known testosterone concentrations, i.e. the calibrators of the standard curve, after logarithmic, semilogarithmic or logit/log transformation, using computer programs usually enclosed in the software of the counter. These mathematical transformations of the readouts permit linearization of the calibration curve over a wide range, allowing accurate calculation of the actual testosterone concentration in the unknowns.

Since testosterone in serum is mostly bound to carrier proteins, which prevents the antibody-antigen reaction by competing with the antiserum, the steroid must be extracted with organic solvents prior to RIA or other immunoassays. Extraction is usually performed by adding 10–20 volumes of diethyl ether to the serum samples. This step is followed by vortexing (5 min) or agitation of the samples on a rotator (30 min) and freezing of the aqueous phase. Testosterone, which is lipophilic, remains in the organic phase which can be decanted, evaporated and reconstituted in assay buffer. This extraction procedure is usually highly efficient ($\approx$90%) and can be monitored by measuring the recovery of trace amounts of radiolabeled testosterone added beforehand. Both testosterone and DHT are extracted by this method. If an accurate quantification of testosterone and DHT is desired, the extracted steroids can be reconstituted in the appropriate diluent and separated by a chromatographic procedure (e.g. HPLC or celite chromatography) before RIA.

The calibrators used in the standard curve are serial dilutions of a sample with known testosterone concentrations dissolved in the same matrix (buffer or serum-based) of the samples measured. In extraction methods, testosterone is weighed, dissolved in ethanol and further diluted in assay buffer. In non-extraction methods the standard is added to steroid-free sera. The maintenance of the same matrix is necessary to ensure parallelism between standards and unknowns.

These assay principles are common to RIA and non-radioactive methods. Unlike the most recent assays for peptidic hormones, the newest technologies which have highly improved sensitivity thanks to the two-site, sandwich approach, cannot be applied to the steroid hormone assays. In the immunoradiometric assays (IRMA) the large protein hormone is first reacted with a capture antibody (in molar excess) coated to the tube walls, the tubes are washed, and a labeled second antibody directed against a second epitope of the hormone is added. Steroid hormones are too small to be reacted simultaneously with two antibodies and the IRMA principle cannot be applied, so that the sensitivity of a testosterone assay can be improved only by

increasing the specific activity of the tracer and/or the affinity and specificity of the antibody.

21.4 Measurement of testosterone

21.4.1 Isotope dilution-mass spectrometry

Unlike protein hormones, which are heterogeneous, steroids can be quantified very accurately in biological samples by means of isotope dilution-mass spectrometry. This procedure allows the absolute identification and quantification of testosterone in blood and other specimens. The procedure is based on the highly specific recognition of the steroid by mass spectrometry coupled to an exact estimation of recovery by addition of labeled testosterone (isotope dilution). According to a well validated method, ^{14}C-labelled testosterone is added to the serum sample and the steroid fraction is extracted with an organic solvent and purified by gel chromatography on Sephadex LH-20. The testosterone-containing fraction is then chemically reacted with heptafluorobutyric anhydride to produce the 3-enol, 17β-diester of testosterone, a step which improves the sensitivity and the specificity of mass fragmentography, since it increases the molecular mass of the steroid and minimizes the probability of spuriously detecting small molecular impurities contained in the sample. The derivative is then injected into the mass spectrometer and multiple ion detection is performed by recording *m/e* 680 and 682. The quantity of testosterone in the sample is then calculated from the ratio of the peak heights or peak areas and from the known amount of ^{14}C-testosterone added (Siekmann 1979). The method is highly precise (1% variation in duplicate determinations) and sensitive (limit of detection: 5 pg) and is the reference method for testosterone determination currently used for measurement of testosterone concentrations in serum pools to be distributed in quality control programs (Thienpont *et al.* 1996).

21.4.2 Radioimmunoassay

The first radioimmunoassay for plasma testosterone was developed at the end of the sixties (Furuyama *et al.* 1970). It was based on an antiserum raised against testosterone coupled to bovine serum albumin at position 3 (T-3-BSA), ^{3}H-labelled testosterone as the tracer and bound/free separation by ammonium sulfate precipitation. Plasma testosterone was extracted and chromatographed on alumina columns prior to immunoassay. Radio- and other immunoassays for plasma and serum testosterone were developed by several investigators e.g. Nieschlag and Loriaux (1972). These authors produced their own antiserum by a novel immunization technique (Vaitukaitis *et al.* 1971) and distributed the antiserum freely to other laboratories so that their method became widely used and their papers were ranked as Citation Classics in 1981. Slowly, kit manufacturers took over

the development of assays, but while the practicability of the assays consistently improved, the overall performance of current immunoassays is not much different from that of 30 years ago. For instance, the early assays already showed a lower detection limit of 3–10 pg, as the current assays do. Since RIAs are most sensitive at low antibody concentrations, when competition between tracer and unknown is high, high affinity antibodies are crucial for sensitive assays. However, usually high affinity is better obtained with polyclonal antisera which display elevated cross-reactivity with DHT. A substantial improvement of the sensitivity of testosterone RIAs (and other immunoassays as well) could be achieved by using highly specific monoclonal antibodies with high affinity, a goal very difficult to reach.

In general the current in-house methods for testosterone RIA are the same as the early assays of the seventies and are still in use mainly for research purposes because they are cheap and accurate. An extraction step is necessary to eliminate serum proteins which do not allow the correct interaction of albumin and SHBG-bound testosterone with the antiserum. Due to the extraction, traditional RIAs are somewhat cumbersome and have been almost completely replaced by non-extraction methods for clinical use.

Most of the current commercially available RIAs for testosterone do not usually require an extraction step and are either based on double antibody separation or are in solid phase, i.e. the antibody is fixed at the wall of the reaction tubes (coated tubes) so that no centrifugation is required, reducing the hands-on time and improving practicability. Serum testosterone is displaced from carrier proteins by chemical agents competing for protein binding, e.g. danazol (Pugeat *et al.* 1981), although the exact nature of the kit components is usually known only to the manufacturers and is protected by property rights. Well-validated non-extraction methods may work well for male serum samples, although inaccurate testosterone concentrations are occasionally measured in individual samples containing abnormal SHBG concentrations or substances (e.g. drugs) interfering with the kit components. However, results obtained with different RIA kits have been repeatedly reported to be poorly comparable (Jockenhövel *et al.* 1992; Boots *et al.* 1998; Taieb *et al.* 2003; Wang *et al.* 2004).

21.4.3 Other immunoassays

The most popular alternatives to radioactive methods are immunoassays based on non-radioactively labeled tracers such as fluroimmunoassay (FIA), chemiluminescent assay (LIA) and enzyme-linked immunosorbent assay (EIA/ELISA). They can be in liquid or in solid phase, whereby solid phase, microtitre plate-based assays are preferred due to easy handling and proneness to automation. Also in these assays it is the antigen that is labeled and competes with the endogenous testosterone for binding to the antiserum. The primary antibody can be poly- or monoclonal and is

usually bound to the tube wall or to the plate well. In EIAs/ELISAs the tracer is represented by testosterone coupled to an enzyme (alkaline phosphatase, β galactosidase, penicillinase, acetylcholinesterase or horse radish peroxidase) which starts a colorimetric reaction upon addition of the substrate at the end of the incubation time. This results in color development which is inversely proportional to the amount of unlabelled testosterone and can be read by a spectrophotometer. The sensitivity of EIA/ELISA is affected by the number of steroid molecules coupled to the enzyme and the proper molar ratio between steroid and enzyme must be carefully validated (Rassaie *et al.* 1992). In FIA the tracer is testosterone coupled to a molecule (e.g. Europium) which fluoresces upon stimulation. The fluorescence is measured by a fluorimeter and, again, is inversely related to the amount of cold testosterone contained in the sample. Critical in EIAs/ELISAs and FIAs are the washing steps, which should be carefully carried out in order to eliminate non-specifically bound substances which would result in poor precision and falsely elevated readouts.

Automatic multianalyzers, mainly based on non-radioactive methods are now available and widely used for the direct and quick measurement of serum testosterone. Some of these systems have been evaluated against the reference method based on mass spectrometry, showing acceptable results at least in male samples (Fitzgerald and Herold 1996; Levesque *et al.* 1998; Gonzales-Sagrado *et al.* 2000). However, inconsistency of results obtained with different methods are reported as well, and some systems seem to suffer from systematic problems, resulting in over- or underestimation of serum testosterone and/or insufficient sensitivity, especially in female samples (Taieb *et al.* 2002, 2003; Wang *et al.* 2004). This is very evident when comparing the results of external quality control trials (Middle 2002). As for every other method, each laboratory should carefully validate the results obtained by the multianalyzer before it is implemented for routine testosterone measurement. In practice, however, validation is limited by the fact that most of the systems are based on a master calibration curve carried out by the manufacturer and not available to customers. Each assay then requires only one or two calibrators to adjust the master curve and parallelism tests cannot be performed. As in the non-automatic assays, differences observed between the kits can be ascribed to differences in the matrix of the calibrators and in the affinity, titer and specificity of the antibodies used.

21.4.4 Assessment of free testosterone

The direct measurement of free testosterone in serum is based on the same principles governing the assay of free thyroid hormones and has been extensively considered and reviewed by R. Ekins in the past (Ekins 1990). As indicated above, serum testosterone exists in an equilibrium between free and protein-bound fractions, an equilibrium which is invariably disturbed by all methods of free hormone measurement, a factor that should be kept in mind when choosing a method and

analyzing the data. The methods of reference for free hormone analysis are equilibrium dialysis and ultrafiltration, which should be used for research purposes and to validate other systems.

21.4.4.1 Equilibrium dialysis

In equilibrium dialysis the serum (dialysand) is put in contact with a buffer (dialysate) though a membrane which allows the passage of low molecular weight compounds (e.g. free hormones) but retains the binding proteins. As a consequence of the passage of free hormone molecules to the dialysate, new hormone molecules will dissociate from the binding proteins until a new equilibrium is reached and the free hormone concentration is the same on the two sides of the membrane. The free hormone can now be measured in the dialysate either directly (e.g. by RIA) or indirectly by knowing the total hormone concentration and assessing the percentage of added labeled hormone passed in the free fraction. Provided that the ion composition of the buffer does not interfere with the equilibrium constant (K), dialysis is thermodynamically equivalent to serum dilution and leads to a reduction of the free hormone concentration and to dissociation of new hormone from the binding proteins until a new equilibrium is established in the system. At equilibrium the original free hormone concentration is therefore only "approximately" maintained because, since the total hormone concentration is constant, the final, measured free hormone concentration will be somewhat diluted and lower than that in the original sample. This effect can be regarded as negligible if the relative free hormone concentration is low, as in the case of free thyroxin (0.02%), but might become relevant in the case of free testosterone (2%) so that the buffer volume against which the sample is dialyzed should be kept to a minimum. In this respect, it is the total volume of dialysand + dialysate which determines the dilution factor while the position of the dialysis membrane between the two compartments, i.e. the individual volume of the two compartments, is irrelevant for the free hormone concentration which, at equilibrium, will be the same on the two sides (Ekins 1990).

21.4.4.2 Ultrafiltration

The problem of sample dilution is avoided in the case of ultrafiltration of undiluted serum, the second reference method in free hormone determination. In this procedure a serum sample is centrifuged through a membrane with an appropriate molecular weight cutoff. Only free hormone and low molecular weight compounds will be collected in the ultrafiltrate at a concentration equal to that in the original sample. The free hormone can be directly assessed by RIA of the ultrafiltrate or by indirect measurement of the relative fraction of labeled hormone added to the original samples which is recovered in the ultrafiltrate (Vlahos *et al.* 1982). Direct

measurement by RIA is preferable both in ultrafiltration and in equilibrium dialysis, since the impurities of the tracers can result in inaccurate estimation of the free fraction. Ultrafiltration devices are commercially available (e.g. Centrifree® micropartition system, Millipore).

Possibly ultrafiltration may be limited if non-filterable binding competitors are present in the sample, or if binding proteins interact with the membrane. This will result in progressive increase of the free hormone concentration in the filtrate. However, since the ultrafiltration time is rather short (one hour or less) compared to dialysis (several hours), the possible changes in the equilibrium ensuing from these and other factors may be assumed to be negligible.

21.4.4.3 Direct free testosterone RIA

These methods are based on the concept that if an antibody is added to a serum sample, only free hormone will bind to it and the antibody occupancy will depend on the free hormone concentration. As a result, however, protein-bound hormone will dissociate and a new equilibrium will be established. Therefore, the antibody concentration should be kept "small" enough to minimize the depletion of the protein-bound pool, i.e. not more than 1% of total hormone should be displaced from the binding proteins to the antibody. Quantification of the antibody-bound hormone, i.e. the free hormone concentration, can be achieved indirectly by knowing the total hormone concentration, adding labeled hormone and measuring the fraction of it which is taken up by the antibody ("labeled hormone antibody uptake"). Alternatively, a two-step approach involves adding the sample to a solid phase antibody, washing off the unbound serum components and adding labeled hormone which will be bound by the residual, unoccupied antibody binding sites. Since the amount of antibody is limited, the higher the free hormone concentration, the lower the number of unoccupied antibody sites at the end of the first incubation, the lower the antibody occupancy by labeled hormone at the end of the second incubation. In order for this method to work, a two-step approach, with removal of serum after the first reaction of the antiserum with the free hormone, is necessary because if the labeled hormone interacts with the serum binding proteins, this would impair the estimation of antibody occupancy by the tracer. The two-step approach, however, is not necessary if one uses a labeled compound which is totally non-reactive with serum proteins, but can be recognized by the solid phase antibody present in a limited amount and which competes for binding with the free hormone in the sample. This is the principle of the free "analog" testosterone assay on which some popular commercially available kits are based.

The direct measurement of free testosterone in serum based on the labeled hormone "analog" is valid, provided that the analog does not interact with the serum proteins, a condition which is currently not met by commercial kits. In fact, neither

the identity of the analog tracer, nor the validation of the kit (showing the absence of interactions with the serum protein) is usually disclosed by the manufacturer. On the contrary, the "analog" principle is often not even mentioned or is misrepresented in the instruction accompanying the kits, which are often validated only against other kits and not against dialysis or ultrafiltration. It should be kept in mind that, in practice, finding a hormone analog totally unreactive with serum proteins is very difficult and several studies have shown that such an interaction indeed occurs, resulting in inaccurate measurements of free testosterone. In this respect it is interesting that serum free testosterone measured by an "analog" method accounts for 0.5–0.65% of total testosterone, while equilibrium dialysis and ultrafiltration give values of 1.5–4%, revealing inconsistencies between the different approaches (Rosner 1997; Winters *et al.* 1998). In a direct comparison, free testosterone values measured by a bestseller "analog" kit were only 20–30% of those measured by equilibrium dialysis (Vermeulen *et al.* 1999). For these reasons it is recommended that, if an "analog" method is to be considered for routine free testosterone determination, in-house validation of the kit should involve comparison with dialysis or ultrafiltration and estimation of the binding of the analog tracer to endogenous proteins e.g. by adding exogenous SHBG (e.g. serum from pregnant women) and by estimating tracer binding to concanavalin A-bound SHBG after chromatography of the serum samples (Winters *et al.* 1998). Unfortunately, the kits for direct free testosterone measurement presently available do not measure what they claim to do and give inaccurate results (Rosner 2001). Their use should be discouraged.

21.4.4.4 Salivary testosterone

Salivary testosterone is considered to be a good index of serum free testosterone and a highly significant correlation with serum total and free testosterone has long been known (Wang *et al.* 1981). As other steroid hormones, free testosterone enters saliva though passive diffusion across the acinar cells of the salivary glands. Testosterone can be measured directly in saliva by RIA or FIA with or without extraction (Wang *et al.* 1981; Schürmeyer *et al.* 1984; Tschöp *et al.* 1998; Granger *et al.* 1999). Monitoring salivary testosterone levels may be particularly useful in studies involving children or subjects poorly compliant with blood withdrawal and is currently widely used in behavioral studies (Granger *et al.* 1999). It can also be used to monitor the pharmacokinetics of testosterone preparations used for substitution therapy without requiring the patient to come to the laboratory frequently (Tschöp *et al.* 1998). Saliva testing of testosterone concentration is also very popular in the internet, where several US companies advertise and sell at-home hormone test kits.

21.4.4.5 Bioavailable testosterone

Several lines of evidence suggest that not only free testosterone but also albumin-bound testosterone is available to the target tissues for biological activity (Manni *et al.* 1985). Therefore, the non SHBG-bound testosterone is called "the bioavailable testosterone". This parameter can be measured based on the property of ammonium sulfate to precipitate SHBG together with the steroids bound to it. Tracer amounts of labeled testosterone are added to the samples and, after allowing for equilibration with endogenous testosterone, an equal volume of a saturated solution of ammonium sulfate is added (final concentration 50%) and SHBG is separated by centrifugation. The percentage of labeled testosterone remaining in the supernatant represents an estimation of bioavailable testosterone, which can be calculated knowing the total testosterone concentration of the sample (Manni *et al.* 1985). Alternatively, testosterone can be measured directly in the supernatant by RIA (Dechaud *et al.* 1989). It has been reported that the ammonium sulfate concentration is critical to proper precipitation of SHBG only, a parameter which should be accurately tested in each laboratory in order to avoid precipitation of albumin as well and underestimation of bioavailable testosterone (Davies *et al.* 2002).

When properly done, bioavailable testosterone correlates quite well with total testosterone and calculated free testosterone. The estimation of bioavailable testosterone might be of value in clinical conditions of mild hypogonadism accompanied by increased SHBG and possibly reduced concentrations of serum albumin, such as in late-onset hypogonadism of the ageing male (Wheeler 1995; Morley *et al.* 2002).

21.4.4.6 Calculated free testosterone

The classical methods for free and bioavailable testosterone measurement reported above, i.e. equilibrium dialysis, ultrafiltration and ammonium sulfate precipitation are too cumbersome for clinical routine. This is the main reason why "analog" methods became so popular. A recent study compared the direct immunoassay of free testosterone by a labeled analog with calculation of free testosterone from total testosterone and immunoassayed SHBG concentrations, bioavailable testosterone assessed by ammonium sulphate precipitation and the free androgen index (ratio 100T/iSHBG). Using sera with a wide range of SHBG capacities, the study showed that calculated free testosterone (FT) values were almost identical to the values obtained by equilibrium dialysis except in sera from pregnant women, which contain high levels of saturated SHBG so that SHBG as determined by immunoassay overestimates its actual binding capacity (Vermeulen *et al.* 1999). The FT value is obtained from total testosterone and immunoassayable SHBG, assuming a constant albumin concentration and considering the equilibrium constants (K) of testosterone binding to SHBG and to albumin.

It was suggested that FT appears to be a rapid, simple, and reliable index of bioavailable testosterone, comparable to dialysis and suitable for clinical routine

except in pregnancy. The formula and some examples of how to calculate FT starting from total testosterone and SHBG levels determined by immunoassay are available at http://www.issam.ch/freetesto.htm.

21.4.5 Bioassay

While immunological methods measure the mass of circulating testosterone, the overall androgenic bioactivity in serum and peripheral tissues results from bioavailable testosterone and other androgens. An in vitro androgen bioassay has been recently developed, based on the androgen-dependent interaction between the ligand-binding domain and the N-terminal region of the androgen receptor, which were fused to Gal4 DNA-binding domain of *Saccharomyces cerevisiae* and to the transcriptional activation domain of herpes simplex VP16 protein, respectively. These plasmids are transfected in COS-1 cells together with the coexpression of AR-interacting protein 3, which amplifies the interaction, and with the reporter plasmid containing five Gal4-binding sites upstream of the luciferase gene. Luciferase activity measured in cell lysates results from androgen bioactivity in serum added to the cell cultures (Raivio *et al.* 2001). This bioassay has a sensitivity of 0.8 nM testosterone equivalents and correlates significantly with total testosterone and free testosterone index. In addition, the bioassay measures androgen levels three to four times lower than total serum testosterone measured by RIA, suggesting that only free and albumin-bound testosterone, i.e. bioavailable testosterone, are detected as bioactive. The use of this novel bioassay is as yet limited to the experimental field (Raivio *et al.* 2002; Raivio *et al.* 2003a and b).

21.5 Measurement of DHT

The principles and the methods for testosterone assay are basically valid for DHT as well. The main problem in DHT measurement is the elevated cross-reactivity of all polyclonal antisera with testosterone, which renders the direct, accurate quantification of DHT in a male serum sample impossible. As a consequence, DHT has to be separated from testosterone before measurement or, alternatively, testosterone must be chemically modified so that it is not recognized by the antibody. At present there are only two ways of quantifying DHT in serum accurately: after chromatographic separation or after oxidation of testosterone. Both methods involve an extraction step.

Various chromatographic procedures to separate serum steroids were validated in the seventies and in the eighties. The most common methods for chromatographic separation of DHT and testosterone are HPLC and celite chromatography. In HPLC the chromatographic medium consists of modified, lipophilic microspheres of silica gel onto which the extracted sample is loaded. DHT and testosterone are then sequentially eluted by an aqueous solution with an increasing

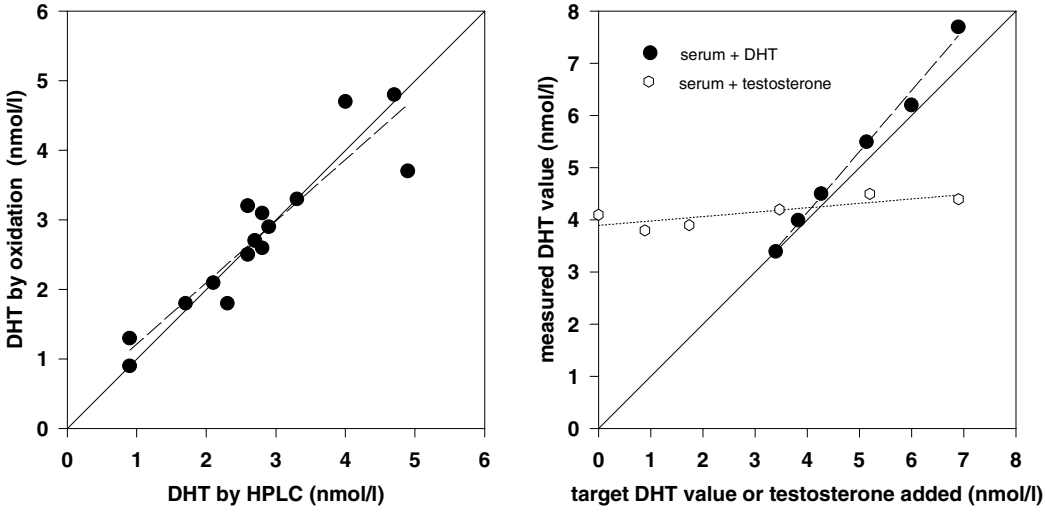


Fig. 21.3 In-house revalidation of a commercial kit for DHT determination based on oxidation of testosterone. Left panel: DHT values in serum samples measured by HPLC and by the oxidative method. Right panel: recovery of DHT after addition of DHT or testosterone to a serum sample.

concentration of the organic solvent acetonitrile. Samples are then evaporated, reconstituted in assay buffer and assayed by RIA (Lerchl and Nieschlag 1995). Celite is a diatomaceous earth widely used for steroid separation. Minicolumns, prepared fresh by packing a few mg celite, are equilibrated with iso-octane and loaded with the extracted samples. The column is then eluted sequentially with 100% iso-octane, 5–10% ethyl acetate in iso-octane (elution of DHT) and 20–50% ethyl acetate in iso-octane (elution of testosterone). Eluates are evaporated and reconstituted in assay buffer for testosterone and DHT determination by RIA (Abraham *et al.* 1975; Werawatgoompa *et al.* 1982). Chromatographic methods are still the first choice for accurate quantification of testosterone and DHT in serum. However, they are cumbersome and time-consuming, especially due to the solvent evaporation time.

In non-chromatographic methods, the serum sample is incubated with potassium permanganate. This oxidizing agent converts the double bond at position 4–5 of testosterone to dihydroxyl alcohol and does not affect DHT. The samples are then extracted with an organic solvent, evaporated, reconstituted in assay buffer and measured by RIA (Werawatgoompa *et al.* 1982). Since it eliminates the chromatographic step, this type of assay is very convenient when large numbers of samples must be measured, provided that the results have been validated against a chromatographic method (Fig. 21.3). The newest, direct, non-extractive methods for DHT measurement, such as microtitre plate-based ELISA which are commercially

available, have not been sufficiently validated. Unless such methods withstand an in-house re-validation against chromatographic methods, they should not be used.

21.6 Quality control

21.6.1 Choice of the kit and assay validation

Presently most laboratories choose to measure serum testosterone and DHT by commercially available kits. In all cases the final adoption of a kit for routine determination should depend on an in-house re-validation of the candidate assay. This re-validation includes an assessment of the sensitivity, specificity, accuracy and intra-assay precision of the kit. The specificity and the accuracy of the assay are checked by performing cross-reactivity, parallelism and recovery tests. These tests are usually carried out by the kit manufacturer beforehand and the data are reported in the kit instructions, but it is good practice to repeat them in one's own laboratory, since experience shows that many systems fail to deliver what they promise. Parallelism and recovery tests are crucial and should be performed with several different individual serum samples, since, especially in direct, non-extraction methods, the matrix in which the calibrators are dissolved may be inappropriate, resulting in non-linearity. The serum-matrix effect can be avoided using extraction methods.

Commercial kits contain control sera. Kit controls usually perform quite well and provide results in the expected range. However, care should be taken to use independent, certified control sera, in which the testosterone concentration has been determined by mass spectrometry. Quite often kit controls are in the expected range, while certified control sera are not. Such kits should not be used. Another criterion for choice is comparison of results in a sufficient number of patient sera to those obtained with a validated method. All these tests should be performed even if the candidate testosterone system is part of an automatic multianalyzer. Companies should be asked to contribute data on the calibrators and to allow complete validation before the choice to adopt that system is made. This is particularly relevant considering the overall poor performance in external quality control trials of most kits and automatic analyzers with sera containing low testosterone concentrations, i.e. from children, women and hypogonadal men.

Laboratories must often face the decision whether to adopt a new methodology. This is due to the large number of kits competing for the market or to the launch of an "improved" kit version, to budgetary necessities calling for automation and/or adoption of a multianalyzer, or to more stringent regulatory and environmental laws. All these aspects must be considered, but converting to a new methodology should never be at the costs of accuracy and reproducibility. Sometimes changes in reagent lots cause major differences in the results obtained with a kit; these become

evident both from the clinical plausibility of the data and from internal quality control. In this case a full re-validation should be performed, the kit manufacturer approached and, if the problem persists, the methodology changed.

21.6.2 Internal quality control

Once a system has been adopted some long-term parameters have to be kept under constant control. These parameters include intra- and inter-assay variability, assay drift, maximal and non-specific binding, and the standard curve characteristics, such as the slope and the dose at 50% curve displacement. Control charts have to be set up and constantly observed in order to distinguish between sporadic and systematic changes in some parameters, the latter being an indication of some modification in the kit (e.g. a change in a reagent lot) or some problem in the equipment. Intra-assay precision is evaluated by calculating the variability of the duplicate determinations. Measuring samples in singlicate does not allow evaluation of this parameter. In our experience testosterone and DHT should always be measured in duplicate, since the intra-duplicate variability at low concentrations is often $>10\%$. Another important parameter is assay drift. By measuring control sera at the beginning and at the end of the assay it can be determined whether the results at the end of the assay have any tendency to be systematically over- or underestimated. This is particularly relevant in ELISAs and methods relying on colorimetric reactions. If such a tendency is discovered, the number of samples which can be measured in one assay should be reduced. As a general rule, if the assay embraces several microtitre plates, calibrators or even entire standard curves should be run in each plate.

Inter-assay precision is checked by using certified control sera with three different testosterone concentrations (low, middle, high) in each assay. Changes in control sera lots have to be recorded. The results obtained in each assay must fall within a pre-determined, allowed range, usually within ± 2 SD of the target value, otherwise repeating the assay should be considered. Control sera can give results outside the allowed range sporadically but ideally their variability should oscillate equally on the two sides of the target value. The persistence of results mainly on one side indicates a systematic problem, which, in the example of Fig. 21.4 was identified in the automatic pipettor.

21.6.3 External quality assessment

In most western countries successful participation in an external quality assessment (EQA) program is a prerequisite to obtain and maintain the license to perform diagnostic tests for many analytes, including testosterone. Participating laboratories receive samples with unknown testosterone concentrations and return the results

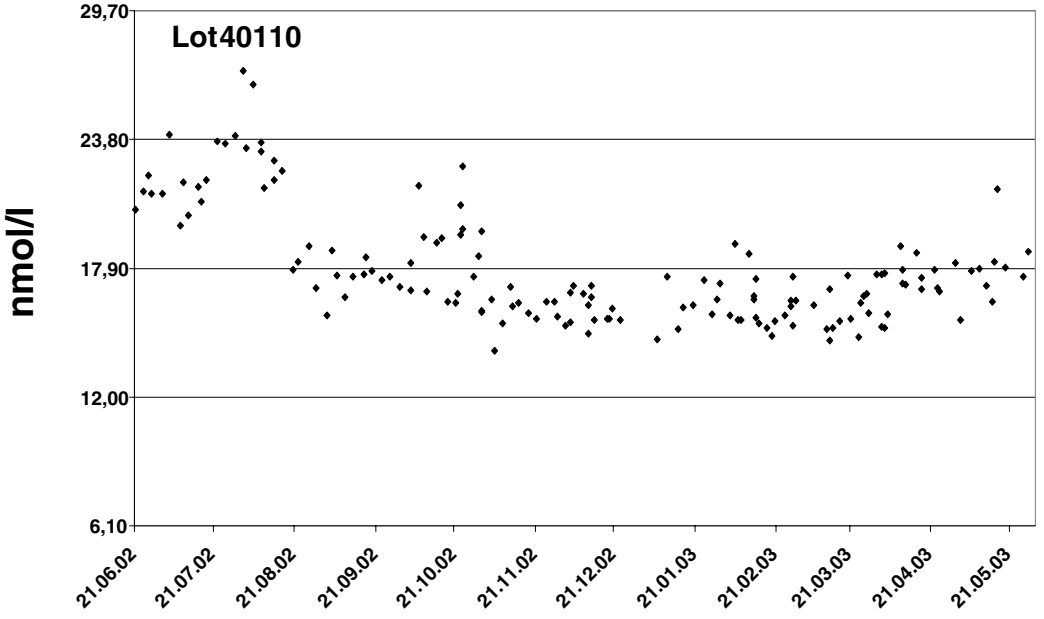


Fig. 21.4 Internal quality control. Results obtained measuring a certified control serum over one year by enzyme immuno assay (EIA). Shortly after introduction of this control serum it became evident that the assay had the tendency to overestimate the target value of this lot (17.9 nmol/l). After servicing of the pipettor/plate reader equipment and re-validation (August 2002) the results improved.

to the scheme organizer. The results are then evaluated and the laboratory receives an assessment of its performance. An example of the results of the external quality assessment survey in Germany, organized by the German Society of Clinical Chemistry is given in Fig. 21.5. This example and the data available from the UK National External Quality Assessment Scheme (UK NEQAS) and from the US College of American Pathologists (CAP) reveal that, overall, less than 15% of the labs still use RIA for testosterone measurement, direct non-extractive methods are employed almost exclusively. More than 60% of the labs perform the analysis using automatic multianalyzers. In the UK NEQAS 2002 and in the US CAP survey 2003 only 3/239 and 3/953 participants, respectively used an extraction/chromatographic method[1]. The data in Fig. 21.5 show the very high inter-lab variability of the results obtained in Germany from the measurement of two serum samples containing testosterone concentrations in the adult male range. Most of the laboratories manage to

[1] College of American Pathologists' 2003 Ligand (Special) Survey Y-A. Participant Summary Report. All conclusions and interpretations in this publication with respect to the College of American Pathologists' database are those of the author and not those of the College.

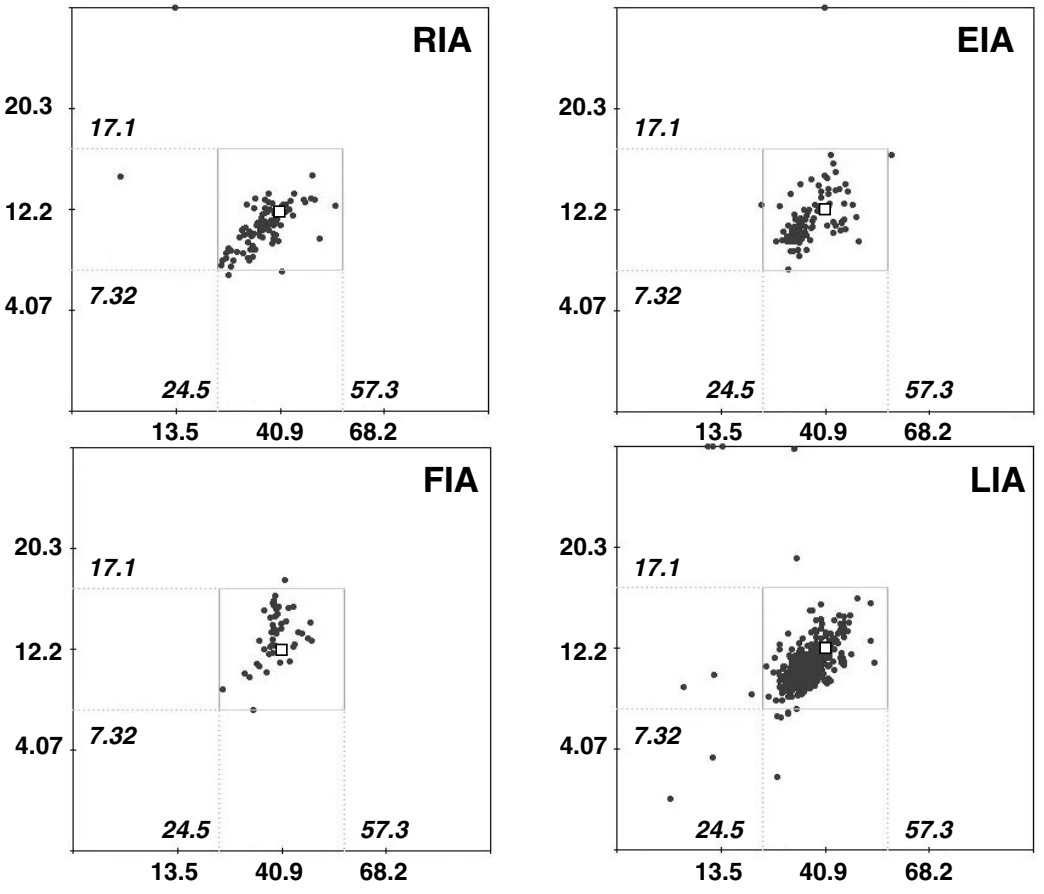


Fig. 21.5 External quality assessment. Results of two control sera with a testosterone concentration of 12.2 nmol/l (sample A, Y axis) and 40.9 nmol/l (sample B, X axis), respectively as measured by mass spectrometry in German laboratories participating in an external quality assessment scheme. Results are grouped by radioimmunoassays (RIA), methods based on enzymatic (EIA), fluorimetric (FIA) or luminescent (LIA) detection systems. Each dot represents results from one laboratory. Target values are indicated by the small white square at the center of each graph. According to current German guidelines the target is considered to be successfully met if the results fall within ±40% of the value measured by mass spectrometry, indicated by the square defined by dotted lines. Results available online from the German Society of Clinical Chemistry (www.dgkc-online.de).

produce results falling within the allowed range, which, according to the actual German guidelines, permits variations of ± 40% of the testosterone value measured by mass spectrometry. This is, of course, a very wide range, such that the measurement of sample A of Fig. 21.5, which has a nominal value of 12.2 nmol/l, is considered successful if the laboratory obtains any value between 7.32 and 17.1 nmol/l.

In practice this means that a man with borderline serum testosterone concentrations has an equal probability of being classified as normal or hypogonadal (i.e. $>$ or $<$ 12 nmol/l) and both diagnoses are correct from the analytical point of view. The comparison between the four method groups shown in Fig. 21.5 does not reveal major differences among them. However, a more accurate analysis of external quality assessment results is usually performed by the UK NEQAS and this permits identification of kits which are less accurate than others (Middle 2002). The UK NEQAS analysis also shows clearly that the performance of testosterone kits is quite bad for the measurement of female samples, whereas the overall bias for male samples (about $\pm$ 15%) is acceptable. Therefore, as long as commercially available kits are used, the accuracy of the serum testosterone determination is very much dependent on the in-house re-validation and of a very strict *internal* quality control, both of which remains up to the individual laboratory.

External quality assessment for SHBG and free testosterone is much less advanced. UK NEQAS and US CAP offer schemes for SHBG, but only few laboratories make use of them. The US CAP survey 2003 reports on 79 laboratories measuring SHBG and 9 laboratories measuring bioavailable testosterone by ammonium sulfate precipitation. As far as free testosterone is concerned, six labs were reported to use equilibrium dialysis, three labs centrifugal ultrafiltration and 70 labs used an "analog" method. In view of the analytical problem of the "analog" kits reported above, it is not surprising that these labs produce free testosterone results much too low compared to those obtained by FT calculation, dialysis or ultrafiltration. No external quality assessment is presently available for DHT.

21.7 Key messages

- Serum testosterone is measured in clinical routine by immunological competitive methods based on polyclonal antisera and labeled hormone.
- The reference method for testosterone measurement is mass spectrometry. Extraction methods should be used for accurate, reproducible testosterone measurements.
- Non-extraction, direct methods based on non-radioactively labeled tracers are currently routinely used. Automatic multianalzers are those systems mostly used for serum testosterone measurement.
- Any testosterone measurement system, including those based on automatic analyzers, should be carefully validated in-house against an extraction method before it is adopted for routine assays.
- So-called "analog" free testosterone methods are unreliable. Calculated free testosterone gives the best estimation of free testosterone as measured by dialysis or ultrafiltration.
- DHT should be measured by chromatographic or oxidative methods.
- Participation in external quality control programs is mandatory. Strict internal quality control is fundamental to ensure accurate measurements.

21.8 REFERENCES

Abraham GE, Manlimos FS, Solis M, Wickman AC (1975) Combined radioimmunoassay of four steroids in one ml of plasma: II. Androgens. Clin Biochem 8:374–378

Anderson DC (1974) Sex-hormone-binding globulin. Clin Endocrinol (Oxf) 3:69–96

Boots LR, Potter S, Potter D, Azziz R (1998) Measurement of total serum testosterone levels using commercially available kits: high degree of between-kit variability. Fertil Steril 69:286–292

Davies R, Collier C, Raymond M, Heaton J, Clark A (2002) Indirect measurement of bioavailable testosterone with the Bayer Immuno 1 system. Clin Chem 48:388–490

Dechaud H, Lejeune H, Garoscio-Cholet M, Mallein R, Pugeat M (1989) Radioimmunoassay of testosterone not bound to sex-steroid-binding protein in plasma. Clin Chem 35:1609–1614

Dunn JF, Nisula BC, Rodbard D (1981) Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53:58–68

Dussault JH, Letarte J, Guyda H, Laberge C (1977) Serum thyroid hormone and TSH concentrations in newborn infants with congenital absence of thyroxine-binding globulin. J Pediatr 90:264–265

Ekins R (1990) Measurement of free hormones in blood. Endocr Rev 11: 5–46

Fitzgerald RL, Herold DA (1996) Serum total testosterone: immunoassay compared with negative chemical ionization gas chromatography-mass spectrometry. Clin Chem 42:749–755

Furuyama S, Mayes DM, Nugent CA (1970) A radioimmunoassay for plasma testosterone. Steroids 16:415–428

Gonzalez-Sagrado M, Martin-Gil FJ, Lopez-Hernandez S, Fernandez-Garcia N, Olmos-Linares A, Arranz-Pena ML (2000) Reference values and methods comparison of a new testosterone assay on the AxSYM system. Clin Biochem 33:175–179

Granger DA, Schwartz EB, Booth A, Arentz M (1999) Salivary testosterone determination in studies of child health and development. Horm Behav 35:18–27

Jockenhövel F, Krüsemann C, Jaeger A, Olbricht T, Reinwein D (1992) Comparability of serum testosterone determination with the ten most commonly used commercial radioimmunoassays and with a new enzyme immunoassay. Klin Lab 38: 81–88

Lerchl A, Nieschlag E (1995) Diurnal variations of serum and testicular testosterone and dihydrotestosterone (DHT) in Djungarian hamsters (*Phodopus sungorus*): testes are the main source for circulating DHT. Gen Comp Endocrinol 98:129–136

Levesque A, Letellier M, Swirski C, Lee C, Grant A (1998) Analytical evaluation of the testosterone assay on the Bayer Immuno 1 system. Clin Biochem 31:23–28

Manni A, Pardridge WM, Cefalu W, Nisula BC, Bardin CW, Santner SJ, Santen RJ (1985) Bioavailability of albumin-bound testosterone. J Clin Endocrinol Metab 61:705–710

Mendel CM, Murai JT, Siiteri PK, Monroe SE, Inoue M (1989) Conservation of free but not total or non-sex-hormone-binding-globulin-bound testosterone in serum from Nagase analbuminemic rats. Endocrinology 124:3128–3130

Mendel CM (1992) The free hormone hypothesis. Distinction from the free hormone transport hypothesis. J Androl 13:107–116

Middle JG (2002) UK NEQAS for Steroid Hormones Annual Review 2002, UK NEQAS, University Hospital Birmingham NHS Trust

Morley JE, Patrick P, Perry HM3rd (2002) Evaluation of assays available to measure free testosterone. Metabolism 51:554–559

Nieschlag E, Loriaux DL (1972) Radioimmunoassay for plasma testosterone. Z Klin Chem Klin Biochem 10:164–168

Pugeat MM, Dunn JF, Nisula BC (1981) Transport of steroid hormones: interaction of 70 drugs with testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53: 69–75

Raivio T, Tapanainen JS, Kunelius P, Janne OA (2002) Serum androgen bioactivity during 5alpha-dihydrotestosterone treatment in elderly men. J Androl 23:919–921

Raivio T, Santti H, Schatzl G, Gsur A, Haidinger G, Palvimo JJ, Janne OA, Madersbacher S (2003a) Reduced circulating androgen bioactivity in patients with prostate cancer. Prostate. 55: 194–198

Raivio T, Palvimo JJ, Dunkel L, Wickman S, Janne OA (2001) Novel assay for determination of androgen bioactivity in human serum. J Clin Endocrinol Metab 86: 1539–1544

Raivio T, Toppari J, Kaleva M, Virtanen H, Haavisto AM, Dunkel L, Janne OA (2003b) Serum androgen bioactivity in cryptorchid and noncryptorchid boys during the postnatal reproductive hormone surge. J Clin Endocrinol Metab 88:2597–2599

Rassaie MJ, Kumari LG, Pandey PK, Gupta N, Kochupillai N, Grover PK (1992) A highly specific heterologous enzyme-linked immunosorbent assay for measuring testosterone in plasma using antibody-coated immunoassay plates or polypropylene tubes. Steroids 57: 288–294

Rosner W (1997) Errors in the measurement of plasma free testosterone. J Clin Endocrinol Metab 82:2014–2015

Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA (1999) Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane. J Steroid Biochem Mol Biol 69:481–485

Rosner W (2001) An extraordinarily inaccurate assay for free testosterone is still with us. J Clin Endocrinol Metab 86:2903

Schurmeyer T, Nieschlag E (1984) Comparative pharmacokinetics of testosterone enanthate and testosterone cyclohexanecarboxylate as assessed by serum and salivary testosterone levels in normal men. Int J Androl 7:181–187

Segre GV, Brown EN (1998) Measurement of hormones. In William's Textbook of Endocrinology. Wilson JD, Foster DW, Kroneneberg HM, Larsen PR (eds) WB Saunders, Philadelphia, pp 43–54

Siekmann L (1979) Determination of steroid hormones by the use of isotope dilution–mass spectrometry: a definitive method in clinical chemistry. J Steroid Biochem 11: 117–123

Simoni M, Baraldi E, Baraghini GF, Boraldi V, Roli L, Seghedoni S, Verlardo A, Montanini V (1988) Twenty-four-hour pattern of plasma SHBG, total proteins and testosterone in young and elderly men. Steroids 52:381–382

Simoni M, Montanini V, Fustini MF, Del Rio G, Cioni K, Marrama P (1992) Circadian rhythm of plasma testosterone in men with idiopathic hypogonadotrophic hypogonadism before

and during pulsatile administration of gonadotropin-releasing hormone. Clin Endocrinol 36: 29–34

Taieb J, Benattar C, Birr AS, Lindenbaum A (2002) Limitations of steroid determination by direct immunoassay. Clin Chem 48:583–585

Taieb J, Mathian B, Millot F, Patricot MC, Mathieu E, Queyrel N, Lacroix I, Somma-Delpero C, Boudou P (2003) Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem 49:1381–1395

Thienpont LM, van Nieuwenhove B, Stockl D, Reinauer H, De Leenheer AP (1996) Determination of reference method values by isotope dilution-gas chromatography/mass spectrometry: a five years' experience of two European Reference Laboratories. Eur J Clin Chem Clin Biochem 34:853–860

Tschöp M, Behre HM, Nieschlag E, Dressendorfer RA, Strasburger CJ (1998) A time-resolved fluorescence immunoassay for the measurement of testosterone in saliva: monitoring of testosterone replacement therapy with testosterone buciclate. Clin Chem Lab Med 36:223–230

Vaitukaitis J, Robbins JB, Nieschlag E, Ross GT (1971) A method for producing specific antisera with small doses of immunogen. J Clin Endocrinol Metab 33:988–991

Vermeulen A, Verdonck L, Kaufman JM (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672

Vlahos I, MacMahon W, Sgoutas D, Bowers W, Thompson J, Trawick W (1982) An improved ultrafiltration method for determining free testosterone in serum. Clin Chem 28:2286–2291

Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS (2004) Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography tandem mass spectrometry. H Clin Endocrinol Metab (in press)

Wang C, Plymate S, Nieschlag E, Paulsen CA (1981) Salivary testosterone in men: further evidence of a direct correlation with free serum testosterone. J Clin Endocrinol Metab 53:1021–1024

Werawatgoompa S, Dusitsin N, Sooksamiti P, Leepipatpaiboon S, Virutamasen P, Boonsiri B (1982) A rapid method for the determination of 5 alpha-dihydrotestosterone in Thai males receiving medroxyprogesterone acetate. Contraception 25:523–533

Wheeler MJ (1995) The determination of bio-available testosterone. Ann Clin Biochem 32:345–357

Winters SJ, Kelley DE, Goodpaster B (1998) The "analog" free testosterone assay: are the results in men clinically useful? Clin Chem 44:2178–2182

22

Synthesis and pharmacological profiling of new orally active steroidal androgens

A. Grootenhuis, M. de Gooyer, J. van der Louw, R. Bursi and D. Leysen

Contents

22.1 Introduction

There is a general need for new orally active androgens, to be used for androgen replacement therapy and male hormonal contraception. As a substitute for low levels of testosterone in hypogonadal men, therapy with the natural androgen testosterone is the first choice. However, testosterone is an androgen with a relatively low affinity for the androgen receptor. It is metabolically relatively unstable which results in poor oral bioavailability. In addition, testosterone is converted by

5α-reductase into the more potent androgen 5α-dihydrotestosterone (5α-DHT). Early attempts to prevent metabolic instability by 17α-alkylation of androgens (like introduction of 17 α-ethynyl in estrogens and progestagens) were not successful, due to liver toxicity or low androgenic activity (Vida 1969). Another approach to circumvent metabolic instability is esterification of the 17β-OH group of testosterone with long chain fatty acids. Testosterone undecanoate formulated in an oily solution (Andriol®/Andriol Testocaps™, dissolved in oleic acid and a mixture of castor oil and polypropylene glycol laurate, respectively) is currently the only orally active testosterone derivative. This testosterone ester is hydrolysed by tissue (liver) esterases and testosterone is released (Bursi *et al.* 2001). However, due to the limited potency of testosterone and limited bioavailability of testosterone undecanoate, relative high doses of Andriol®/Andriol Testocaps™ are required twice a day for human androgen replacement (total dose 160 –240 mg).

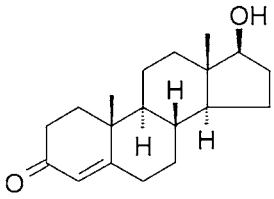
Although no side effects of testosterone replacement on the prostate have been reported, androgens which are not potentiated upon 5α-reduction in the prostate and skin, like 7α-methyl-19-nortestosterone (MENT) and 19-nortestosterone (nandrolone), are considered prostate-safe androgens (Kumar *et al.* 1992; Cummings *et al.* 1998). MENT however, has low oral activity, like testosterone, and is therefore currently being developed as a 17β-O-acetate for parenteral application.

An important metabolite of testosterone is estradiol, which is formed by the enzyme aromatase, a product of the cytochrome P450 gene family CYP19 (Simpson *et al.* 2002).

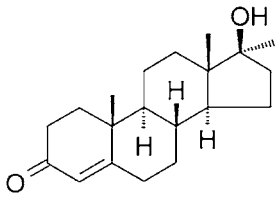
Since oral application is the preferred route of administration, a systematic search for more potent, metabolically stable androgens that are substrates of the aromatase enzyme, was initiated at N. V. Organon. As part of the project we investigated a series of 7α-substituted 19-nortestosterone derivatives. It was hypothesised that chain elongation and, optionally, unsaturation of the 7α-substituent (Fig. 22.1) would result in compounds with higher metabolic stability. The above mentioned androgens were characterised in vivo for their pharmacokinetic profile and their potency to suppress/prevent castration-induced LH and FSH increase, trabecular bone mineral density (BMD) loss, and the effect on prostate weight of male rats. In addition, effects on endogenous testosterone levels of intact male monkeys (*Macaca arctoides*) were studied. Testosterone undecanoate was used as the orally active reference androgen throughout all these in vivo *studies.*

22.2 Synthesis of nandrolone derivatives and reference steroids

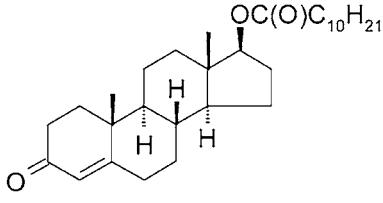
Structures of nandrolone derivatives and reference steroids are shown in Fig. 22.1.



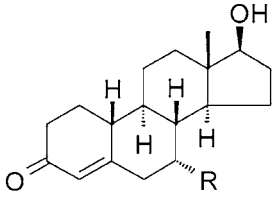
Testosterone (**1**)



17α-Methyltestosterone (**2**)



Testosterone undecanoate (**3**)



R = H: Nandrolone (**4a**)
R = CH_3: MENT (**4b**)
R = CH_2CH_3: 7α-Ethylnandrolone (**4c**)
R = $CH{=}CH_2$: 7α-Vinylnandrolone (**4d**)
R = C≡CH: 7α-Ethynylnandrolone (**4e**)

Fig. 22.1 Structures of nandrolone derivatives and reference steroids.

22.2.1 Preparation of nandrolone, MENT and reference steroids

For several years, diosgenine was one of the most important starting materials for sex hormones, including testosterone and derivatives. Nowadays, it has been superseded by other natural products, for instance sitosterol, cholesterol and other sterols (Kirk-Otmar 1983; Zeelen 1990). Sitosterol can be converted, by microbiological degradation, to androstenedione (Fig. 22.2). The latter may serve as starting material for the production of testosterone (**1**) and derivatives. Depending on the microbes used, sitosterol can also be converted to sitolactone (Fig. 22.3). The latter serves as starting material for the production of 19-norandrostenedione which can be used for the preparation of nandrolone (**4a**) and derivatives. 19-Norandrostenedione may also be converted to *e.g.* Δ^6-nandrolone acetate, which is one of the precursors for 7α-substituted nandrolones (Anonymous 1962).

22.2.2 Preparation of nandrolone derivatives

7α-Ethylnandrolone (**4c**) and 7α-vinylnandrolone (**4d**) were prepared by 1,6-addition of Et_2CuLi or vinylmagnesium chloride/copper(I), respectively, to Δ^6-nandrolone derivatives **7a** and **7b**, followed by acidic hydrolysis and/or

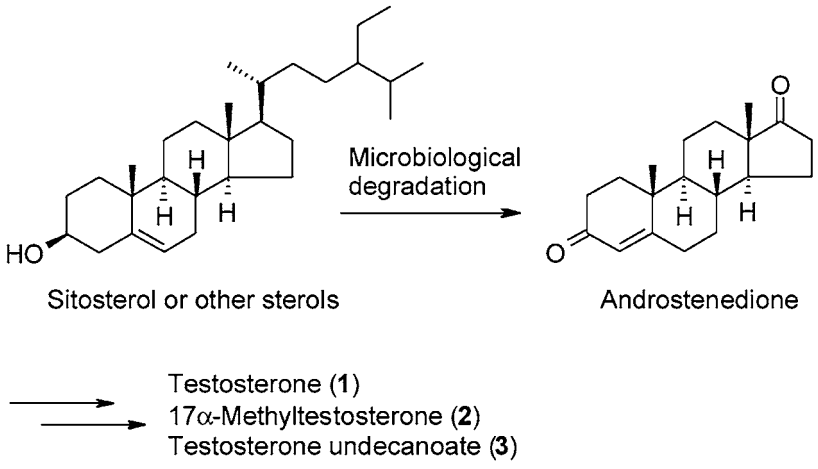


Fig. 22.2 Preparation of testosterone and derivatives.

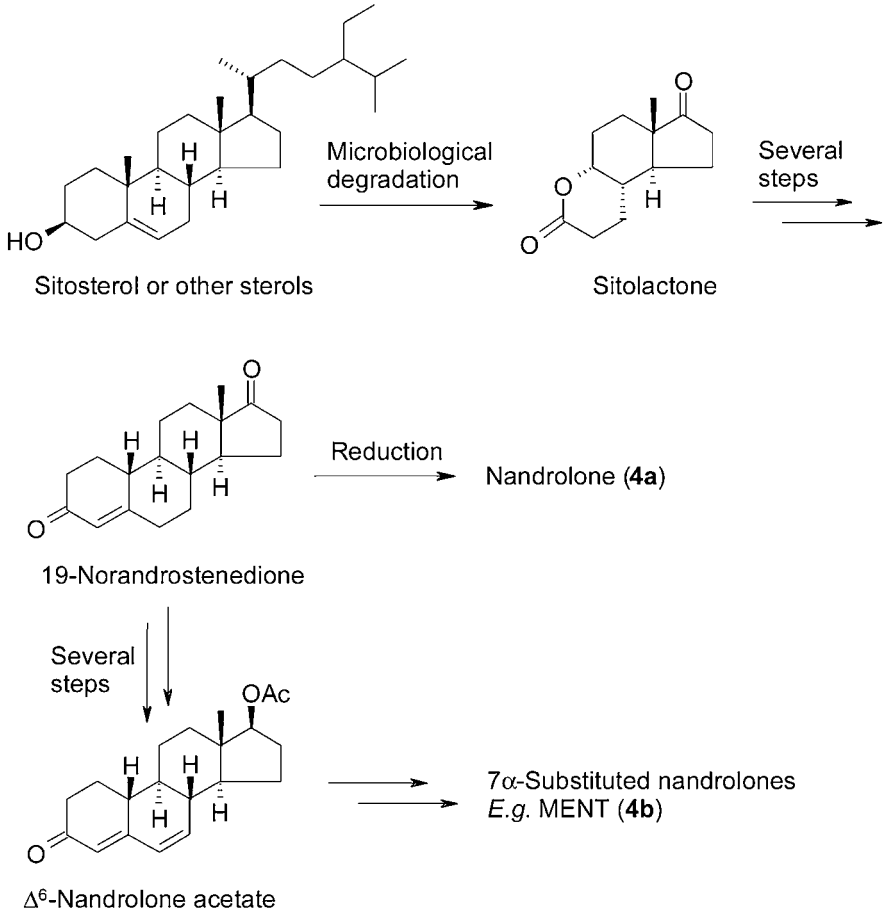
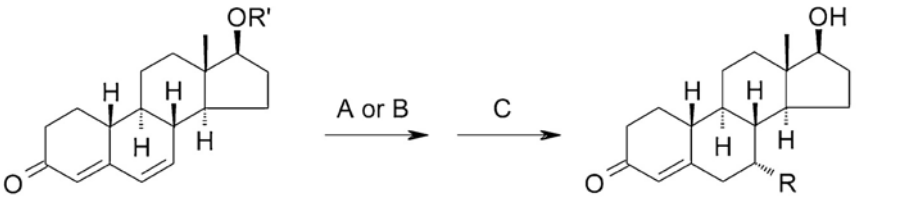


Fig. 22.3 Preparation of nandrolone derivatives **4a** and **4b**.



7a R' = TBS
7b R' = Ac

4c R = CH_2CH_3, 28%
4d R = $CH{=}CH_2$, 59%

Experimental conditions:
A: 1) **7a**, Et_2CuLi, ether, THF, -30 °C; 2) TMSCl, -30 °C → -10 °C; 3) NH_4Cl (aq).
B: 1) **7b**, $CH_2{=}CHMgCl$, CuBr.Me_2S (0.1 eq), LiSPh (0.1 eq), LiBr (0.1 eq), THF, -15°C; 2) NH_4Cl (aq).
C: 1) HCl, acetone, RT; 2) KOH, MeOH, THF, H_2O, RT (R' = Ac).

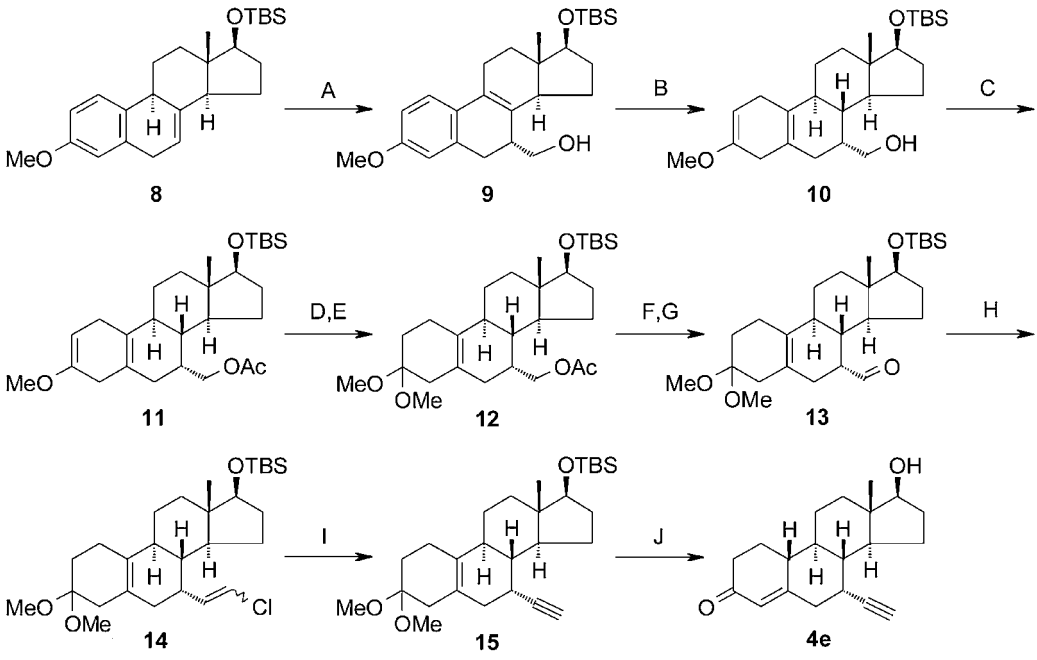
Fig. 22.4 Preparation of nandrolone derivatives **4c** and **4d**.

saponification (R = Ac) (Fig. 22.4) (van der Louw *et al.* 2003). Introduction of ethynyl by 1,6-addition is not possible. Instead, 7α-ethynylnandrolone (**4e**) was obtained from alcohol **9**, which was produced from the Δ^7-steroid derivative **8** by the method of Künzer *et al.* (2003) (Fig. 22.5). Birch reduction of **9** resulted in diene **10** which was converted by standard procedures to aldehyde **13**. Wittig reaction with $ClCH_2PPh_3Cl$/*t*-BuONa and elimination of chlorovinyl compound **14** with *n*-BuLi produced ethynyl compound **15**. Treatment of the latter with hydrochloric acid produced nandrolone derivative **4e**. Detailed information on the synthesis of 7α-ethylnandrolone (**4c**), 7α-vinylnandrolone (**4d**) and 7α-ethynylnandrolone (**4e**) can be found elsewhere (van der Louw *et al.* 2003). Also included in this study is the new 7α-alkylated nandrolone derivative Org X, the structure of which will be reported later.

22.3 Methods of pharmacological evaluation

22.3.1 Androgen receptor transactivation and binding

Steroid receptor activity is dependent on three factors: the concentration of the ligand, the receptor, and co-regulatory proteins. The composition and presence of steroid receptors in the cell determine the response of the ligand and the transactivation of target genes by ligand-occupied receptors is modulated by the presence of nuclear co-activators and co-repressors (Katzenellenbogen *et al.* 1996). For the determination of the androgenic activity of compounds, androgen receptor binding assays and androgen receptor transactivation assays can be used. With the binding assay the affinity of a compound for the androgen receptor can be determined but



Experimental conditions:

A: $(CH_2O)_n$, Me_2AlCl, CH_2Cl_2, hexane, -10°C, 45 %.
B: 1) Li, liquid NH_3, THF, -33°C; 2) EtOH, 98 %.
C: Ac_2O, pyridine, RT, 100 %.
D: Oxalic acid, MeOH, H_2O, 40°C, 40 %.
E: $(MeO)_3CH$, MeOH, TsOH, 0°C, 84 %.
F: $LiAlH_4$, THF, 0°C, 97 %.
G: Pr_4NRuO_4, NMO, acetone, 99 %.
H: $ClCH_2PPh_3Cl$, *t*-BuONa, THF, 0°C → RT, 53 % (*E* : *Z* = 3 : 2).
I: *n*-BuLi, THF, hexane, -15°C → RT, 63 %.
J: HCl, acetone, RT, 89 %.

Fig. 22.5 Preparation of nandrolone derivative **4e**.

the biological effect of that compound cannot be predicted. The biological effect can be estimated in agonistic and antagonistic transactivation assays. To study the agonistic activity of androgens, Chinese hamster ovary (CHO) cells were transfected with the human androgen receptor and the mouse mammary tumor virus (MMTV) promotor in front of the firefly luciferase reporter gene. This cell line is named CHO-AR-MMTV-LUC (clone 1G12-A5-CA). The agonistic activity for the androgen receptor was determined according to the procedure described by Schoonen *et al.* (1998). In brief, 5×10^4 cells/well were seeded into a 96-well plate and incubated with test compounds (final ethanol content: 1% v/v) for 16 h in

medium with 5% charcoal-treated bovine calf serum supplement at 37°C in a humidified atmosphere of air supplemented with 5% CO_2. Thereafter, of the total 250 μl incubation volume, 200 μl was removed, and 50 μl LucLite was added for cell lysis and luciferase measurement. Luciferase activity was measured in a Topcount luminescence counter (Canberra Packard, Meridan, USA). Relative agonistic activity (RAA) studies were carried out with various concentrations (1:2:4 dilutions) of the standard and compounds of interest. From these curves the EC50 values were determined. The RAA of the different compounds tested was expressed as a percentage of the EC50 value of the reference compound 5α-dihydrotestosterone (EC50 of 5α-DHT=100%).

The binding affinity to the androgen receptor was determined according to the procedure described by Bergink *et al.* (1983). For displacement analysis CHO-AR-MMTV-LUC (clone 1G12-A5-CA) cells were used. Cells were cultured and harvested, and cytosolic preparations prepared as described previously (Bergink *et al.* 1983). Before use, cytosol equivalent to 1 g of cells was diluted with buffer at a ratio of 1:15. Ethanolic solutions of compounds were pipetted (10 μl) into 96-well plates, the ethanol was evaporated and the residue was dissolved in buffer containing radiolabeled 5α-DHT (50 μl). After mixing thoroughly, 50 μl of ice cold cytosolic preparation containing human androgen receptor was added, thoroughly mixed and incubated over night at 4°C. The free and receptor bound 5α-DHT was separated by a dextran-coated charcoal precipitation. Finally, 100 μl supernatant was counted in a Topcount microplate scintillation counter (Canberra Packard, Meridan, USA). Specific binding was determined by subtracting non-specific from total binding. Relative binding affinity (RBA) studies were carried out with various concentrations of the standards (1:2:4 dilutions) and compounds of interest. The RBA was calculated relative to 5α-DHT of which the EC50 was set at 100%.

22.3.2 Determination of metabolic stability with human hepatocytes

Compounds used for oral application can be converted during first-pass metabolism in the intestine and the liver. In contrast to testosterone the androgen 17α-methyltestosterone is an orally active androgen. To predict the oral availability of nandrolone derivatives the metabolic stability was assessed in cryopreserved human male hepatocytes and compared to the metabolically stable reference 17α-methyltestosterone (MT, compound **2** in Fig. 22.1) and the unstable reference testosterone (compound **1**, in Fig. 22.1). The nandrolone derivatives and reference compounds were tested at a concentration of 10 nmol/l. Cryopreserved human hepatocytes from healthy young men (25–45 years) were obtained from In Vitro Technologies (Baltimore, MD, USA). The hepatocytes were thawed at 37°C and immediately placed on ice. The cells were washed twice in cold medium (William's

medium E without phenol red, supplemented with glutamax I, gentamycin (50 μg/ml), insulin (1 μmol/l) and hydrocorticosterone (10 μmol/l)). The hepatocytes were seeded at a density of 5×10^5 viable cells/well in a 12-well plate. The incubations were started by the addition of a test compound in medium. The incubations were performed at 37°C in an atmosphere of air/O_2/CO_2 (55/40/5) for different interval times (0, 30, 60, and 180 minutes). The incubations were terminated by pipetting the incubation mixture into one volume of acetone on ice. The acetone was evaporated under a stream of nitrogen, the volume was adjusted to 1.5 ml and centrifuged at 10,000 g during 30 min at 4°C. The supernatants were collected for LC-MS/MS analysis.

The parent compound was determined using a Supelcosil LC-8-DB (33 × 4.0 mm) column and a gradient of methanol in 20 mmol/l ammonium acetate dissolved in formic acid (0.1 mol/l) at a flow-rate of 1 ml/min. All mass measurements were performed at an API-3000 mass spectrometer (Perkin-Elmer Sciex, Toronto, Canada).

22.3.3 Aromatase susceptibility of new androgens

The experiments were performed as described by de Gooyer *et al.* (2003). In brief, the nandrolone derivatives, testosterone and 17α-methyltestosterone were incubated with supersomes containing human aromatase (Gentest, Woburn, MA, USA) for 0, 15, 30, 60, and 120 min. The final reaction mixture in a phosphate buffer (pH 7.4) contained 100 nmol/l compound, 20 nmol/l cytochrome P450 and 1 mmol/l NADPH. The incubations were terminated by extraction with ethylacetate. The ethylacetate was evaporated and the residue was dissolved in 1 ml 100% ethanol. The ethanolic solution was added to CHO cells stably transfected with either the human androgen receptor (AR) or human estrogen receptor α (ERα) and a promotor gene in front of a firefly luciferase reporter gene. The final concentration of test compounds in the receptor transactivation assay was maximally 1 nmol/l. After an incubation of 18h the luciferase activity was measured using the LucLite assay kit (Canberra Packard) and the luminescence signal was measured on a Topcount 96-well plate scintillation counter (Canberra Packard).

22.3.4 Effects on bone, LH/FSH, ventral prostate and muscle in castrated male rats

Screening for oral in vivo activity was performed in male castrated rats which were treated once daily for 4 days with gelatin/mannitol suspension or arachis oil solution, as has been reported in a restoration study by Kumar *et al.* (1992). Three hours after the last oral application, blood was collected from the tail and serum LH was determined with a sensitive immunofluometrix method (van Casteren *et al.* 2000). The minimal active dose (MAD) was defined as the dose that results in a statistically significant suppression of 65% (±10%) of serum LH. For effects on

bone, rats were castrated and directly treated for 6 weeks. Effects on trabecular bone mineral density (BMD) in the distal femur were determined as described by (Ederveen and Kloosterboer 2001). Androgenic/anabolic effects were determined by the increase of the wet weight of the ventral prostate (VP) and musculus levator ani (MLA), respectively (Herschberger *et al.* 1953).

22.3.5 Effects on plasma testosterone of intact male monkeys

Intact male *Macaca arctoides* were treated orally once daily for seven successive days with different doses of Org X in arachis oil solution. In 2–3 pre-treatment samples plasma testosterone was determined on day 7 and 15 with a commercial immunoassay (Immunolite, DPC). Plasma levels of Org X were determined in blood sampled before each oral administration (trough levels) and determined by LCMSMS.

22.3.6 Pharmacokinetic evaluation in different animals

Single-dose pharmacokinetic (SD-PK) evaluation of the different androgens was performed in castrated male rats, castrated rabbits and intact female monkeys (*Macaca arctoides*) after a single oral administration of 10, 10, and 5 mg/kg, respectively. Blood was collected at eight different time points. The amount of applied androgen was determined after sample pre-treatment (C18 separation) and detected with a Sciex API 4000 MSMS detector as reported by de Gooyer *et al.* (2003). Most important PK parameters such as elimination half-life ($t^1/_2$), area under curve (AUC) expressed as nmol*hours after complete elimination (infinitive, called AUCinf) and maximal concentration (Cmax) are reported. Values were normalised to 1 mg/kg for the dose given, therefore nAUCinf and nCmax will be used throughout the text.

22.4 Pharmacological profile

22.4.1 Androgen receptor binding and transactivation

Table 22.1 summarizes the results for androgen receptor binding and agonistic activity. The binding activity shows that introduction of a 7α-methyl or ethyl group to nandrolone increases the relative binding affinity (RBA) while a 7α-vinyl or ethynyl did not increase the RBA. Org X has a RBA that is 3-fold higher than nandrolone and 9-fold higher than testosterone. The relative agonistic activity (RAA), however, of all the 7α- derivatives of nandrolone is higher than would be expected from the RBA. The 7α-substituents increase the agonistic activity with the highest increase for the methyl substituent. Org X has an agonistic androgen activity which is 4-fold higher than that of nandrolone and 14-fold higher than that of testosterone.

Table 22.1 The relative binding affinity (RBA) and relative agonistic transactivation activity (RAA) of 7α-substituted nandrolone derivatives for the human androgen receptor

R	RBA	RAA
7α-H	50.7 ± 5.3% (12)	55.0 ± 14.4% (3)
7α-methyl	146.3 ± 5.0% (4)	269.4 ± 15.6% (16)
7α-ethyl	133.5 ± 86.3% (2)	152.0 ± 42.9% (2)
7α-vinyl	54.0 ± 2.0% (2)	190.0 ± 11.5% (3)
7α-ethynyl	40.0 ± 6.0% (2)	132.8 ± 27.3% (1)
Org X	164.0 ± 6.0% (2)	228.3 ± 41.3% (4)
testosterone	17.6 ± 1.2% (34)	16.5 ± 0.5% (30)
17α-methyltestosterone	27.4 ± 8.0% (3)	19.5 ± 0.5% (2)

5α-dihydrotestosterone was used as reference compound and set at 100%. Data are mean ± SEM, number of experiments between brackets.

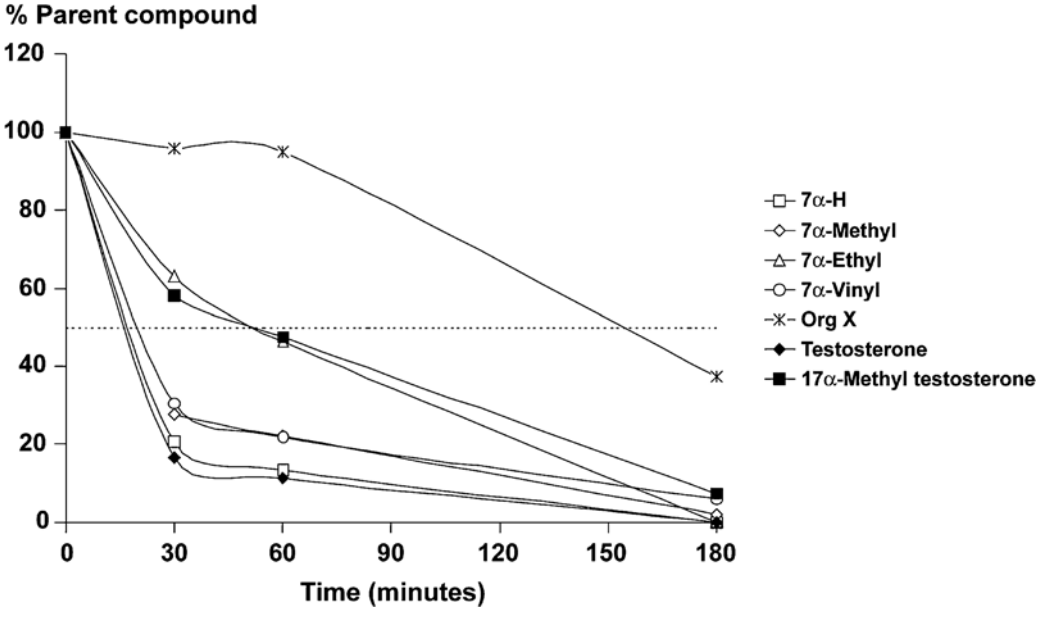


Fig. 22.6 Metabolic stability of nandrolone derivatives in cryopreserved human male hepatocytes. Testosterone and 17α-methyltestosterone were used as metabolically unstable and stable reference compounds, respectively.

22.4.2 Metabolic stability in human hepatocytes

The metabolic stability of the nandrolone derivatives in human hepatocytes are shown in Fig. 22.6. Testosterone is rapidly metabolized in human cryopreserved hepatocytes while the metabolism of the orally active 17α-methyltestosterone is much slower. The metabolic stability of 7α-ethylnandrolone and Org X is

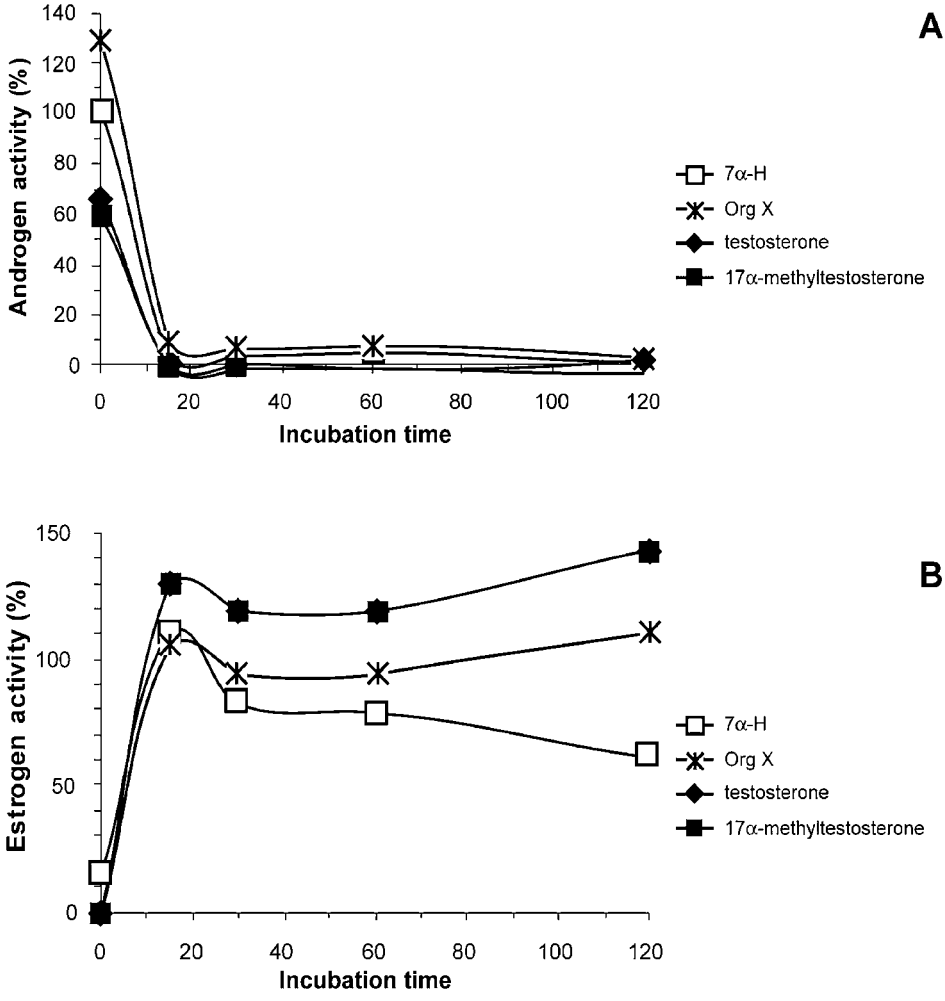


Fig. 22.7 Susceptibility of androgens for human recombinant aromatase as determined with bioassays for androgen receptor (panel A) and estrogen receptor(α) activation (panel B) (see also Table 22.2).

comparable to or much better than that of 17α-methyltestosterone, respectively. Nandrolone, MENT and 7α-vinyl nandrolone have a metabolic stability comparable to the metabolically unstable testosterone.

22.4.3 Susceptibility for human aromatase

To evaluate whether 7α-substituted nandrolone derivatives are substrates for human aromatase, compounds were incubated with recombinant human aromatase. The conversion of the compounds was determined with bioassays for androgenic and estrogenic (ERα) activity. The results for testosterone, 17α-methyltestosterone, nandrolone, and Org X are shown in Fig. 22.7. These

Table 22.2 Susceptibility of 7α-substituted nandrolone derivatives to aromatization by human recombinant aromatase (see also Fig. 22.7)

R	Converted by human aromatase
7α-H	Yes
7α-methyl	Yes
7α-ethyl	Yes
7α-vinyl	Yes
7α-ethynyl	Yes
Org X	Yes
testosterone	Yes
17α-methyltestosterone	Yes

Table 22.3 Oral activity of 7α-substituted nandrolone derivatives and reference compounds as determined by their ability to suppress serum LH (3 hours after last dose) in male castrated rats

R	LH suppression MAD p.o. mg/kg
7α-H	∼12.5 (1)
7α-methyl	∼15 (1)
7α-ethyl	∼3.5 (4)
7α-vinyl	∼5.5 (4)
7α-ethynyl	>5 (1)
Org X	0.32 (6)
testosterone	∼70 (1)
17α-methyltestosterone	31 (2)
testosterone-undecanoate	29 (3)

Rats were treated once daily for 4 days with different doses and the minimal active dose (MAD) (resulting in 65% ± 10% suppression of LH) is indicated. Data are means of different independent experiments (indicated between brackets).

compounds are rapidly converted by human aromatase as shown by a sharp decrease in androgenic activity and a concomitant rapid increase in estrogenic activity. All 7α-substituted nandrolone derivatives can be converted by human aromatase to their phenolic A-ring derivatives (Table 22.2).

22.4.4 Efficacy in castrated rats: effects on bone, LH/FSH, ventral prostate and muscle

The relative in vivo potency was determined after 4 days of treatment of castrated rats by assessing the suppressing effect on serum LH (Table 22.3). Org X was the

most active compound in this test. On average only a daily dose of 0.32 mg/kg was needed for 65% suppression of serum LH. This means that Org X is about 100 times more potent than testosterone undecanoate, the current oral standard for human use. The nandrolone derivatives were more active than testosterone (3.5–12.5 and 70 mg/kg, respectively). 17α-methyltestosterone is relatively inactive in suppressing serum LH in the rat; approximately 31 mg/kg was required.

The most promising compounds were evaluated in the rat osteoporosis test, in which androgens were given orally for six weeks to male castrated rats. Six weeks following castration a consistent and statistically significant loss in trabecular BMD was observed in the distal femur (Fig. 22.8). With the reference androgen, testosterone undecanoate, daily oral treatment with 80 mg/kg per day is required to maintain trabecular BMD at intact levels (A). With this dose LH (D) was completely suppressed and FSH (E) levels were suppressed to intact levels. At 80 mg/kg per day the ventral prostate weight (B) reached intact levels (90% from the placebo intact group). At a dose of 20 mg/kg anabolic effects were already found, the musculus levator ani (MLA) was maintained at intact levels with this dose (C).

With Org X, with as low a daily dose of 2 mg/kg, trabecular BMD was maintained at intact levels and LH and FSH were suppressed towards intact levels. At this dose a clear prostate-sparing effect was observed, i.e. the ventral prostate weight was only 40% of the placebo-treated intact rats. Anabolic activity, however, was already complete at the lowest tested daily dose of 0.25 mg/kg.

In conclusion, Org X is around 40 times more potent than the reference androgen testosterone undecanoate in the prevention of castration-induced BMD loss and at the same time has a prostate-sparing effect.

22.4.5 Efficacy in intact monkeys: effects on serum testosterone

For the evaluation of efficacy of the most promising orally active androgens the non-seasonal, Old World primate model *Macaca arctoides* was used. The pharmacological efficacy parameter which is most easily tested is suppression of serum testosterone. Three pre-treatment blood samples were collected from each monkey and the average pre-treatment testosterone level was set at 100% for each individual monkey. The normal range of total testosterone in these animals is 15–40 nmol/l. Once-daily oral treatment of intact male monkeys *Macaca arctoides* for seven days with different doses of Org X resulted in a dose-dependent suppression of serum testosterone at day 7 (24 hours after the last oral treatment (Fig. 22.9)). A daily dose of 8 μg/kg yielded 60% suppression of endogenous testosterone. Nine days after the last dose, plasma testosterone levels had returned to the normal range (8 to 40 μg/kg/day) at 200 μg/kg/day probably more time is required for recovery. Org X is 80 times more potent than 17α-methyltestosterone, since ~660 μg/kg is required for similar suppression of plasma testosterone in this model (Ubink *et al.* 2003).

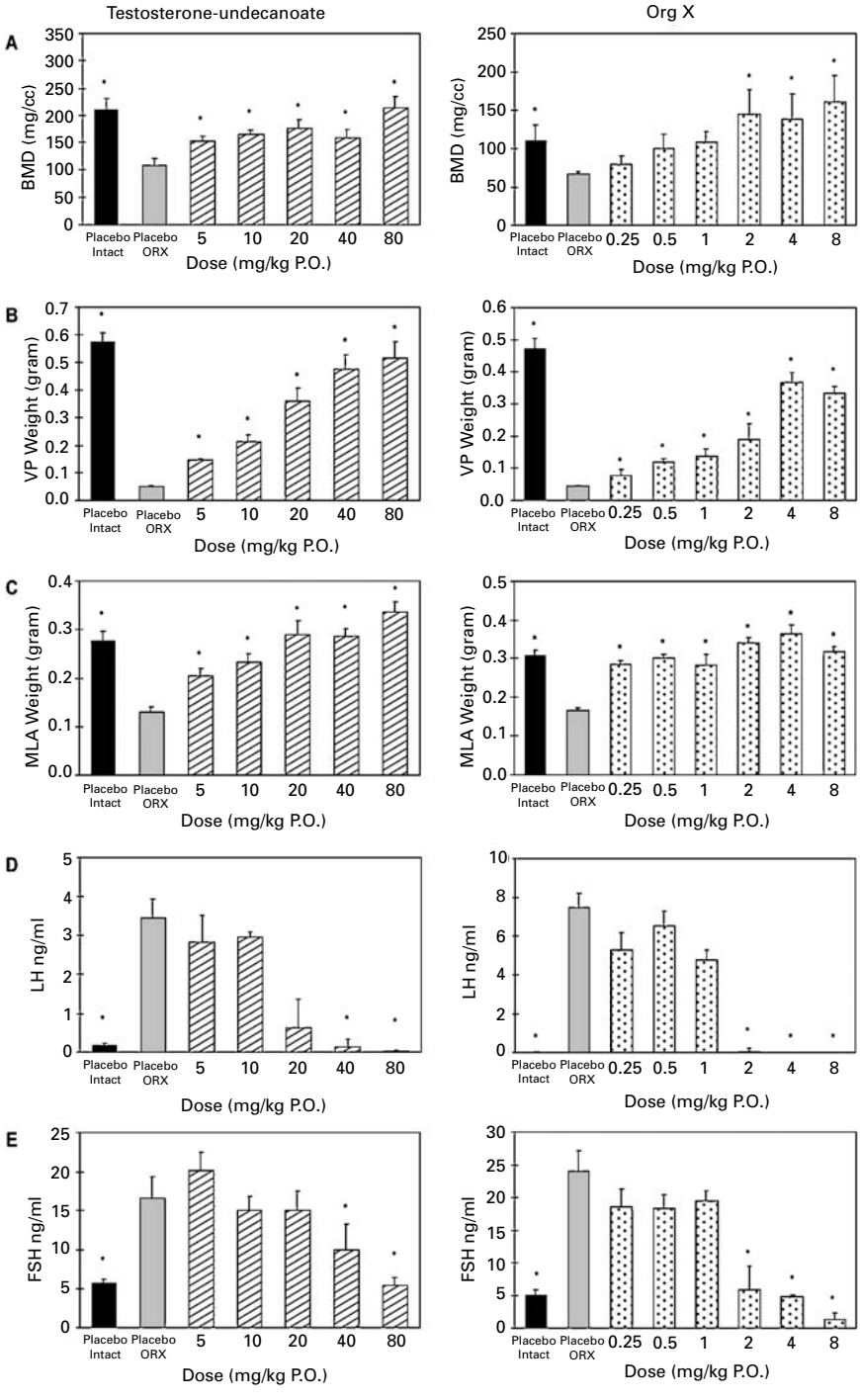


Fig. 22.8 Effect of once-daily (for 6 weeks) oral treatment of castrated male rats with several doses of testosterone undecanoate (TU, left panel) and Org X (right panel) on trabecular bone mineral density (BMD) (**A**), ventral prostate (VP) weight (**B**), musculus levator ani (MLA) weight (**C**), serum LH (**D**) and serum FSH (**E**). Placebo-treated intact and placebo-treated castrated rats are included (Placebo Int and Placebo ORX, respectively). Data represent mean ± SEM (N = 5), * significantly different from Orx placebo ($p < 0.05$).

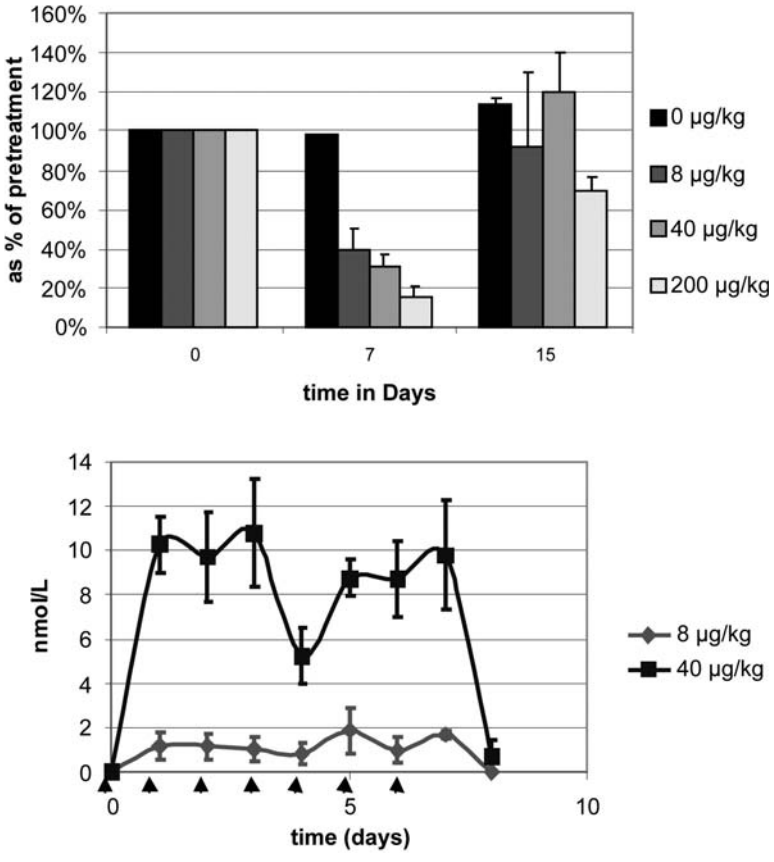


Fig. 22.9 Effect of once-daily (for 7 days) oral treatment of intact *Macaca arctoides* with indicated doses of Org X on plasma testosterone on day 7 (24 hours after last application) and day 15 (day 9 of recovery) (upper panel) and trough (nadir) plasma levels of Org X (lower panel). Data represents mean ± SEM (N = 3). Arrows indicate oral application.

Plasma levels of Org X were determined throughout the experiment. Trough levels (just before a new oral application, 24 hours after application of the compound) were in the range 1.8−10 nmol/l at doses of 8 and 40 µg/kg/day, respectively (Fig. 22.9). Since the relative agonistic activity of Org X is 14-fold higher than that of testosterone (see 22.4.1), at a dose of 8 µg/kg 25 nmol/l (1.8*14) testosterone equivalents are present in the circulation. In addition, 16.2 nmol/l testosterone is still present in this trough sample at day 7 (40% of an average pre-treatment level of 38.1 nmol/l); therefore a total of 41 nmol/l testosterone equivalents is present. This total amount of testosterone equivalents is just in the normal range (15–40 nmol/l) of testosterone. The effect of long-term application of Org X is currently under investigation.

Table 22.4 Overview of single-dose PK data obtained after oral application of indicated steroids to female monkeys (5 mg/kg), rabbit and rat (10 mg/kg)

	Monkey			Rabbit			Rat		
R	$t^1/_2$	nAUCinf	nCmax	$t^1/_2$	nAUCinf	nCmax	$t^1/_2$	nAUCinf	nCmax
7α-H		ND			ND			ND	
7α-methyl	2.0	12.3	2.5	2.13	1.7	0.7	1.45	1.7	0.8
7α-ethyl	1.9	5.3	0.8			0.6	1.2		3.4
7α-vinyl	1.4	3.3	1.0	2.2	0.7	0.4	1.1		6.2
7α-ethynyl		ND			ND			ND	
Org X	16.9	882	39.76	6.9	32.9	2.1	1.0	3.4	1.8
testosterone	1.3	101	23	1.4	12.2	3.5	1.4	1.1	0.3
17α-methyl testosterone	3.9	173	23.1	3.2	165	16.5	1.1	2.1	0.9
testosterone-undecanoate[a]	variable	51	4	2.9	6.7	2.6	1.3	19.5	8.6

[a] PK data of testosterone are given

$T^1/_2$ (in hours), normalized Area Under Curve (to 1 mg/kg) extrapolated to infinity (nAUCinf, h*nmol/l) and normalized maximal concentration (nCmax, nmol/l) are presented.

22.4.6 Pharmacokinetic evaluation in different species

Since at the start of the project the preferred species for the evaluation of single-dose pharmacokinetics (SD-PK) was not known, most of the compounds were evaluated in rat, rabbit, and monkey after oral administration (Table 22.4). In the rat $t^1/_2$ was rather similar for the androgens tested and there was no direct relationship with the observed minimal active dose (MAD) for LH suppression after 4 days treatment of male castrated rats (section 22.4.4. and Table 22.3).

In the rabbit clear differences between compounds were observed; 17α-methyltestosterone resulted in longer $t^1/_2$ and higher nAUCinf than testosterone. With Org X a longer $t^1/_2$ was found compared to the 7α-alkylated nandrolone derivatives.

In the female monkey most pronounced differences between the different compounds were observed. 17α-methyltestosterone resulted in a longer $t^1/_2$ than testosterone (3.9 and 1.33 hours, respectively). With the 7α-methyl/ethyl/vinyl nandrolones relatively low exposures (nAUCinf) compared to testosterone were found. The optimal kinetic profile was observed with Org X, a $t^1/_2$ and nAUCinf of 16.9 hours and 882 nmol*hours were found, respectively.

22.5 Interpretation of results

For androgen replacement therapy and male hormonal contraception there is a need for orally active androgens which can substitute the total spectrum of physiological effects of testosterone, preferably at a lower dose. In the search for new orally active androgens, a combination of in vitro and in vivo assays has been used with the aim to select androgens with higher androgen receptor affinity and less metabolic instability than the reference androgen, testosterone. Additional prerequisites of new androgens are some aromatization towards estrogenic metabolites and the absence of androgen receptor activation upon 5α-reduction (like testosterone) to obtain a prostate-sparing effect.

We exploited the fact that nandrolone and 7α-methyl nandrolone (MENT) have 3- and 10-fold increased androgen receptor binding affinity as compared to testosterone, respectively (Bergink *et al.* 1985; Kumar *et al.* 1999). However, these androgens have not been developed further for oral application due to their limited oral efficacy. The oral efficacy of MENT was expected since both high androgen receptor activity and high metabolic stability have been reported using rat liver microsomes (Agarwal and Monder 1988). Our metabolic stability assay, using intact human hepatocytes, indicates that there is no difference in the metabolism between nandrolone, MENT and the metabolically unstable reference androgen, testosterone. A plausible explanation for this difference is that the cofactor (NAD) for the most important androgen inactivating enzyme, the microsomal enzyme 17β-hydroxysteroid dehydrogenase type 2, is not added to the microsomal preparation (Puranen *et al.* 1999). In addition, the low metabolic stability of MENT is also more in line with the pharmacokinetic data in different species and the relative low oral efficacy of MENT in the LH-suppression test using castrated male rats. Our hypothesis was that 7α-alkyl elongation/unsaturation of nandrolone would improve metabolic stability while maintaining androgen receptor affinity. Indeed, 7α-ethylnandrolone is a metabolically relative stable androgen having a good androgen receptor binding affinity. This androgen resulted in good LH suppression in the rat, 4-fold more potent than MENT. Since the pharmacokinetic data in rat, rabbit and monkey indicated low exposure after oral application, this compound was not evaluated further.

Of all nandrolone derivatives tested, Org X is the most metabolically stable androgen. The excellent in vitro data of Org X (high receptor activity and metabolic stability) was confirmed by several in vivo tests. After a single oral dose of Org X pharmacokinetic evaluation revealed that both in the rabbit and monkey good kinetics were found. In general, the more or less similar pharmacokinetic behaviour of steroids after oral application in the rabbit and monkey differs from that in the rat. Efficacy studies also indicated that Org X is a potent oral androgen. In the LH-suppression

test using male castrated rats, the oral activity of Org X is 0.3 mg/kg, which is 200, 100 and 45-fold better than the oral efficacy of testosterone, testosterone-undecanoate and MENT, respectively. In the rat osteoporosis test, Org X is around 40-fold more active than testosterone in maintaining trabecular BMD at intact levels. A similar increase in efficacy of Org X for LH/FSH suppression was observed. At this BMD/LH/FSH replacement dose of Org X a clear prostate-sparing effect was observed. The high potency of Org X was confirmed in intact male monkeys. A once-daily dose of only 8 μg/kg was needed for 60% suppression of endogenous testosterone. Even at this low dose, trough levels (24 hours after application) of 1.8 nmol/l of Org X were found. The relatively low efficacy of 17α-methyltestosterone in the rat LH suppression test was confirmed by the relative high dose (666 μg/kg) required for testosterone suppression in the male monkey (Ubink *et al.* 2003). On the basis of ventral prostate weight stimulation in rats, a 50-fold higher potency of 17α-methyltestosterone compared with testosterone was expected (Segaloff 1963). These data indicate that LH suppression in rats is far more indicative of the required profile of a new androgen than ventral prostate stimulation.

22.6 Key messages

- 7α-alkylation of nandrolone increases the AR agonistic activity.
- Org X is a novel 7α-alkylated nandrolone derivative.
- 7α-ethylnandrolone and Org X are metabolically stable compounds as determined after incubation with human hepatocytes.
- 7α-substituted nandrolone derivatives and Org X can be converted in vitro by recombinant human aromatase to active estrogens, just like the natural androgen testosterone.
- Org X is around 40 times more potent than testosterone undecanoate in the castrated male rat in maintaining trabecular mineral bone density, LH and FSH suppression and anabolic effects. At the bone-protecting dose, a prostate-sparing effect of Org X was observed.
- With a once daily oral dose for seven days of 8 μg/kg of Org X suppression of endogenous plasma testosterone in intact male monkeys was observed.
- Pharmacokinetic data obtained after one single oral dose revealed no differences in the rat between the different nandrolone derivatives.
- Pharmacokinetic parameters after single oral application of the different nandrolone derivatives and reference compounds revealed that the rabbit is more predictive for the monkey than the rat.
- LH suppression in the rat is more indicative of the required profile of a new androgen than ventral prostate stimulation.

22.7 REFERENCES

Agarwal AK, Monder C (1988) In vitro metabolism of 7 alpha-methyl-19-nortestosterone by rat liver, prostate, and epididymis. Endocrinology 123:2187–2193

Anonymous(1962) 7-methyltestosterone and derivatives (BE 610385), 1–25 16–5–1962 (Patent)

Bergink EW, Janssen PS, Turpijn EW, van der Vies J (1985) Comparison of the receptor binding properties of nandrolone and testosterone under in vitro and in vivo conditions. J Steroid Biochem 22:831–836

Bergink EW, van Meel F, Turpijn EW (1983) Binding of progestagens to receptor proteins in MCF-7 cells. J Steroid Biochem 19:1563–1570

Bursi R, de Gooyer ME, Grootenhuis AJ, Jacobs PL, van der LJ, Leysen D (2001) (Q) SAR study on the metabolic stability of steroidal androgens. J Mol Graph Model 19:552–558

Cummings DE, Kumar N, Bardin CW, Sundaram K, Bremner WJ (1998) Prostate-sparing effects in primates of the potent androgen 7alpha- methyl-19-nortestosterone: a potential alternative to testosterone for androgen replacement and male contraception. J Clin Endocrinol Metab 83:4212–4219

de Gooyer ME, Oppers-Tiemissen HM, Leysen D, Verheul HA, Kloosterboer HJ (2003) Tibolone is not converted by human aromatase to 7alpha-methyl-17alpha-ethynylestradiol (7alpha-MEE): Analyses with sensitive bioassays for estrogens and androgens and with LC-MSMS. Steroids 68:235–243

Ederveen AG, Kloosterboer HJ (2001) Tibolone exerts its protective effect on trabecular bone loss through the estrogen receptor. J Bone Miner Res 16:1651–1657

Herschberger LG, Shipley EV, Meyer RK (1953) Myotrophic activity of 19-Nortestosterone and other steroids determined by modified levator ani muscle method. Proc Soc Exper Biol Med 83:175–180

Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS (1996) Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Mol Endocrinol 10:119–131

Kirk-Otmar (1983) Steroids. In: Encyclopedia of chemical technology Silver and silver alloys to sulfolanes and sulfones (Kirk-Otmar), John Wiley & Sons pp 645–728

Kumar N, Crozat A, Li F, Catterall JF, Bardin CW, Sundaram K (1999) 7alpha-methyl-19-nortestosterone, a synthetic androgen with high potency: structure-activity comparisons with other androgens. J Steroid Biochem Mol Biol 71:213–222

Kumar N, Didolkar AK, Monder C, Bardin CW, Sundaram K (1992) The biological activity of 7 alpha-methyl-19-nortestosterone is not amplified in male reproductive tract as is that of testosterone. Endocrinology 130:3677–3683

Kunzer H, Sauer G, Wiechert R Tetrahedron (2003) (Letter) 32:743

Puranen TJ, Kurkela RM, Lakkakorpi JT, Poutanen MH, Itaranta PV, Melis JP, Ghosh D, Vihko RK, Vihko PT (1999) Characterization of molecular and catalytic properties of intact and truncated human 17beta-hydroxysteroid dehydrogenase type 2 enzymes: intracellular localization of the wild-type enzyme in the endoplasmic reticulum. Endocrinology 140:3334–3341

Schoonen WG, de Ries RJ, Joosten JW, Mathijssen-Mommers GJ, Kloosterboer HJ (1998) Development of a high-throughput in vitro bioassay to assess potencies of progestagenic compounds using Chinese hamster ovary cells stably transfected with the human progesterone receptor and a luciferase reporter system. Analyt Biochem 261:222–224

Segaloff A (1963) The enhanced local androgenic activity of 19-nor steroids and stabilization of their structure by 7alpha- and 17alpha-methyl substituents to highly potent androgens by any route of administration. Steroids 1:299–315

Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, Speed C, Jones M (2002) Aromatase–a brief overview. Annu Rev Physiol 64:93–127

Ubink R, Kop W, Grootenhuis AJ (2003) Suppression of endogenous testosterone with a new orally active androgen in male *Maccaca arctoides*. (in preparation)

van Casteren JI, Schoonen WG, Kloosterboer HJ (2000) Development of time-resolved immunofluorometric assays for rat follicle-stimulating hormone and luteinizing hormone and application on sera of cycling rats. Biol Reprod 62:886–894

van der Louw J, Leysen D, Buma-Bursi H (2003) Orally active androgens (WO 00/59920) (Patent)

Vida JA (1969) Androgens and anabolic agents. Academic Press, New York, London, pp 1–327

Zeelen FJ (1990) Steroid synthesis. In: FJ Zeelen, Medicinal chemistry of steroids, Elsevier, Amsterdam, pp 315–325

23

Hormonal male contraception: the essential role of testosterone

E. Nieschlag, A. Kamischke and H.M. Behre

Contents

23.1 General prospects

23.1.1 Why male contraception at all?

The invention of the "pill" for women was undoubtedly one of the most significant medical and cultural events of the twentieth century. Nature has sweetened procreation with the pleasures of sex to guarantee human reproduction. The pill was the culmination of a millennial-long development of methods to disentangle procreation from sex, and has had a substantial impact on society – e.g. on family planning, morality and demography, not to mention economic and political impact. An equivalent pharmacological male method is not yet available.

Female contraception is very effective. Nevertheless, 50% of the 1,000,000 conceptions occurring every day worldwide remain unplanned, of which 150,000 are terminated by abortion, an intervention that will end fatally for 500 of these women. Although improved distribution and utilization of female contraceptive methods might ameliorate this situation, the contribution of a male contraceptive is well worth considering. Men enjoy the pleasures of sex, but can do little to contribute to the tasks of family planning – a pharmacological male contraceptive is perhaps long overdue. In addition, the risks of contraception would also be more fairly shared between women and men. Representative surveys have shown that a pharmacological male contraceptive would be acceptable to large segments of the population in industrial nations, and would thus contribute to further stabilization of population dynamics. It might also help developing countries whose exponential population growth endangers economic, social, and medical progress. Last but not least, male contraception can be considered an outstanding issue in the political field of gender equality.

23.1.2 Existing methods

For the male there are ways to eliminate both procreation and sex at the same time. Such methods have been used in the past and are still being practiced on a limited scale. Castration has been employed since ancient time to destroy enemies by abolishing their ability to reproduce and transmit their genes. Until the end of the imperial period in China (1912), men were willing to sacrifice their testicles (and often with them their lives) in return for high-ranking positions and political influence at the emperor's court. Meanwhile, in the West, up until almost the same time, some promising boys were forced to give up their manhood for the sake of preserving their prepubertal voice and achieving fame as singers, often without success. Abstinence is a less bloody means of eliminating procreation, but few men are willing to give up both sex and procreation for extended periods of time, let alone their entire lives.

Traditional male methods of contraception such as periodic abstinence or coitus interruptus are associated with a relatively high rate of unwanted pregnancy and also cause a disturbance in sexual activity. Condoms are the oldest barrier method available. However, when using condoms conception rates are relatively high, with 12 out of 100 couples conceiving during the first year of use (Pearl index = 12). Condom use has increased since the beginning of the AIDS epidemic, but more for protection from HIV infection and other sexually transmitted diseases than for contraceptive purposes.

Vasectomy is a safe and surgically relatively simple method for male contraception. The rate of unwanted pregnancies after vasectomy is less than 1%. The drawback to vasectomy is that it is not easily reversible. Achieving fatherhood after vasectomy requires either surgical reversal or sperm extraction from a testicular biopsy and intracytoplasmatic sperm injection into the ovum. Only about 50% of these men will become fathers in the end.

Given the disadvantages of these mechanical male methods, what then are the prerequisites for an ideal male contraceptive? It should

- be applied independently of the sexual act
- be acceptable for both partners
- not interfere with libido, potency, or sexual activity
- have neither short- nor long-term toxic side effects
- have no impact on eventual offspring
- be rapidly effective and fully reversible
- be as effective as comparable female methods

23.1.3 New approaches to male contraception

Despite attempts to improve the existing methods, e.g., vas occlusion instead of surgical dissection, or the introduction of new materials (e.g. polyurethane) for condoms, the inherent disadvantages of these methods preventing sperm transport into the female tract persists, and must be replaced and/or supplemented by pharmacological methods. Posttesticular approaches to male contraception are still in the preclinical phase. By investigating the molecular physiology of sperm maturation, epididymal function and fertilization, the aim is to identify processes that might be blocked by specific pharmacological agents with rapid onset of action. However, all substances investigated so far have shown toxic side effects when interfering effectively with sperm function. At the moment then, only hormonal methods fulfill most of the requirements for a male contraceptive and are currently under clinical development.

All hormonal male contraceptives clinically tested to date are based on testosterone, either on testosterone alone or on a combination of testosterone with other hormones, in particular with either gestagens or GnRH analogues. Because of the

essential role of testosterone, it is appropriate to include an overview on current hormonal approaches to male contraception in this volume.

23.2 Principle of hormonal male contraception

The testes have an endocrine and an exocrine function: the production of androgens and of male gametes. Suppression of gamete production or interference with gamete function without affecting the endocrine function is the goal of endocrine approaches to male fertility regulation. However, since the two functions of the testes are interdependent, it has remained impossible so far to suppress spermatogenesis exclusively and reversibly without significantly affecting androgen synthesis.

FSH and LH/testosterone are responsible for the maintenance of fully normal spermatogenesis (for review see Chapter 5, also Weinbauer and Nieschlag 1996). If only one of the two is eliminated, spermatogenesis will be reduced, but only in quantitative terms, i.e. fewer but normal sperm will be produced and azoospermia will not be achieved. This has been demonstrated in monkeys by the elimination of FSH by immunoneutralization, resulting in reduced sperm numbers but not in complete azoospermia (Srinath *et al.* 1983), which – at least until quite recently – was considered to be required for an effective male method. Therefore, even if new modalities for the selective suppression of FSH or FSH action should become available, it remains doubtful whether they would lead to a method for male contraception (Nieschlag 1986). However, in bonnet monkeys immunization against FSH or the FSH receptor led to an impairment of the fertilizing capacity of sperm (Moudgal *et al.* 1992; 1997a). To date it has remained equivocal whether similar effects can be obtained in humans (Moudgal *et al.* 1997b; 1997c).

Until such results become available, the concept of azoospermia remains valid as a prerequisite for effective hormonal male contraception. However, as it is very difficult to achieve azoospermia uniformly in all volunteers participating in clinical trials for hormonal contraception and the pregnancy rates appear to be acceptably low if sperm counts drop below 1 mill/ml, investigators active in the field reached a consensus that azoospermia or at least oligozoospermia <1 mill/ml sperm should be the goal for an effective hormonal method (Nieschlag 2002). To achieve this goal not only FSH must be suppressed, but also intratesticular testosterone must be drastically reduced. Since testosterone alone can maintain spermatogenesis and much lower testosterone concentrations appear to be necessary for maintenance of spermatogenesis than previously considered, intratesticular testosterone must be depleted to such an extent that peripheral serum concentrations drop into the hypogonadal range. In order to maintain androgenicity, including libido, potency, male sex characteristics, psychotropic effects, protein anabolism, bone structure and hematopoesis, testosterone levels in the general circulation have to be replaced,

while the testes themselves are depleted of testosterone. However, even testes of volunteers achieving azoospermia show measurable testosterone concentrations, although reduced to 2% of normal, and volunteers developing azoospermia have low intratesticular levels similar to those suppressing only to oligozoospermia (McLachlan *et al.* 2002). Therefore, other factors must be of additional importance. Interestingly, the macaque monkey suppressed to azoospermia shows hardly any decrease in intratesticular testosterone and elimination of FSH action appears to be more important than intratesticular testosterone (Narula *et al.* 2001; Weinbauer *et al.* 2001). For some time it was thought that the intratesticular conversion of testosterone to DHT is of importance in the maintenance of spermatogenesis and should be interfered with. However, the application of a 5α-reductase inhibitor did not additionally effect the suppression of spermatogenesis by testosterone alone (McLachlan *et al.* 2000). Recently, the number of CAG repeats in exon 1 of the androgen receptor has been found to determine the suppressibility of spermatogenesis, provided FSH and LH are well suppressed (von Eckardstein *et al.* 2002).

This leads to the general principle of hormonal male contraception, namely the suppression of FSH and LH, resulting in depletion of intratesticular testosterone and cessation of spermatogenesis, while at the same time, peripheral testosterone is substituted with an androgen preparation. This can be achieved by testosterone alone. However, since testosterone alone does not lead to azoospermia or severe oligozoospermia (<1 mill/ml) in all individuals tested, testosterone needs to be combined with other substances suppressing pituitary gonadotropin secretion. As in female hormonal contraceptives, gestagens as pituitary-suppressing agents are being tested in men in combination with androgens. GnRH agonists, as well as antagonists are also being explored as further possible combinations with androgens.

Recommendations for Regulatory Approval for Hormonal Male Contraception

(Int J Androl 25:375 (2002)

The investigators at the 6th Summit Meeting on Hormonal Male Contraception, Petersberg, Germany, held on July 7–9th, 2002 recognized the need for standardized clinical trials to develop a hormonal male method and drafted the following recommendations: The goal of hormonal male contraception is the reversible suppression of spermatogenesis to a level compatible with infertility. In principle this can be achieved by using an androgen alone or an androgen in combination with a gestagen or a GnRH-antagonist. The success of this principle in terms of lowering sperm counts in semen to azoospermia or to very low counts has been demonstrated in a multitude of trials. Some trials demonstrated the contraceptive efficacy of this approach when couples used no other method of contraception. Investigators agree that information gained from preliminary studies on male contraception have reached a stage that hormonal contraceptive products for men should now be proposed for development for general use.

In order to bring a hormonal method to the market, larger scale clinical trials are required. As no pharmacological method for male contraception is currently available, this represents a novel effort requiring new recommendations for testing.

The investigators agreed that the following criteria should be fulfilled:

1. **In phase II dose-finding studies, the suppression of spermatogenesis can be used as the main parameter.**
   As the surrogate parameter, sperm concentrations, measured according to WHO criteria, can be used and the goal should be $\leq$ 1 million/mL.
   After cessation of treatment, the return to normal values should be ascertained, i.e. $\geq$ 20 million/mL.
2. **In the efficacy trials, pregnancy rate will be the endpoint, using the efficacy rate of condoms as a reference. For contraceptive efficacy, two independent phase III trials for 1 year should be completed by 200 men/couples per trial. Alternatively, the number of subjects that can establish a significant improvement against condom use could be investigated.**
3. **For safety assurance for a new chemical entity, trials are required involving at least 300–600 men for 6 months at the intended combination and dose, 100 men exposed for 1 year and a total of 1500 men in phase I– III studies at the minimum.**
4. **Long-term safety will be monitored by post-marketing surveillance.**

The necessary laboratory investigations, especially semen analysis, need to be made under strict quality control.

These recommendations were drafted and approved by:

Prof. Dr. Eberhard Nieschlag (Organizer of the Summit Meeting) (University of Muenster, Germany), Dr. Richard A. Anderson (University of Edinburgh, Scotland), Dr. Dan Apter (Family Federation of Finland, Helsinki, Finland), Dr. Kiagus M. Arsyad (University of Sriwijaya, Palembang, Indonesia), Prof. Dr. David Baird (University of Edinburgh, Scotland), Prof. Dr. Hermann M. Behre (University of Halle, Germany), Prof. Dr. William J. Bremner (University of Washington, Seattle, WA, USA), Doug Colvard (CONRAD, Arlington, VA, USA), Dr. T. G. Cooper (University of Muenster, Germany), Dr. Gu Yi-Qun (National Research Institute for Family Planning, Beijing, China), Prof. Dr. Mike Harper (CONRAD, Arlington, VA, USA), Prof. Dr. Ilpo Huhtaniemi (University of Turku, Finland), Dr. Axel Kamischke (University of Muenster, Germany), Dr. Peter Liu (University of Sydney, Australia), Dr. Robert McLachlan (Monash University, Melbourne, Australia), Dr. M. Cristina Meriggiola (University of Bologna, Italy), Prof. Dr. Dr. Nukman Moeloek (University of Indonesia, Jakarta, Indonesia), Prof. Dr. Somnath Roy (National Institute of Health and Family Welfare, New Delhi, India), Dr. Régine Sitruk-Ware (Population Council, New York, NY, USA), Dr. Kalyan Sundaram (Population Council, New York, NY, USA), Prof. Dr. Ronald S. Swerdloff (University of California, Torrance, CA, USA), Prof. Dr. Geoffrey M. H. Waites (St. Jean de Gonville, France), Prof. Dr. Christina C. L. Wang (University of California, Torrance, CA, USA), Dr. Xing-Hai Wang (Jiangsu Family Planning Research Institute, Nanjing, China), Prof. Dr. Frederick C. W. Wu (University of Manchester, UK), Dr. Michael Zitzmann (University of Muenster, Germany).

Present at the Summit Meeting were also representatives of Schering/Jenapharm (Dr. Ulrich Gottwald, Dr. Doris Huebler, Dr. Albert Radlmaier, Dr. Farid Saad, Dr. Rolf Schuermann) and Organon (Dr. Thom

Dieben, Dr. AJ Grootenhuis, Dr. Wendy Kersemaekers, Dr. Mirjam L. P. J. Mol-Arts, Dr. Gerrit Voortman), Dr. Robert Spirtas (National Institutes of Health, Bethesda, MD, USA), Dr. Judy Manning (USAID, Washington, DC, USA), and WHO-HRP Dr. Michael T. Mbizvo (WHO, Geneva, Switzerland), and Dr. Kirsten Vogelsong (WHO, Geneva, Switzerland).

23.3 Testosterone alone

23.3.1 Testosterone enanthate

According to the principle outlined above, testosterone should be the first choice for hormonal male contraception since it not only suppresses pituitary LH and FSH secretion, but also replaces testosterone. Indeed, since the 1970s various investigations have been undertaken to suppress spermatogenesis with testosterone alone (Reddy and Rao 1972; Patanelli 1978) (Table 23.1). Not until 1990 was an initial study testing this form of male contraception published by the WHO, the first study ever performed on the efficacy of hormonal male contraception (WHO 1990). Volunteers in ten centers on four continents participated and received 200 mg testosterone enanthate intramuscularly per week. Those volunteers developing azoospermia within the first six months continued to receive injections for a further year. In this period (efficacy phase) couples refrained from using any further contraceptive methods. A total of 137 men reached the efficacy phase. During this period only one pregnancy occurred. This high rate of efficacy is well comparable to that of established female methods. This was a very encouraging result. However, only about two-thirds of all participants developed azoospermia. The other volunteers showed strong suppression of spermatogenesis, as evidenced by oligozoospermia (Waites 2003).

In order to answer the question of whether men developing oligozoospermia can be considered infertile, a second worldwide multicenter study followed (WHO 1996). In this study azoospermia again proved to be a most effective prerequisite for contraception. If sperm concentrations, however, failed to drop below 3×10^6/ml, resulting pregnancy rates were higher than when using condoms. When sperm concentrations decreased below 3×10^6/ml, which was the case in 98% of the participants, protection was not as effective as for azoospermic men, but was better than that offered by condoms.

Even if these WHO studies represented a breakthrough by confirming a principle of action (Waites 2003), they did not offer a practicable method. For a method requiring weekly intramuscular injections is not acceptable for broad use. Moreover, several months (on average four) were required before sperm production reached significant suppression. For this reason current research is concentrating

on the development of long-acting testosterone preparations and on substances to improve overall effectiveness.

The WHO multicenter studies revealed an interesting phenomenon: the rate of azoospermia was greater in East Asian than in Caucasian men (WHO 1990; 1996). This finding was also confirmed by independent studies using testosterone enanthate injections in men in Indonesia (Arysad 1993), Thailand (Aribarg *et al.* 1996) and China (Cao *et al.* 1996).

23.3.2 Testosterone buciclate

Under the auspices of WHO a synthesis program identified testosterone buciclate as a testosterone ester with long-lasting effectiveness. First tested in monkeys and then in hypogonadal patients, it showed a long effective phase of 3–4 months after a single injection (see Chapter 11). A single injection of 1200 mg in a contraceptive study resulted in suppression of spermatogenesis comparable to that of weekly enanthate injections (Behre *et al.* 1995). Unfortunately, WHO and the NIH, which jointly hold the patent on testosterone buciclate, were unable to find an industrial partner to further develop this promising ester for general use, so that its potential for male contraception has never been fully explored.

23.3.3 Testosterone undecanoate

If testosterone is suited for contraception, the orally effective testosterone undecanoate should provide the male contraceptive "pill". This possibility was tested in the early phase of development. However, even when high doses of 3×80 mg were taken daily for 12 weeks, only one of seven volunteers developed azoospermia (Nieschlag *et al.* 1978). Although this result was disappointing, the study demonstrated that stable levels of testosterone in serum are important to suppress pituitary gonadotropins.

While testosterone undecanoate was used as an oral preparation solely in the West, in China it has been marketed as an intramuscular preparation for use in hypogonadism (see Chapter 14) and has more recently also been tested for male contraception. In a large multicenter efficacy study – the first completed since the WHO studies – involving 308 Chinese men given monthly injections of 500 mg testosterone undecanoate after a loading dose of 1,000 mg, only 3% of the subjects did not suppress to azoospermia or severe oligozoospermia, and the remaining 97% induced no pregnancy (Gu *et al.* 2003). This highly successful trial encouraged the Chinese investigators to undertake a phase III trial involving 1,000 couples for an efficacy phase of two years which is currently underway (Handelsman 2003). If successful, testosterone undecanoate may become the first registered hormonal male contraceptive – in China.

In Caucasian men intramuscular testosterone undecanoate has not only been tested successfully for the treatment of male hypogonadism (von Eckardstein and Nieschlag 2002), but also for male contraception. Applying an improved galenic preparation of testosterone undecanoate (using castor oil instead of Chinese tea seed oil as vehicle) injection intervals could be spaced to six weeks, but, as with testosterone enanthate in weekly injections, only 2/3 of the volunteers achieved azoospermia (Kamischke *et al.* 2000b). Extrapolating from the kinetic profiles it appears that the injection intervals might be further extended, but the rate of sperm suppression remains in the range of other testosterone preparations so that other substances need to be added to achieve higher success in Caucasians.

23.3.4 Testosterone pellets

Pellets consisting of pure testosterone are used for substitution in hypogonadism in some countries (see Chapter 14). In male contraceptive studies a one-time application showed efficacy comparable to weekly testosterone enanthate injections (Handelsman *et al.* 1992). The disadvantage of minor surgery required for insertion under the abdominal skin is compensated by their low price and long duration of action. In further studies testosterone pellets have only been used in combination with other substances (see below).

23.3.5 19-Nortestosterone

When searching for preparations with longer-lasting effectiveness, 19-nortestosterone-hexoxyphenylpropionate was tested whose spectrum of effects is very similar to that of testosterone, and which has been used as an anabolic since the 1960s (molecular structure in Fig. 23.1). This 19-nortestosterone ester injected every three weeks enabled azoospermia to be reached by as many men as by testosterone enanthate. Thus the 19-nortestosterone ester is as effective as testosterone enanthate but allows a longer injection interval (Behre *et al.* 2001; Knuth *et al.* 1985; Schürmeyer *et al.* 1984). However, 19-nortestosterone is not fully equivalent to testosterone as it is converted to estrogens to a lesser degree than testosterone. Although no side effects were detected in the trials using 19-nortestosterone, long-term untoward effects, e.g. on bones, cannot be excluded. In the light of newer long-acting testosterone preparations, 19-nortestosterone appears less attractive for contraception.

23.3.6 7α-Methyl-19-nortestosterone (MENT)

Another synthetic androgen with possible application in hypogonadism (see Chapter 13) and in male contraception is 7α-methyl-19-nortestosterone (MENT). It has been tested in three doses of subdermal silastic implants in a multicenter study by the Population Council. In a dose-dependent fashion azoospermia can be

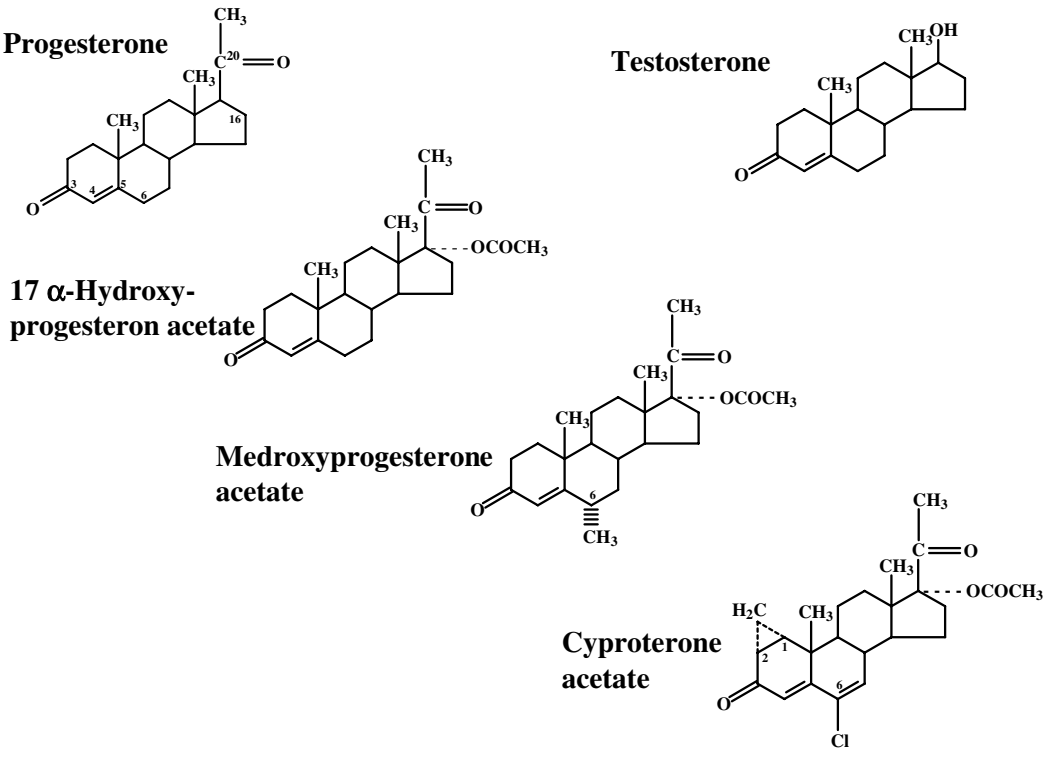


Fig. 23.1 Progestins derived from 17-hydroxyprogesterone tested in hormonal male contraception.

achieved at the same rate as other testosterone preparations alone, i.e. in about 2/3 of the tested subjects, the advantage being that the effect of one set of implants may last for as long as one year (von Eckardstein *et al.* 2003).

23.4 Testosterone combined with progestins

An overview of all studies using testosterone in combination with a progestin performed to date is given in Table 23.1.

23.4.1 Testosterone or 19-nortestosterone plus DMPA

19-Norethisterone (norethindrone), medroxyprogesterone acetate (MPA), depot-MPA (DMPA), 17-hydroxyprogesterone capronate and megestrol acetate have been used in clinical trials initiated by the WHO (1972–1983) and the Population Council (Schearer *et al.* 1978) (molecular structures in Figs. 23.1 and 23.2). The most favorable combination was the monthly intramuscular injection of 200 mg DMPA plus 200 mg testosterone enanthate or testosterone cypionate; this combination gave the best results in suppressing spermatogenesis and the incidence of untoward side

Table 23.1 Overview of studies on hormonal male contraception using testosterone either alone or in combination with progestins

Reference	Number of subjects	Ethnic origin	Androgen dose	Progestin dose	Azoospermia (n)	Severe oligozoospermia below 1 mill/ml (n)	Oligozoo-spermia below 3 mill/ml (n)
Testosterone alone							
WHO 1990	271	mixed	TE 200 mg. i.m. / week	None	157	??	??
Handelsman *et al.* 1992	9	unknown	T-Pellets 1200 mg	None	5	4	0
Handelsman *et al.* 1996	10	unknown	T-Pellets 400 mg	None	0	0	0
Handelsman *et al.* 1996	10	unknown	T-Pellets 800 mg	None	4	0	0
Meriggiola *et al.* 1996	5	Caucasian	TE 100 mg. i.m. / week	None	5	0	0
Bebb *et al.* 1996	18	Caucasian	TE 100 mg. i.m. / week	None	6	4	1
WHO 1996	225	mixed	TE 200 mg. i.m. / week	None	157	29	8
Zhang *et al.* 1999	12	Chinese	TU 500 mg i.m./ 4 weeks	None	11	1	0
Zhang *et al.* 1999	12	Chinese	TU 1000 mg i.m./ 4 weeks	None	12	0	0
Kamischke *et al.* 2000b	14	Caucasian	TU 1000 mg i.m. / 6 weeks	None	7	4	1
McLachlan *et al.* 2002	5	unknown	TE 200 mg. i.m. / week	None	4= < 0.1 or azoospermia	4	0
Gu *et al.* 2003	305	Chinese	TU 500 mg i.m./ 4 weeks	None	284	6	6
Depot medroyprogesterone acetate							
Alvarez-Sanchez *et al.* 1977	8	Dominican Republic	TE 250 mg. i.m. / week	DMPA 150 mg/4 weeks	4	3	1
Alvarez-Sanchez *et al.* 1977	10	Dominican Republic	TE 250 mg. i.m. / week	DMPA 300 mg/4 weeks	7	2	0
Brenner *et al.* 1977	6	Caucasian	TE 200 mg. i.m. / week	DMPA 100 mg / 4 weeks	1	2	1
Brenner *et al.* 1977	3	Caucasian	TE 200 mg. i.m. / week	DMPA 150 mg / 4 weeks	1	0	0
Frick *et al.* 1977	12	Caucasian	TE 250 mg. i.m. / week	DMPA 100 mg i.m. / 4 weeks	6	4	0
Frick *et al.* 1977	6	Caucasian	T-Propionate 4 rods	DMPA 100 mg i.m./4 weeks	2	0	0
Melo and Coutinho 1977	11	Brasilian	TE 200 mg. i.m. / week	DMPA 100–150 mg i.m/ 4 weeks	11= < 0.1 or azoospermia	0	?

(*cont.*)

Table 23.1 (*cont.*)

Reference	Number of subjects	Ethnic origin	Androgen dose	Progestin dose	Azoospermia (n)	Severe oligozoospermia below 1 mill/ml (n)	Oligozoo-spermia below 3 mill/ml (n)
Faundes *et al.* 1981	10	Dominican Republic	TE 500 mg i.m. / week	DMPA 150 mg/4 weeks	8	1	0
Frick *et al.* 1982	4	Caucasian	TE 500 mg/4 weeks	150 mg/4 weeks	4	0	0
Frick *et al.* 1982	5	Caucasian	TE 250 mg/2 weeks	75 mg/2 weeks	5	0	0
WHO 1993	45	Indonesian	19-Nortestosterone 200 mg i.m. / 3 weeks	DMPA 250 mg i.m./ 6 weeks	44	1	0
WHO 1993	45	Indonesian	TE 200 mg i.m. / 3 weeks	DMPA 250 mg i.m./ 6 weeks	43	2	0
Knuth *et al.* 1989	12	Caucasian	19-Nortestosterone 200 mg i.m. / 3 weeks	DMPA 250 mg i.m./ 6 weeks	8	3	1
Wu and Aitken 1989	10	Caucasian	TE 250 mg i.m. / week	DMPA 200 mg/4 weeks	6	0	4
Pangkahila 1991	10	Indonesian	TE 100 mg i.m. / week	DMPA 100 mg / 4 weeks	10	0	0
Pangkahila 1991	10	Indonesian	TE 250 mg i.m. / week	DMPA 200 mg/4 weeks	10	0	0
Handelsman *et al.* 1996	10	unknown	T-Pellets 800 mg	DMPA once 300 mg i.m.	9	0	1
McLachlan *et al.* 2002	5	unknown	TE 200 mg i.m. / week	DMPA once 300 mg i.m.	5 = < 0,1 or azoospermia	5	0
Turner *et al.* 2003	53	unknown	T-Pellets 800 mg/ 16 weeks	DMPA 300 mg i.m. /12 weeks	49	2	0
Levonorgestrel							
Fogh *et al.* 1980	5	Caucasian	TE 200 mg / 4 weeks	LNG 250 µg p.o. / day	1	?	1
Fogh *et al.* 1980	5	Caucasian	TE 200 mg i.m. / 4 weeks	LNG 500 µg p.o. / day	2	?	?
Bebb *et al.* 1996	18	Caucasian	TE 100 mg i.m. / week	LNG 500 µg p.o. / day	12	2	3
Anawalt *et al.* 1999	18	Caucasian	TE 100 mg i.m. / week	LNG 125 µg p.o. / day	11	5	1
Anawalt *et al.* 1999	18	Caucasian	TE 100 mg i.m. / week	LNG 250 µg p.o. / day	14	2	0
Ersheng *et al.* 1999	16	Chinese	TU 250 mg i.m./ 4 weeks	Sino-Implant 2 rods	6	0	1
Kamischke *et al.* 2000b	14	Caucasian	TU 1000 mg i.m. / 6 weeks	LNG 250 µg p.o. / day	8	4	2
Gaw Gonzalo *et al.* 2002	20	Mixed	Testoderm TTS 2 patches / day	Norplant II 4 rods	7	5	2

Gaw Gonzalo *et al.* 2002	15	Mixed	Testoderm TTS 2 patches / day	LNG 125 μg p.o. / day	5	1	1
Gaw Gonzalo *et al.* 2002	14	Mixed	TE 100 mg i.m. / week	Norplant II 4 rods	13	1	0
Pöllänen *et al.* 2001	5	Caucasian	DHT-Gel 250 mg / day	LNG 30 μg p.o./day	0	0	1
Pöllänen *et al.* 2001	5	Caucasian	DHT-Gel 250 mg / day	Jardelle (LNG) 1 rod	0	0	0
Pöllänen *et al.* 2001	8	Caucasian	DHT-Gel 500 mg / day	Jardelle (LNG) 2 rods	0	0	0
Pöllänen *et al.* 2001	7	Caucasian	DHT-Gel 250 mg / day	Jardelle (LNG) 4 rods	0	0	0
Norethisterone							
Kamischke *et al.* 2001	14	Caucasian	TU 1000 mg i.m. / 6 weeks	NETE 200 mg / 6 weeks	13	0	0
Kamischke *et al.* 2002	14	Caucasian	TU 1000 mg i.m. / 6 weeks	NETE 200 mg / 6 weeks	13	1	0
Kamischke *et al.* 2002	14	Caucasian	TU 1000 mg i.m. / 6 weeks	NETE 400 mg / 6 weeks	13	1	0
Kamischke *et al.* 2002	14	Caucasian	TU 1000 mg i.m. / 6 weeks	NETA 10 mg p.o. / day	12	2	0
Cyproterone acetate							
Meriggiola *et al.* 1996	5	Caucasian	TE 100 mg i.m. / week	CPA 50 mg p.o/day	3	0	1
Meriggiola *et al.* 1996	5	Caucasian	TE 100 mg i.m. / week	CPA 100 mg p.o/day	5	0	0
Meriggiola *et al.* 1998	5	Caucasian	TE 100 mg i.m. / week	CPA 12.5 mg p.o/day	3	2	0
Meriggiola *et al.* 1998	5	Caucasian	TE 100 mg i.m. / week	CPA 25 mg p.o/day	5	0	0
Meriggiola *et al.* 2002b	9	Caucasian	TE 100 mg i.m. / week	CPA 5 mg p.o/day	6	3	0
Meriggiola *et al.* 2002b	7	Caucasian	TE 200 mg i.m. / week	CPA 5 mg p.o/day	0	4	2
Desogestrel or etonorgestrel							
Wu *et al.* 1999	8	Caucasian	TE 50 mg i.m. / week	DSG 300 μg p.o. / day	8	0	0
Wu *et al.* 1999	7	Caucasian	TE 100 mg i.m. / week	DSG 150 μg p.o. / day	4	3	0
Wu *et al.* 1999	8	Caucasian	TE 100 mg i.m. / week	DSG 300 μg p.o. / day	6	0	1
Anawalt *et al.* 2000	7	Caucasian	TE 50 mg i.m. / week	DSG 150 μg p.o. / day	4	1	0
Anawalt *et al.* 2000	8	Caucasian	TE 100 mg i.m. / week	DSG 150 μg p.o. / day	8	0	0
Anawalt *et al.* 2000	8	Caucasian	TE 100 mg i.m. / week	DSG 300 μg p.o. / day	7	1	0
Kinniburgh *et al.* 2001	8	Caucasian	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	6	2	0
Kinniburgh *et al.* 2001	7	Caucasian	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	5	1	0
Anderson *et al.* 2002b	9	Black	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	9	0	0

(*cont.*)

Table 23.1 (*cont.*)

Reference	Number of subjects	Ethnic origin	Androgen dose	Progestin dose	Azoospermia (n)	Severe oligozoospermia below 1 mill/ml (n)	Oligozoo-spermia below 3 mill/ml (n)
Anderson *et al.* 2002b	11	Mixed	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	9	0	1
Anderson *et al.* 2002b	8	Black	T-Pellets 400 mg / 12 weeks	DSG 300 μg p.o. / day	8	0	0
Anderson *et al.* 2002b	12	Mixed	T-Pellets 400 mg / 12 weeks	DSG 300 μg p.o. / day	8	0	0
Anderson *et al.* 2002a	14	Caucasian	T-Pellets 400 mg / 12 weeks	Implanon (ENG) 1 rod	9	1	3
Anderson *et al.* 2002a	14	Caucasian	T-Pellets 400 mg / 12 weeks	Implanon (ENG) 2 rods	9	4	0
Kinniburgh *et al.* 2002b	15	Caucasian	T-Pellets 400 mg / 12 weeks	DSG 300 μg p.o. / day	15	0	0
Kinniburgh *et al.* 2002b	18	Asian	T-Pellets 400 mg / 12 weeks	DSG 300 μg p.o/day	18	0	0
Kinniburgh *et al.* 2002b	18	Asian	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	11	2	2
Kinniburgh *et al.* 2002b	13	Caucasian	T-Pellets 400 mg / 12 weeks	DSG 150 μg p.o. / day	11	2	0
Self-applicable							
Nieschlag *et al.* 1978	7	Caucasian	Andriol 240 mg p.o. / day	None	1	0	0
Guerin and Rollet. 1988	13	Caucasian	Andriol 160 mg p.o. / day	NETA 10 mg p.o./day	7	2	3
Guerin and Rollet. 1988	5	Caucasian	T gel 250 mg / day	NETA 5 mg p.o. / day	4	1	0
Guerin and Rollet. 1988	5	Caucasian	T gel 250 mg / day	NETA 10 mg p.o. / day	5	0	0
Guerin and Rollet. 1988	8	Caucasian	T gel 250 mg / day	MPA 20 mg p.o. / day	5	0	1
Meriggiola *et al.* 1997	8	Caucasian	Andriol 80 mg p.o. /day	CPA 12.5 mg p.o./ day	1	3	2
Hair *et al.* 1999	4	Caucasian	Andropatch 2 patches / day	DSG 75 μg p.o / day	0	1	0
Hair *et al.* 1999	6	Caucasian	Andropatch 2 patches / day	DSG 150 μg p.o,/ day	3	0	0
Hair *et al.* 1999	7	Caucasian	Andropatch 2 patches / day	DSG 300 μg p.o. / day	4	1	0
Büchter *et al.* 2000	12	Caucasian	Testoderm TTS 2 patches / day	LNG 250 μg p.o. later 500 μg	2	3	0
Gaw Gonzalo *et al.* 2002	19	Mixed	Testoderm TTS 2 patches / day	None	5	0	1
Pöllänen *et al.* 2002	2	Caucasian	DHT-Gel 250 mg / day	None	0	0	0

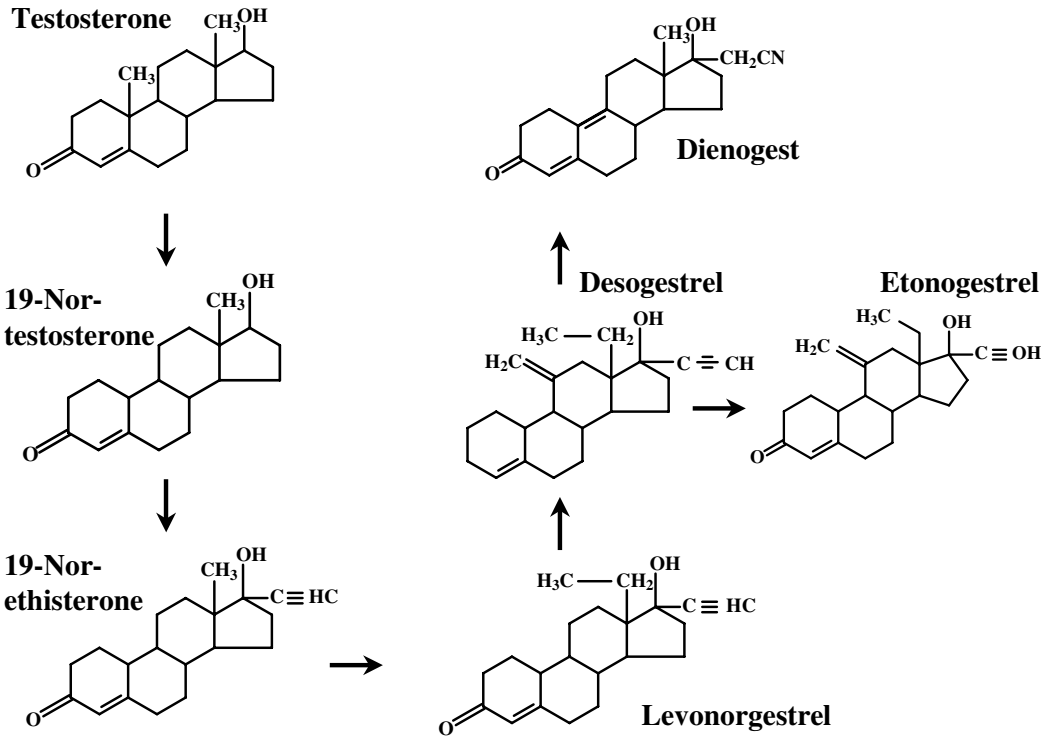


Fig. 23.2 Progestins derived from 19-nortestosterone tested in hormonal male contraception.

effects was low. However, this combination did not produce azoospermia uniformly and its possible efficacy remained uncertain.

Since monotherapy with the long-acting androgen ester 19-nortestosterone-hexoxyphenylproprionate injected every three weeks resulted in effective suppression of spermatogenesis to azoospermia in about 70% of the volunteers (Schürmeyer *et al.* 1984) the possibility of even more complete suppression of spermatogenesis was tested (Knuth *et al.* 1989). Twelve volunteers were injected weekly with 200 mg 19-nortestosterone hexoxyphenylpropionate, followed by injections with the same dose every three weeks up to week 15. In addition, the volunteers were injected with 250 mg DMPA in weeks 0, 6 and 9. Azoospermia was achieved in eight of twelve volunteers during the study course, while in three of the remaining four volunteers, spermatogenesis was suppressed to single sperm, and, in one volunteer, to a sperm concentration of 1.4 mill/ml.

The promising results prompted the WHO Task Force on the Regulation of Male Fertility to launch a large-scale multicenter trial in five centers in Indonesia, comparing the effectiveness of testosterone enanthate, or 19-nortestosterone hexyoxyphenylpropionate, in combination with DMPA (WHO 1993). Surprisingly,

43/45 and 44/45 subjects in the testosterone and the 19-nortestosterone groups respectively suppressed to azoospermia. Unfortunately, this study had failed to include groups treated with the androgens alone, so that it remained unclear whether the azoospermia rates of 97% and 98% were due to the combined treatment or could also be achieved by the androgens alone.

The latter possibility appears likely in the light of the ethnic differences between Caucasian and East Asian men described above. Although ultimately effective, the disadvantage remains that it took almost 20 weeks to reach azoospermia or the lowest sperm counts in these volunteers. Thus, more rapid onset of sperm suppression is required.

A recent study using either 200 mg testosterone enanthate given alone in weekly intramuscular injections or in combination with an injection of 300 mg DMPA showed that the suppression rate was not greater when DMPA was added (McLachlan *et al.* 2002). However, when subcutaneous testosterone implants of 200 mg were applied every 4 or 6 months in combination with 300 mg DMPA given every three months, 51/53 men achieved azoospermia or suppression below 1×10^6 sperm/ml. During a twelve-month efficacy phase with otherwise unprotected intercourse no pregnancy occurred (35.5 person years) (Turner *et al.* 2003). The differences between the studies highlight the fact that obviously the kinetics of testosterone are very important, since the implants produce very stable serum levels and the testosterone enanthate injections cause high peaks and troughs. Although the combination of an implant with an injection every three months may not be ideal, this study is the first to demonstrate the contraceptive efficacy of a testosterone + progestin combination.

Recovery to baseline semen parameters appears to be rather slow in studies employing DMPA. This may be due to secondary depots of this progestin formed in the subcutaneous and abdominal fat and requires special attention should studies be extended over several years.

23.4.2 Testosterone plus levonorgestrel

Levonorgestrel has been widely used for contraception in females either orally or as an implant and has proved safe and effective. Although early studies combining 0.5 mg levonorgestrel given orally with testosterone enanthate were not very encouraging (Fogh *et al.* 1980), more recent trials comparing testosterone enanthate (100 mg/week) alone with testosterone enanthate in combination with 0.5 mg levonorgestrel given orally showed that the combination resulted in more pronounced suppression of spermatogenesis than testosterone enanthate alone (Bebb *et al.* 1996).

Encouraged by the renewed interest in levonorgestrel we conducted a trial combining oral levonorgestrel with a transdermal testosterone patch applied to the trunk

(Büchter *et al.* 1999). The advantage of such a combination is that it is completely self-administered and thus independent of medical personnel. Unfortunately the results were disappointing, as suppression of spermatogenesis was insufficient. We presume that the testosterone dose absorbed from the transdermal systems was too low and often impeded by inadequate adhesiveness to the skin of the systems (Büchter *et al.* 1999). The study again emphasizes the need for steady serum testosterone levels to suppress gonadotropins, even when co-administered with a potent gestagen.

Similarly it was shown that the combination of 0.5 mg levonorgestrel given orally with transdermal DHT was quite ineffective, nor did the combination of transdermal DHT with levonorgestrel implants lead to sufficient suppression of spermatogenesis (Pöllänen *et al.* 2001).

Finally, when the long-acting testosterone preparation testosterone undecanoate (in castor oil) given at six weeks intervals was combined with oral levonorgestrel, the progestin did not enhance the effect of testosterone undecanoate alone (Kamischke *et al.* 2000). However, when levonorgestrel was administered in 4 capsules delivering about 160 μg levonorgestrel (Norplant II = Jadelle)/per day together with weekly injections of 100 mg testosterone enanthate, 93% of the subjects achieved azoospermia and all suppressed to oligozoospermia below 1×10^6/ml sperm (Gaw Gonzalo *et al.* 2002). As effective as this combination may be, it brings us back to weekly testosterone injections, making the approach impractical for general use. The combination of levonorgestrel implants with long-acting testosterone preparations (ideally also implants) might be a solution and requires investigation.

23.4.3 Testosterone plus cyproterone acetate

Animal studies and studies in sexual delinquents have shown that the antiandrogen cyproterone acetate, which can be considered a potent progestin, suppresses spermatogenesis, an effect exerted through suppression of pituitary gonadotropin secretion. In clinical trials using 5 to 20 mg cyproterone acetate per day for up to 16 weeks, sperm counts and motility were reduced markedly (Fogh *et al.* 1979; Moltz *et al.* 1980; Wang and Yeung 1980). Thus, cyproterone acetate appeared to be a possibility for male fertility control. However, decreases in serum testosterone levels to below normal were also observed. Some of the volunteers complained of fatigue, lassitude and decrease in libido and potency attributable to the diminished testosterone levels.

When later cyproterone acetate was combined with testosterone enanthate injections at even higher doses of 50 and 100 mg, it effectively suppressed spermatogenesis (Meriggiola *et al.* 1996), but even when lower doses of cyproterone acetate were administered, antiandrogenic effects prevailed and the volunteers showed decreased red blood, preventing this antiandrogenic gestagen from being an attractive

combination for male contraception (Meriggiola *et al.* 1998). Although the attempt to create a male pill by co-administration of oral testosterone undecanoate with oral cyproterone acetate led to suppression of spermatogenesis, it had to be discontinued because of a decrease in hemoglobin and hematocrit caused by the antiandrogen (Meriggiola *et al.* 1997).

23.4.4 Testosterone plus 19-norethisterone

19-noresthisterone, one of the earliest progestins derived from testosterone (Djerassi *et al.* 1954), is characterized by some undesirable androgenicity when given to women, but might be of advantage when administered to men. An early study with only few volunteers using a combination of orally effective 19-norethisterone acetate with either a transdermal testosterone gel or oral testosterone undecanoate led to azoospermia in all volunteers (Guerin and Rollet 1988). Considering its properties and these promising results, it was surprising that it took another ten years to investigate the use of 19-norethisterone more systematically.

In a pharmacokinetic study single injections of 200 mg 19-norethisterone enanthate led to a marked suppression of the gonadotropins (FSH for 29 days), testosterone, SHBG and sperm (Kamischke *et al.* 2000a). When testosterone undecanoate became available in the form of intramuscular depot injections it was combined with noresthisterone enanthate and volunteers achieved azoospermia or severe oligozoospermia in all but one. The additive effect to testosterone undecanoate alone was striking. An injected dose of 200 mg 19-norethisterone enanthate every 6 weeks was as effective as 400 mg, so that 200 mg appears to be a useful dose. Although 19-noresthisterone acetate 10 mg given orally daily in combination with intramuscular testosterone undecanoate is as effective as injected noresthisterone enanthate, the combination of the two steroids in one injection appears quite attractive (Kamischke *et al.* 2000b; 2001; 2002b). The effectiveness of various combinations is shown in Figure 23.3. Larger-scale trials should now be performed in order to test the contraceptive efficacy of this combination.

23.4.5 Testosterone plus desogestrel or etonogestrel

Orally administered desogestrel, a levonorgestrel derivative, was evaluated in clinical trials using 300 μg/day combined with weekly injections of 50 or 100 mg testosterone enanthate for 24 weeks. A third group received 150 μg/day desogestrel and 100 mg testosterone enanthate per week intramuscularly. While the group receiving 50 mg testosterone enanthate showed complete suppression of spermatogenesis i.e. azoospermia, the other groups achieved only incomplete suppression. In the most effective group, total serum testosterone levels were found in the range of the lower limit of normal men and this may explain the volunteers complaints of decreased sex drive, depression, fatigue and nocturnal sweating (Wu *et al.* 1999).

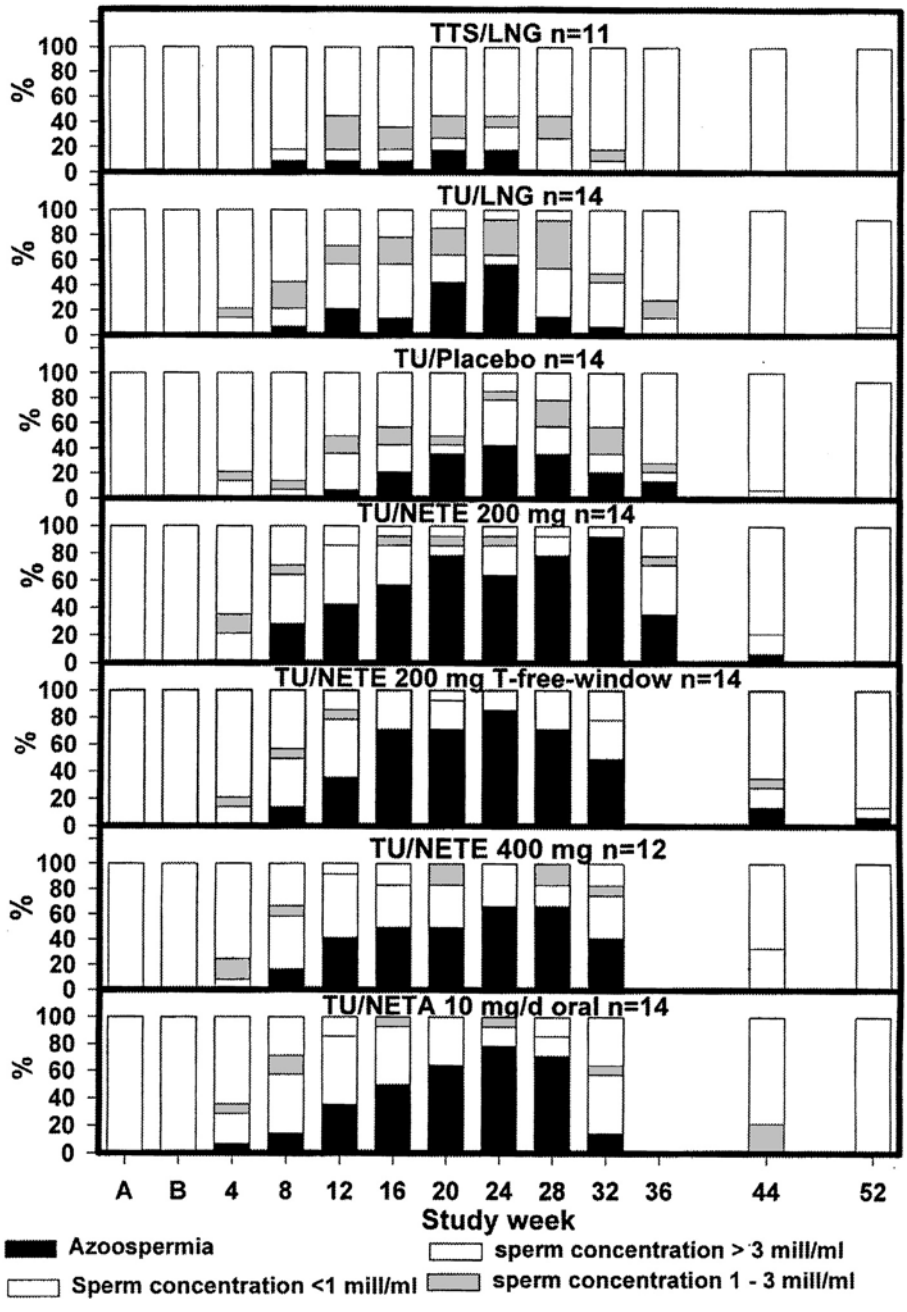


Fig. 23.3 Effectiveness of various testosterone (T) and progestin combinations in terms of suppression of spermatogenesis (data from Büchter *et al*. 1999; Kamischke *et al*. 2000; 2001; 2002b). TTS = transdermal T, LNG = Levonorgestrel, TU = T undecanoate intramuscular, NETE = norethisterone enanthate intramuscular, NETA = norethisterone acetate.

In a two-center study in Edinburgh and Shanghai testosterone pellets (400 mg every 3 months) were combined with either 150 or 300 μg desogestrel/day orally (Kinniburgh *et al.* 2002a). Azoospermia was achieved in all 33/33 men receiving the 300 μg desogestrel dose. Disregarding the fact that a combination of an implant with an oral pill might not offer a highly attractive option, these results are quite promising.

This group continued their investigations using etonogestrel, the active metabolite of orally active desogestrel, as an implant which was recently licensed for use as a female contraceptive (Implanon). 28 men received one or two etonogestrel implants which provide contraceptive protection in females for three years, but the implants were removed from the volunteers after six months. In addition, they received 400 mg testosterone pellets at the beginning of the study and after three months. Nine men in each group achieved azoospermia and in the group with 2 implants sperm counts fell to 0.1×10^6/ml in 13/14 men (Anderson *et al.* 2002).

23.4.6 Testosterone plus dienogest

The latest progestin to be tested for male contraceptive purposes is the orally effective dienogest. This is another 19-norprogestin in which position 17 is not substituted by the common ethinyl group, but by a cyanomethyl group and a double bond is introduced in ring B. When given at 2, 5 or 10 mg doses over 21 days, 10 mg resulted in a suppression of gonadotropins comparable to 10 mg of cyproterone acetate. Semen parameters were not affected, as one would expect with this short application period (Meriggiola *et al.* 2002a). As dienogest displays only mild antiandrogenic activity, this substance may be a possible candidate for future trials.

23.5 Testosterone plus GnRH analogues

23.5.1 Testosterone plus GnRH agonists

In contrast to naturally occurring GnRH, GnRH agonists – after producing an initial stimulation of gonadotropin release for approximately two weeks – lead to GnRH receptor down-regulation and thereby to suppression of LH and FSH synthesis and secretion.

Between 1979 and 1992, 12 trials for hormonal male contraception using GnRH agonists, mostly in combination with testosterone were published (for review Nieschlag *et al.* 1992). Altogether 106 volunteers participated in these trials. The GnRH agonists decapeptyl, buserelin and nafarelin were administered at daily doses of 5–500 μg/volunteer for periods of 10–30 weeks. In about 30% of men, sperm production could be suppressed below 5×10^6/ml and azoospermia occurred in 21 men, while in the remaining volunteers, sperm numbers were only slightly reduced or remained unaffected. One explanation for the ineffectiveness of GnRH

agonist plus androgen is the escape of FSH suppression after several weeks of GnRH agonist treatment (Behre *et al.* 1992; Bhasin *et al.* 1994).

Altogether, GnRH agonists in combination with testosterone did not prove useful in male contraception. At times it has been suggested that higher doses of the GnRH agonists should be used, but currently no further clinical studies appear to be under way.

23.5.2 Testosterone plus GnRH antagonists

In contrast to GnRH agonists, GnRH antagonists produce a precipitous and prolonged fall of LH and FSH serum levels in men (e.g. Behre *et al.* 1994; Pavlou *et al.* 1989). It took much longer to develop GnRH antagonists that were suitable for clinical application than it did for GnRH agonists, and clinical trials using GnRH antagonists for male contraception started some 12 years later than those using agonists. To date, results have become available from five clinical trials using GnRH antagonists for male contraception (for review Nieschlag and Behre 1996; Swerdloff *et al.* 1998; Behre *et al.* 2001).

Overall, 35 of the 40 volunteers (88%) who took part in these studies became azoospermic, most within three months. This is a much better rate of complete suppression than that produced by the administration of testosterone enanthate alone. Although studies in monkeys had suggested that delayed testosterone administration would increase the effectiveness of GnRH antagonists (Weinbauer *et al.* 1987; 1989) – in men GnRH administration followed by delayed testosterone administration (azoospermia in 20/22 men) offered little advantage over concomitant GnRH and testosterone administration (azoospermia in 15/18 men). It should also be noted that, in the later studies with concomitant administration, all 14 volunteers became azoospermic (Behre *et al.* 2001; Pavlou *et al.* 1994). The major advantage of using GnRH antagonists is the short time required to achieve azoospermia, i.e., within 6–8 weeks, which is considerably shorter than the mean of 17 weeks that is required in Caucasian men when testosterone alone was used (WHO 1995).

These results are promising. However, the antagonists and regimen tested to date require daily injections which makes them unacceptable for contraceptive purposes. The development of depot formulations is therefore anticipated with great interest, but such development appears to be much more difficult than it had been for GnRH agonists. Furthermore, it could be argued that the high price of GnRH antagonists may preclude their development as male contraceptives which need to be affordable and in the same price range as comparable female methods. In order to shorten the period of use of GnRH antagonists, two studies have investigated the possibility of applying GnRH antagonists only in an initial suppression phase, and then continuing with the androgen alone (Swerdloff *et al.* 1998; Behre *et al.*

2001). Although successful in the monkey model (Weinbauer *et al.* 1994) studies in men produced contradicting results so that this approach requires further experimentation.

23.6 Side effects and acceptability

Possible side effects of hormonal male contraception might be caused by too high or too low testosterone levels or by additional substances. Decreased testicular volumes reflecting suppression of spermatogenesis is inherent to all hormonal methods, but is not considered a serious effect by the volunteers as long as sexual function remains unaltered. Weight gain is most likely an anabolic effect of testosterone. Due to the high peak serum testosterone levels caused by testosterone enanthate in the earlier studies, acne and mild gynecomastia could be observed in individual cases. Except for local skin reactions, side effects of GnRH analogues are mainly attributable to decreased testosterone levels, not sufficiently compensated for by testosterone supplementation. Sweating and in particular, nocturnal sweating is a feature of some added progestins (see Table 23.2).

Depending on the type and doses of progestin, significant decreases are observed in sex hormone binding globulin. This indicates the influence of progestins on liver function and may enhance the androgenicity of the testosterone preparation, since the unbound free fraction of testosterone in circulation may increase. Some of the effects seen when progestins are added may be due to this phenomenon. When adding levonorgestrel or 19-noresthisterone acetate or enanthate an increase in prolactin is seen, which remains without biological significance. An increase in red blood was more pronounced when progestins were added to testosterone than when testosterone was given alone. Hemostasis is affected by testosterone alone (down-regulation) and by progestin (in this case norethisterone) alone (up-regulation), but given in combination, the effects appear to be neutralized (Zitzmann *et al.* 2002).

Overall, very few subjects left the trials due to side effects, but it has to be kept in mind that all studies so far were of relatively short duration and long-term effects need to be investigated. This should best be done with a combination showing enough contraceptive efficacy to become marketed.

Similarly, the acceptability of hormonal male contraception can only be assessed when a final product becomes available. Nevertheless, interviews with volunteers in contraceptive trials and systematic opinion polls in different cultural settings indicate that a substantial proportion of men would be ready to take a hormonal male contraceptive, preferably a pill, but injections or implants would also attract users (Martin *et al.* 2000; Weston *et al.* 2002). Above all, the female partners would be quite in favour of the men using a contraceptive (Glasier *et al.* 2000).

Table 23.2 Side effects noted in three trials for male contraception using testosterone undecanoate intramuscular alone (1000 mg/6 weeks) or in combination with oral levonorgestrel (0.5 mg/day) or intramuscular norethisterone enanthate intramuscular (200 or 400 mg/6 weeks) or oral norethisterone acetate (10 mg/day)

	TU alone n = 14	TU + LNG n = 14	TU + NETE n = 40	TU + NETA n = 14
Body weight	(↑)	↑	↑	↑
Testis volume	↓	↓	↓	↓
Prostate volume	–	–	–	–
PSA	–	–	–	–
Blood pressure	–	–	–	–
Libido/sexual function	–	–	–	–
(Mild) acne	+	+	+	–
Nocturnal sweating	–	+	++	++
SHBG	(↓)	↓↓	↓↓	↓
Prolactin	(↑)	↑	↑	↑
Erys, Hb, Hk[a]	(↑)	↑	↑	(↑)
HDL cholesterol	↓	↓	↓	↓↓
LDL cholesterol	–	–	(↑)	–
Lp (a)	–	↓	↓	↓
ApoA-1	↓	↓	↓	↓
Glucose basal	–	–	–	–
Glucose tolerance	–	–		
Plasmin α_2-anti-Plasmin complex (PAP)	↓	–	–	

[a] Erys = erythrocytes, Hb = hemoglobin, Hk = hematocrit

Kamischke 2000, 2001, 2002; Zitzmann *et al.* 2002

23.7 Outlook

Initially research in hormonal male contraception was predominantly driven by the WHO Human Reproduction Programme (Waites 2003), by the Population Council, the NIH and USAID/CONRAD. While these organisations still play an important role in the development of the field, investigators initiated complementary research and tapped the national research councils and other organisations for support. When pressure from the public also increased, the pharmaceutical industry finally succumbed to the organisations' and investigators' demand for involvement, since without their input no marketable contraceptive can be developed. The

pharmaceutical industry has now become a partner in development: its pace now rests with industry – and the regulatory agencies. Cooperation as well as competition between the companies may both spur development of a hormonal male contraceptive. The prospect of China being the first country to have a hormonal male contraceptive will further accelerate efforts in the West.

23.8 Key messages

- Testosterone-induced azoospermia leads to effective, safe and reversible male contraception.
- Suppression of spermatogenesis to below 1 mill/ml sperm may still be compatible with protection from pregnancy.
- About two thirds of Caucasian and almost all East Asian men reach azoospermia when given weekly testosterone enanthate injections or 4 to 6 weekly injections of testosterone undecanoate.
- In order to speed up suppression of spermatogenesis and increase the rate of azoospermia, testosterone is combined with either progestins or GnRH antagonists.
- All effective approaches tested so far require injections or implantations. Self-administered modalities (oral or transdermal) did not yet prove to be effective.
- Side effects of hormonal contraception are rare and tolerable. Long-term effects require further investigation.
- Acceptability of a hormonal method as assessed by opinion polls is high.
- After academic research established the principle of hormonal male contraception, the pace of development is now dictated by impetus on the part of the pharmaceutical industry.

23.9 REFERENCES

Alvarez-Sanchez F, Faundes A, Brache V, Leon P (1977) Attainment and maintenance of azoospermia with combined monthly injections of depot medroxyprogesterone acetate and testosterone enanthate. Contraception 15:635–648.

Anawalt BD, Bebb RA, Bremner WJ, Matsumoto AM (1999) A lower dosage levonorgestrel and testosterone combination effectively suppresses spermatogenesis and circulating gonadotrophin levels with fewer metabolic effects than higher dosage combinations. J Androl 20: 407–414.

Anawalt BD, Herbst KL, Matsumoto AM, Mulders TM, Coelingh-Bennink HJ, Bremner WJ (2000) Desogestrel plus testosterone effectively suppresses spermatogenesis but also causes modest weight gain and high-density lipoprotein suppression. Fertil Steril 74:707–714

Anderson RA, Kinniburgh D, Baird DT (2002a) Suppression of spermatogenesis by etonogestrel implants with depot testosterone: potential for long-acting male contraception. J Clin Endocr Metab 87:3640–3649

Anderson RA, Van Der Spuy ZM, Dada OA, Tregoning SK, Zinn PM, Adeniji OA, Fakoya TA, Smith KB, Baird DT (2002b) Investigation of hormonal male contraception in African men:

suppression of spermatogenesis by oral desogestrel with depot testosterone. Hum Reprod 17:2869–2877

Aribarg A, Sukcharoen N, Chanprasit Y, Ngeamvijawat J, Kriangsinyos R (1996) Suppression of spermatogenesis by testosterone enanthate in Thai men. J Med Assoc Thai 79:624–629

Arysad, KM (1993) Sperm function in Indonesian men treated with testosterone enanthate. Int J Androl 16:355–361

Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM (1996) Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. J Clin Endocr Metab 81:757–762

Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E (1995) Potential of testosterone buciclate for male contraception: endocrine differences between responders and nonresponders. J Clin Endocr Metab 80:2394–2403

Behre HM, Böckers A, Schlingheider A, Nieschlag E (1994) Sustained suppression of serum LH, FSH and testosterone and increase of high-density lipoprotein cholesterol by daily injections of the GnRH antagonist Cetrorelix over 8 days in normal men. Clin Endocr 40:241–248

Behre HM, Kliesch S, Lemcke B, Nieschlag E (2001) Suppression of spermatogenesis to azoospermia by combined administration of GnRH antagonist and 19-nortestosterone cannot be maintained by 19-nortestosterone alone in normal men. Hum Reprod 16:2570–2577

Behre HM, Nashan D, Hubert W, Nieschlag E (1992) Depot gonadotropin-releasing hormone agonist blunts the androgen-induced suppression of spermatogenesis in a clinical trial of male contraception. J Clin Endocr Metab 74:84–90

Bhasin S, Berman N, Swerdloff RS (1994) Follicle-stimulating hormone (FSH) escape during chronic gonadotropin-releasing hormone (GnRH) agonist and testosterone treatment. J Androl 15:386–39

Brenner PF, Mishell DR Jr, Bernstein GS, Ortiz A, *et al.* (1977) Study of medroxyprogesterone acetate and testosterone enanthate as a male contraceptive. Contraception 15:679–691

Büchter D, von Eckardstein S, von Eckardstein A, Kamischke A, Simoni M, Behre HM, Nieschlag E (1999) Clinical trial of a transdermal testosterone and oral levonorgestrel for male contraception. J Clin Endocr Metab 84:1244–1249

Cao J, Yuan J, Jin W (1996) Clinical trial of an anti-fertility method with testosterone enanthate in normal men. Chung Hua I Hsueh Tsa Chih 76:335–337

Djerassi C, Miramontes L, Rosenkranz G, Sonheimer F (1954) Steroids LIV Synthesis of 19-nor-17α-ethinyltestosterone and 19-nor-17α-methyltestosterone. J Am Chem Soc 76:4092–4094

Ersheng G, Cuihong L, Youlun G, Lamei L, Changhai H (1999) Inhibiting effects of sino-implant plus testosterone undecanoate on spermatogenesis in Chinese men. Reprod Contracept 10:98–105

Faundes A, Brache V, Leon P, Schmidt F, Alvarez-Sanchez F (1981) Sperm suppression with monthly injections of medroxyprogesterone acetate combined with testosterone enanthate at a high dose (500 mg). Int J Androl 4:235–245

Fogh M, Corker CS, Hunter WM, McLean H, Philip J, Schon G, Skakkebaek NE (1979) The effects of low doses of cyproterone acetate on some functions of the reproductive system in normal men. Acta Endocrinol 91:545

Fogh M, Corker CS, McLean H (1980) Clinical trial with levonorgestrel and testosterone enanthate for male fertility control. Acta Endocrinol 95:251–257

Frick J, Bartsch G, Weiske WH (1977) The effect of monthly depot medroxyprogesterone acetate and testosterone on human spermatogenesis. II. High initial dose. Contraception 15:669–677

Frick J, Danner C, Kunit G, Joos H, Kohle R (1982) Spermatogenesis in men treated with injections of medroxyprogesterone acetate combined with testosterone enanthate. Int J Androl 5:246–252

Gaw Gonzalo IT, Swerdloff RS, Nelson AL, Clevenger B, Garcia R, Berman N, Wang C (2002) Levonorgestrel implants (Norplant II) for male contraception clinical trials: combination with transdermal and injectable testosterone. J Clin Endocr Metab 87:3562–3572

Glasier AF, Anakwe R, Everington D, Martin CW, van der Spuy Z, Cheng L, Ho PC, Anderson RA (2000) Would women trust their partners to use a male pill? Hum Reprod 15:646–649

Gu Y-Q, Wang X-H, Xu D, Peng L, Cheng L-F, Huang M-K, Huang Z-J, Zhang G-Y (2003) A multicenter contraceptive efficacy study of injectable testosterone undecanoate in healthy Chinese men. J Clin Endocr Metab 88:562–568

Guerin JF, Rollet J. (1988) Inhibition of spermatogenesis in men using various combinations of oral progestagens and percutaneous or oral androgens. Int J Androl 11:187–199

Hair WM, Kitteridge K, Wu FCW (1999) A new male contraceptive pill/patch combination- oral desogestrel and transdermal testosterone: Suppression of gonadotropins and spermatogenesis in men. In: The Endocrine Society's 81st Annual Meeting; 1999 June 12–15; San Diego, CA, USA; Poster P3–374

Hamilton DW, Waites GMH (eds) (1989) Scientific basis of fertility regulation: Cellular and molecular events in spermatogenesis. Cambridge University Press, pp 315–321

Handelsman DJ (2003) Editorial: Hormonal male contraception – lessons from the East when the Western market fails. J Clin Endocr Metab 88:559–561

Handelsman DJ, Conway AJ, Boylan LM (1992) Suppression of human spermatogenesis by testosterone implants. J Clin Endocr Metab 75:1326–1332

Handelsman DJ, Conway AJ, Howe CJ, Turner L, Mackey MA (1996) Establishing the minimum effective dose and additive effects of depot progestin in suppression of human spermatogenesis by a testosterone depot. J Clin Endocrinol Metab 81:4113–4121

Kamischke A, Diebäcker J, Nieschlag E (2000a) Potential of norethisterone enanthate for male contraception: pharmacokinetics and suppression of pituitary and gonadal function. Clin Endocrinol 53:351–358

Kamischke A, Plöger D, Venherm S, von Eckardstein S, von Eckardstein A, Nieschlag E (2000b) Intramuscular testosterone undecanoate with or without oral levonorgestrel: a randomized placebo-controlled feasibility study for male contraception. Clin Endocrinol 53:43–52

Kamischke A, Heuermann T, Krüger K, von Eckardstein S, Schellschmidt I, Rübig A, Nieschlag E (2002) An effective hormonal male contraceptive using testosterone undecanoate with oral or injectable norethisterone preparations. J Clin Endocr Metab 87:530–539

Kamischke A, Venherm S, Plöger D, von Eckardstein S, Nieschlag E (2001) Intramuscular testosterone undecanoate and norethisterone enanthate in a clinical trial for male contraception. J Clin Endocr Metab 86:303–309

Kinniburgh D, Anderson RA, Baird DT (2002a) Suppression of spermatogenesis with desogestrel and testosterone pellets is not enhanced by addition of finasteride. J Androl 22:88–95

Kinniburgh D, Zhu H, Cheng L, Kicman AT, Baird DT, Anderson RA (2002b) Oral desogestrel with testosterone pellets induces consistent suppression of spermatogenesis to azoospermia in both Caucasian and Chinese men. Hum Reprod 17:1490–1501

Knuth UA, Behre, HM, Belkien L, Bents H, Nieschlag E (1985) Clinical trial of 19-nortestosterone-hexoxyphenylpropionate (Anadur) for male fertility regulation. Fertil Steril 44:814–821

Knuth UA, Yeung CH, Nieschlag E (1989) Combination of 19-nortestosterone-hexyoxyphenylpropionate (Anadur) and depot-medroxyprogesterone-acetate (Clinovir) for male contraception: Fertil Steril 51:1011–1018

Martin CW, Anderson RA, Cheng L, Ho PC, v. d. Spuy Z, Smith KB, Glasier AF, Everington D, Baird DT (2000) Potential impact of hormonal male contraception: cross-cultural implications for development of novel preparations. Hum Reprod 15:637–345

McLachlan RI, McDonald J, Rushford D, Robertson DM, Garrett C, Baker HW (2000) Efficacy and acceptability of testosterone implants, alone or in combination with a 5α-reductase inhibitor, for male hormonal contraception. Contraception 62:73–78

McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, DeKretser DM, Robertson DM (2002) Effects of testosterone enanthate plus medroxyprogesterone acetate on semen quality, reproductive hormones and germ cell populations in normal young men. J Clin Endocrinol Metab 87:546–556

Melo JF, Coutinho EM (1977) Inhibition of spermatogenesis in men with monthly injections of medroxyprogesterone acetate and testosterone enanthate. Contraception 15:627–634

Meriggiola MC, Bremner WJ, Paulsen CA, Valdiserri A, Incorvala L, Motta R, Pavani A, Capelli M, Flamigni C (1996) A combined regimen of cyproterone acetate and testosterone enanthate as a potentially highly effective male contraceptive. J Clin Endocr Metab 81:3018–3023

Meriggiola MC, Bremner WJ, Costantino A, Pavani A, Capelli M, Flamigni C (1997) An oral regimen of cyproterone acetate and testosterone undecanoate for spermatogenic suppression in men. Fertil Steril 68:844–850

Meriggiola MC, Bremner WJ, Costantino A, Di Cintio G, Flamigni C (1998) Low dose of cyproterone acetate and testosterone enanthate for contraception in men. Hum Reprod 13:1225–1229

Meriggiola MC, Bremner WJ, Costantino A, Bertaccini A, Morselli-Labate AM, Huebler D, Kaufmann G, Oettel M, Flamigni C (2002a) Twenty-one day administration of dienogest reversibly suppresses gonadotropins and testosterone in normal men. J Clin Endocrinol Metab 87:2107–2113

Meriggiola MC, Costantino A, Bremner WJ, Morselli-Labate AM (2002b) Higher testosterone dose impairs sperm suppression induced by a combined androgen-progestin regimen. J Androl 23:684–690

Moltz L, Römmler A, Post K, Schwartz U, Hammerstein J (1980) Medium dose cyproterone acetate (CPA): effects on hormone secretion and on spermatogenesis in man. Contraception 21:393

Moudgal NR, Ravindrath N, Murthy GS, Dighe RR, Aravindan GR, Martin F (1992) Long-term contraceptive efficacy of vaccine of ovine follicle-stimulating hormone in male bonnet monkeys (Macaca radiata). J Reprod Fertil 96:91–102

Moudgal NR, Sairam MR, Krishnamurthy HN (1997a) Immunization of male bonnet monkeys (Macaca radiata) with a recombinant FSH receptor preparation affects testicular function and fertility. Endocrinology 138:3065–3068

Moudgal NR, Jeyakumar M, Kishnamurthy HN, Sridhar S, Krishnamurthy H, Martin F (1997b) Development of male contraceptive vaccine – a perspective. Hum Reprod Update 3:335–346

Moudgal NR, Murthy GS, Prasanna Kumur KM, Martin F, Suresh R, Medhamurthy R, Patil S, Sehgal S, Saxena B (1997c) Responsiveness of human male volunteers to immunization with ovine follicle stimulating hormone vaccine: results of a pilot study. Hum Reprod 12:457–463

Narula A, Gu Y-Q, O'Donnell L, Stanton PG, Robertson DM, McLachlan RI, Bremner WJ (2002) Variability in sperm suppression during testosterone administration to adult monkeys is related to follicle stimulating hormone suppression and not to intratesticular androgens. J Clin Endocrinol Metab 87:3399–3406

Nieschlag E (1986) Reasons for abandoning immunization against FSH as an approach to male fertility regulation. In Zatuchni GI, Goldsmith A, Spieler JM, Sciarra J (eds) Male contraception: Advances and future prospects. Harper & Row, Philadelphia, pp 395–400

Nieschlag E (2002) Sixth summit meeting on hormonal male contraception consensus: recommendations for regulatory approval for hormonal male contraception. Int J Androl 25:375

Nieschlag E, Behre HM (1996) Hormonal male contraception: Suppression of spermatogenesis with GnRH antagonists and testosterone. In Filicori M, Flamigni C (eds) Treatment with GnRH analogs: Controversies and perspectives. Parthenon, London, pp 243–248

Nieschlag E, Behre HM, Weinbauer GF (1992) Hormonal male contraception: A real chance? In: Nieschlag E, Habenicht UF (eds) Spermatogenesis – fertilization – contraception. Molecular, cellular and endocrine events in male reproduction. Heidelberg: Springer, pp 477–501

Nieschlag E, Hoogen H, Bölk M, Schuster H, Wickings EJ (1978) Clinical trial with testosterone undecanoate for male fertility control. Contraception 18:607–614

Pangkahila W (1991) Reversible azoospermia induced by an androgen-progestin combination regimen in Indonesian men. Int J Androl 14:248–256

Patanelli DJ (ed) (1978) Hormonal control of male fertility (workshop proceedings). DHEW Publ. No. (NIH) 78-1097, Bethesda, MD

Pavlou SN, Wakefield G, Schlechter NL, Lindner J, Souza KH, Kamilaris TC, Konidaris S, Rivier JE, Vale WW, Toglia M (1989) Mode of suppression of pituitary and gonadal function after acute or prolonged administration of a luteinizing hormone-releasing hormone antagonist in normal men. J Clin Endocrinol Metab 73:1360–1369

Pavlou SN, Herodotou D, Curtain M, Minaretzis D (1994) Complete suppression of spermatogenesis by co-administration of a GnRH antagonist plus a physiologic dose of testosterone. Proceedings of the 76th meeting of the Endocrine Society, Anaheim, CA, p 1324

Pöllänen P, Nikkanen V, Huhtaniemi I (2001) Combination of subcutaneous levonorgestrel implants and transdermal dihydrotestosterone gel for male hormonal contraception. Int J Androl 24:369–380

Reddy PR, Rao JM (1972) Reversible antifertility action of testosterone propionate in human males. Contraception 5:295–301

Schearer SB, Alvarez-Sanches F, Anselmo G, Brenner P, Coutinho E, Lathen-Faundes A, Frick J, Heinild B, Johansson EDB (1978) Hormonal contraception for men. Int J Androl 2 (Suppl. 2) 680–712

Schürmeyer T, Knuth UA, Belkien L, Nieschlag E (1984) Reversible azoospermia induced by the anabolic steroid 19-nortestosterone. Lancet 25:417–420

Srinath BR, Wickings EJ, Witting CH, Nieschlag E (1983) Active immunization with follicle stimulating hormone for fertility control: a 4 1/2-year study in male rhesus monkeys. Fertil Steril 40:110–117

Swerdloff RS, Bagatell CJ, Wang C, Anawalt BD, Berman N, Steiner B, Bremner WJ (1998) Suppression of spermatogenesis in man induced by Nal-Glu gonadotropin releasing hormone antagonist and testosterone enanthate (TE) is maintained by TE alone. J Clin Endocr Metab 83:3527–3533

Turner L, Wishart S, Conway AJ, Liu PY, Forbes E, McLachlan RI, Handelsman DJ (2003) Contraceptive efficacy of a depot progestin and androgen combination in men. J Clin Endocr Metab 88:4659–4667

von Eckardstein S, Nieschlag E (2002) Treatment of male hypogonadism with testosterone undecanoate injected at extended intervals of 12 weeks. J Androl 23:419–425

von Eckardstein S, Schmidt A, Kamischke A, Simoni M, Gromoll J, Nieschlag E (2002) CAG repeat length in the androgen receptor gene and gonadotropin suppression influence the effectiveness of hormonal male contraception. Clin Endocrinol 57:647–655

von Eckardstein S, Noé G, Brache V, Nieschlag E, Croxatto H, Alvarez F, Moo-Young A, Sivin I, Kumar N, Small M, Sundaram K (2003) A clinical trial of 7α-methyl-19-nortestosterone implants for possible use as a long acting contraceptive for men. J Clin Endocrinol Metab 88:5232–5239

Waites GM (2003) Development of methods of male contraception: the impact of the World Health Organization Task Force. Fertil Steril 80:1–15

Wang C, Yeung KK (1980) Use of a low dosage oral cyproterone acetate as a male contraceptive. Contraception 21:245

Weinbauer GF, Nieschlag E (1996) The Leydig cell as a target for male contraception. In: Payne AH, Hardy MP, Russell LD (eds) The Leydig Cell. Vienna, Cache River Press, pp 629–662

Weinbauer GF, Surmann FJ, Nieschlag E (1987) Suppression of spermatogenesis in a nonhuman primate (*Macaca fascicularis*) by concomitant gonadotropin-releasing hormone (GnRH) antagonist and testosterone treatment. Acta Endocrinol 114:138–146

Weinbauer GF, Khurshid S, Fingscheidt U, Nieschlag E (1989) Sustained inhibition of sperm production and inhibin secretion induced by a gonadotropin-releasing hormone antagonist and delayed testosterone substitution in non-human primates (*Macaca fascicularis*). J Endocr 123:303–310

Weinbauer GF, Limberger A, Behre HM, Nieschlag E (1994) Can testosterone alone maintain the gonadotropin-releasing hormone antagonist-induced suppression of spermatogenesis in the non-human primate? J Endocrinol 142:485–495

Weinbauer GF, Schlatt S, Walter V, Nieschlag E (2001) Testosterone-induced inhibition of spermatogenesis is more closely related to suppression of FSH than to testicular androgen levels in the cynomolgus monkey model (*Macaca fascicularis*). J Endocr 168:25–38

Weston GC, Schlipalius ML, Bhuinneain MN, Vollenhoven BJ (2002) Will Australian men use male hormonal contraception? A survey of a postpartum population. Med J Aust 176:204–205

World Health Organization (1972–1995) Special Programme of Research, Development and Research Training in Human Reproduction. Annual and Biannual Reports, WHO, Geneva

World Health Organization Task Force on Methods for the Regulation of Male Fertility (1990) Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet 336:955–959

World Health Organization Task Force on Methods for the Regulation of Male Fertility (1993) Comparison of two androgens plus depot-medroxyprogesterone acetate for suppression to azoospermia in Indonesian men. Fertil Steril 60:1062–1068

World Health Organization Task Force on Methods for the Regulation of Male Fertility (1995) Rates of testosterone-induced suppression to severe oligozoospermia or azoospermia in two multinational clinical studies. Int J Androl 18:157–165

World Health Organization Task Force on Methods for the Regulation of Male Fertility (1996) Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril 65:821–829

Wu FC, Aitken RJ (1989) Suppression of sperm function by depot medroxyprogesterone acetate and testosterone enanthate in steroid male contraception. Fertil Steril 51:691–698

Wu FCW, Balasubramanian R, Mulders TMT, Coelingh-Bennink HJT (1999) Oral progestogen combined with testosterone as a potential male contraceptive: additive effects between desogestrel and testosterone enanthate in suppression of spermatogenesis, pituitary-testicular axis, and lipid metabolism. J Clin Endocr Metab 84:12–122

Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ (1999) A clinical trial of injectable testosterone undecanoate as a potential male contraceptive in normal Chinese men. J Clin Endocrinol Metab 84:3642–3647

Zitzmann M, Junker R, Kamischke A, Nieschlag E (2002) Contraceptive steroids influence the hemostatic activation state in healthy men. J Androl 23:03–511

24

Abuse of androgens and detection of illegal use

W. Schänzer

Contents

24.1 Introduction

Anabolic androgenic steroids (AAS) are known to be misused both in competitive and in non-competitive sports (Haupt and Rovere 1984; Wilson 1988; Yesalis *et al.* 1993). Moreover, it seems that AAS are becoming "social drugs", as even young people apply them as an expression of an improved "life-style".

The misuse of AAS in athletics has been observed for more than 40 years. The first rumours dated from 1954 and were attributed to weightlifters who seemed to have used testosterone (Wade 1972). By 1965 synthetic AAS had become widely

popular among bodybuilders and weightlifters, but were also applied in other forms of sports.

By using these steroids athletes hoped to increase muscle strength. Such improvements in muscle strength to increase physical performance in sport are naturally an essential effect of training. As AAS stimulate protein synthesis in muscle cells, athletes expect performance-enhancing effects beyond that brought about by training alone. At the end of the sixties the first anti-doping rules were established by international sport federations (1967 International Cycling Union, UCI, and 1968 International Olympic Committee, IOC), but only stimulants and narcotics were banned (Clasing 1992). At that time the Medical Commission (MC) of the IOC was already aware of widespread misuse of AAS in sports. They were not banned because no reliable method was available to detect them (Beckett and Cowan 1979). Under these circumstances the first methods for AAS detection were developed (Brooks *et al.* 1975; Ward *et al.* 1975) and in 1974 the MC of the IOC and the International Amateur Athletic Federation (IAAF) first banned the use of AAS. This prohibition encompassed only synthetic steroids, such as metandienone, stanozolol etc. and the misuse of endogenous steroids, e.g. testosterone, was not restricted. At that time athletes misused only synthetic AAS. The reason was that AAS were used in human medicine to treat catabolic conditions, scientific data obtained from animal studies led to the conclusion that synthetic AAS are more anabolic and less androgenic than testosterone itself (Kochakian 1976).

Whether athletes experience a positive performance-enhancing effect or not when using AAS has been discussed controversially for many years. Nowadays it is known that androgens have muscle growth-promoting effects in boys, in women and in hypogonadal men. It has never been proven that androgens, when administered in therapeutical doses, have positive effects on muscle growth in adult men. The assumption that AAS have less effect on muscle growth in males is based on the fact that the androgen receptor in men is nearly completely saturated (Wilson 1996). However, high doses of androgens have been reported to exert muscle mass enhancing effects (Bhasin *et al.* 1996). An unethical and secret program of hormonal doping of athletes in the former German Democratic Republic was reported (Franke and Berendonk 1997) and performance-improving effects of AAS were elucidated.

To control the (mis)use of synthetic AAS urine samples of athletes collected after competition events were tested. As AAS are not used directly during competition but rather during training to increase muscle strength, athletes stopped administration of AAS before competition, switching to those AAS (e.g. stanozolol) they believed could not be detected and to endogenous androgens, such as testosterone, which were not banned. Investigations of test samples from the Olympic Summer Games 1980 in Moscow showed that 2.1% of male and 7.1% of female athletes

had elevated testosterone levels (Zimmermann 1986) in urine and the highest urinary testosterone levels were detected for women in swimming and track and field events (Donike 1983). These results could only be explained by exogenous application of testosterone. Donike developed a gas chromatographic/mass spectrometric (GC-MS) method for detection of testosterone and epitestosterone excreted in urine and proved that the ratio of testosterone to epitestosterone is significantly increased after application of testosterone (Donike *et al.* 1983). Based on these results sport federations also banned testosterone in 1984 and applied the T/E ratio measurements (cutoff level of six) to their rules. In addition to the ban of testosterone other endogenous androgens such as dihydrotestosterone and prohormones of testosterone, dihydrotestosterone and nortestosterone were added to the list of prohibited AAS during recent years.

24.2 Frequency of steroid hormone misuse

Considering this misuse of AAS, the question arises to what extent AAS are really misused? Is it restricted to a few high-level performing athletes in sports or is it so extensive as to be regarded as a social drug problem? Available data derive

1. from positive findings resulting from checking AAS doping in competition sports and
2. from results of questionnaires concerning the use of AAS in non-competitive sports.

24.2.1 Androgen misuse in controlled competition sports

Androgen misuse in competition sports is investigated by laboratories which are accredited by the IOC/World Anti-Doping Agency (WADA). Each year the accredited laboratories (30 laboratories in 2003 worldwide) report the positive findings from the A-samples. The annual testing frequency was about 120,000 samples for all laboratories. From 1992–2001 a total number of 1.034.131 doping tests were analyzed by the IOC accredited laboratories for olympic and non-olympic sports and 8,737 AAS were reported in different types of sport. Fig. 24.1 shows the chemical structure of misused steroids. The data indicate that the misuse of AAS is limited to a number of well-known AAS. Among the most frequent steroid hormones misused in controlled competitive sports are the synthetic steroids nortestosterone, stanozolol, metandienone and methenolone and the endogenous steroid testosterone.

24.2.2 Androgen misuse in non-controlled sports

In comparison to officially controlled competition sports, no analytical data from laboratories concerning the misuse of AAS are available for those areas of athletics

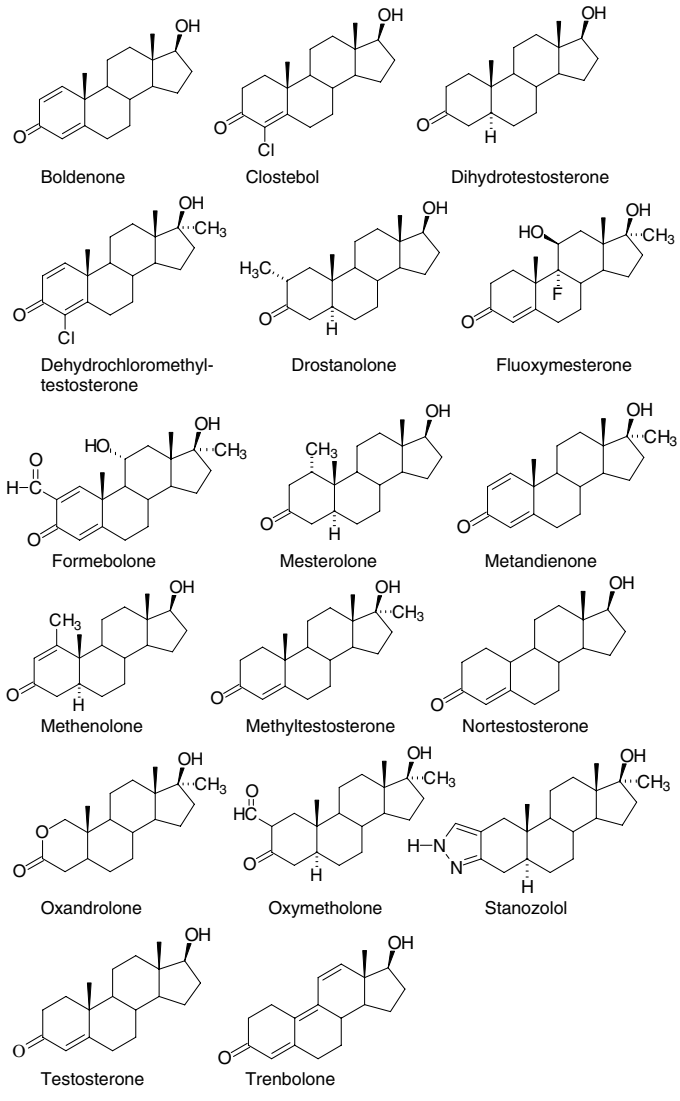


Fig. 24.1 Structure formula of AAS which have been internationally detected in doping tests in sports.

and private life where no tests are performed. To overcome this lack of information scientists have performed surveys; however, only a few publications are available. Yesalis *et al.* (1993) published results of investigations in the United States and calculated that 1 million Americans had used AAS sometime in their lives, including about 250,000 in the past year. In 1993 a Canadian study (Canadian Centre for Drug-Free Sport 1993) confirmed that in Canada 80,000 young people between the ages of 13 and 18 had applied AAS. A self-report questionnaire about the misuse of AAS among 13,355 Australian high school students reported 3.2% of male and 1.2% of

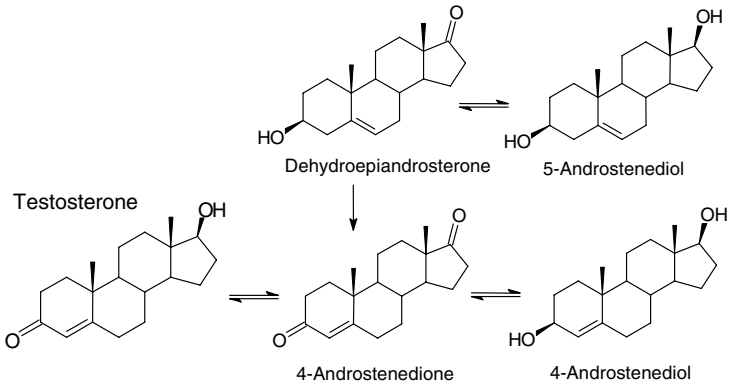


Fig. 24.2 Structure formula of prohormones of testosterone.

female users (Handelsman and Gupta 1997). Questionnaires from Switzerland were summarized by Kamber (1995), who concluded that AAS are a serious problem. A questionnaire from 16,000 recruits in Switzerland and 3,700 women of the same age showed that 1.8% of the recruits and 0.3% of the women had administered AAS in 1993.

Data concerning the most commonly misused AAS in non-controlled sports are only available via recommendations in magazines for bodybuilders, via "underground" handbooks (Taylor 1982; Duchaine 1989; Grundig and Bachmann 1995), the internet and via confiscated, smuggled substances and those obtained from black market sources. Frequently recommended AAS include boldenone undecylenate, drostanolone propionate, fluoxymesterone, mesterolone, metandienone, methandriol dipropionate, methenolone enanthate, methyltestosterone, nortestosterone decanoate and other esters (cypionate, hexylphanylpropionate, laurate), oxandrolone, oxymetholone, stanozolol, testosterone in the form of different esters (cypionate, decanoate, enanthate, isocaproate, hexanoate, isohexanoate, phenylpropionate, propionate and undecanoate) and trenbolone, trenbolone acetate.

These substances are largely identical with those products which were confiscated and distributed by the black market.

24.3 Prohormones of androgens

Since 1999 prohormones of testosterone (Fig. 24.2), dihydrotestosterone (Fig. 24.3), steroids with 5α-androst-1-ene structure (Fig. 24.3), and prohormones of nortestosterone (Fig. 24.4) have been marketed in the United States as nutritional supplements. Prohormones are advertised as having effects similar to testosterone, dihydrodestosterone and nortestosterone because of a "high conversion rate" of prohormones to the physiologically effective steroids in the human body after oral,

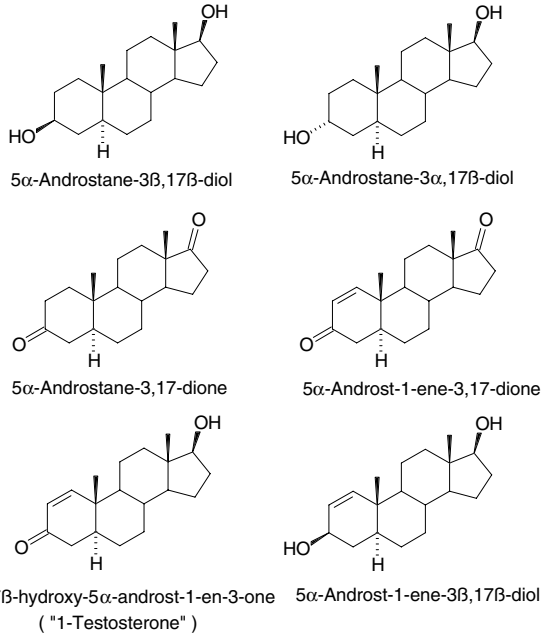


Fig. 24.3 Structure formula of prohormones of dihydrotestosterone and prohormones with 1-ene structure.

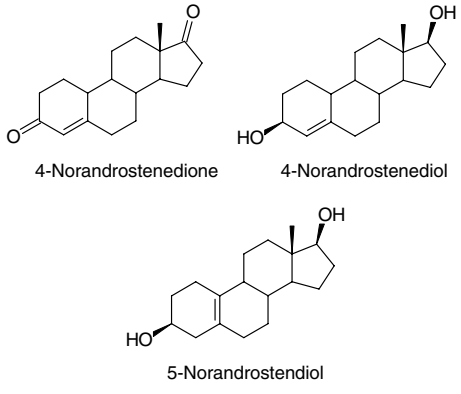


Fig. 24.4 Structure formula of prohormones of nortestosterone.

sublingual or buccal application. In contrast to such incorrect advertisements, only small amounts of the applied prohormone maybe converted to the effective steroid. Indeed, for medical treatment prohormones are useless. Therefore companies providing prohormones recommend application several times per day, especially before training or competition [high amounts (100 mg and more) of oral preparations of single prohormones, combinations of different prohormones and sublingual

preparations]. Published data (Leder *et al.* 2000) demonstrate that in male persons e.g. androstenedione 100 mg/day orally applied for seven days yielded no significant changes in serum testosterone levels compared to the control group whereas 300 mg/day of androstenedione for seven days showed a significant increase in peak and AUC (area under curve) serum testosterone (AUC 34%), but with high interindividual variation. As prohormones are also used by females and adolescents lower amounts of prohormones may yield physiological changes in serum testosterone levels, e.g. 100 mg androstenedione applied orally to females yielded an increase in serum testosterone concentrations up to 0.8 ng/ml (Kicman 2003).

The distribution of prohormones in the United States is not restricted by the Food and Drug Administration (FDA) because these products are not marketed as medications. In Europe prohormones are considered unlicensed medications and their distribution is banned. Nevertheless, control of the misuse of prohormones is difficult, as products enter the European market via neighbour states, directly via airports and by internet or postal orders.

Additional new products are entering the market with a 1-ene structure such as androsta-1,4-diene-3,17-dione which is considered as a prohormone of the synthetic AAS boldenone (17ß-hydroxyandrosta-1,4-dien-3-one) and steroids with a 5α-androst-1-ene structure (Fig. 24.3) which are marketed as prohormones.

24.4 Contamination of nutritional supplements with prohormones

Since 1999, the same time when prohormones were entering the market, nutritional supplements became sporadically "contaminated" with prohormones. "Contamination" in this context signifies that a supplement contains substances which are not declared on the label. As the amount of "contamination" is low, less than 1 per mill of the product, it is assumed that the prohormones are not intentionally added to the supplements but "contamination" may occur due to poor quality control during the production of nutritional supplements and prohormones. Several athletes have been tested in the past with positive results for norandrosterone (main metabolite of nortestosterone and the main metabolite of the prohormones of nortestosterone such as norandrost-4-ene-3,17-dione and norandrost-4-ene-3ß,17ß-diol), where the use of nutritional supplements which were containing low traces of prohormones led to positive results. In such a case the athlete has not intentionally applied a doping substance but the sports federations consider the presence of norandrosterone in the urine sample as a doping offence for which the athlete is fully responsible.

To what extent nutritional supplements may be contaminated has been shown in different studies and by an international study supported by the IOC (Geyer *et al.* 2003). The latter study investigated 630 different nutritional supplements

from 13 countries including the United States, Italy, France, Germany and Great Britain. Out of the 634 samples analysed 94 (14.8%) contained prohormones not declared on the label ("positive supplements"). Of these 94 positive supplements 23 samples (24.5%) contained prohormones of nortestosterone and testosterone, 64 samples (68.1%) only contained prohormones of testosterone, 7 samples (7.5%) only contained prohormones of nortestosterone.

In relation to the total number of products purchased per country most of the positive supplements (84%) originate from companies located in the United States. The positive supplements showed anabolic androgenic steroid concentrations of 0.01 μg/g up to 190 μg/g. Excretion studies with application of supplements containing nortestosterone prohormones corresponding to a total uptake of more than 1 μg resulted in urinary concentrations of the nortestosterone metabolite norandrosterone above the cut-off limit (2 ng/ml urine for male) for several hours (positive doping result). Positive doping tests caused by "contaminated" supplements with prohormones of only testosterone or dihydrotestosterone have not been proved which is explainable by the low amounts of contamination and the applied tests which have to differentiate between endogenous and exogenous origin of testosterone and which will not be influenced by ingestion of low amounts of prohormones or testosterone itself.

24.5 Detection of misuse of anabolic androgenic steroid hormones

24.5.1 Organization of doping tests

Doping control is organized by national and international sport federations and by the WADA for the different types of sports. Increasingly national anti-doping programs are organizing dope control by one overall organization. This strategy seems to be the most effective testing action as any possible intention by individual sport federations to hide positive cases and to protect their athletes can be excluded. The IOC only performed doping tests during the Olympic winter and summer games and it has no "out of competition testing program". This lack has now been compensated by WADA.

For a doping test athletes are selected according to the rules of the responsible sports federation. The doping test is carried out in two steps. The first step includes the sample-taking procedure and transportation of the urine specimens to an IOC accredited laboratory. In the following step the laboratory analyzes the sample for banned drugs. The sample-taking procedure is an important step. To avoid any manipulation athletes have to deliver a urine sample under visual inspection by an accredited supervisor. The urine is divided into an A- and a B-sample, both samples are sealed and then transported to the laboratory. All steps during this procedure are documented and the athlete has to sign a protocol of the

sample-taking procedure and sealing of samples. All handling of a urine specimen (sample-taking, transportation containers and laboratory tests) must be documented and is designated as "chain of custody". The laboratory is not in possession of the athlete's name corresponding to the urine sample. For this reason all samples have code numbers. The reason for dividing the urine specimen into A- and B-samples is to guarantee the best chain of custody: if the A-sample is tested positive, the B-sample will be analyzed in the presence of the athlete and his advisers. If the B-analysis confirms the A-result, the samples are considered as positive. Based on this result the federation can impose sanctions on the athlete.

Doping test samples are analyzed by WADA accredited laboratories. Laboratories seeking accreditation have to comply with the requirements for doping drug testing set by the WADA World-Anti-Doping Code. Additionally the laboratory has to be accredited by a national accreditation body following the standard of ISO 17025. The laboratory must show that it has the capability to analyze all banned substances below the specified concentration limits within a controlled quality system.

The prerequisite for this accreditation system is a standardization of analytical techniques and detection limits of banned substances among the different laboratories. Information concerning new doping drugs and doping techniques is rapidly distributed in order to deal with new problems in a co-ordinated manner. Especially for the detection of synthetic AAS, which are misused mainly during training periods, the laboratory has to use highly sensitive methods.

At the present time 30 laboratories all over the world (18 in Europe, 2 in North America, 2 in South America, 5 in Asia, 1 in Australia and 2 in Africa) are accredited.

24.5.2 Detection and identification of misused anabolic androgenic steroids

Synthetic AAS were first banned in 1974. As no comprehensive analytical method for the detection of AAS in human urine was available at the beginning of the seventies, new methods had to be developed. The first methods were based on radioimmunoassay (RIA) techniques, e.g. Brooks *et al.* (1975) developed an antiserum for metandienone with some cross-reactivity to other 17α-methyl steroids. The RIA techniques were discouraging for several reasons: the method did not consider the high degree of metabolism of AAS (therefore screening for the parent steroid was less successful), the antisera had only limited sensitivity for other steroids and the possibility of false positives, which was not acceptable for routine analysis. As early as 1977 Ward *et al.* presented a gas chromatography / mass spectrometry (GC-MS) method for the detection of the AAS metandienone, nortestosterone, norethandrolone and stanozolol. Nevertheless, the RIA technique was used as a screening method during the 1976 Olympic Games in Montreal and in Moscow in 1980, but confirmation of suspicious samples was performed by GC-MS. After

1981 all IOC accredited laboratories used GC-MS as the main analytical tool for AAS identification (Donike *et al.* 1984; Massé *et al.* 1989; Schänzer and Donike 1993).

Analysis of AAS can be divided and described in two steps: first a sample preparation is performed with the aim to separate the banned substances from the biological matrix (urine) and to reduce biological interference (biological background). Sample preparation for AAS also includes a chemical modification (derivatization) of the isolated substances to improve their analytical detectability. The second step covers the analytical measurement, which is based on a physical principle, mainly on gas chromatography in combination with mass spectrometry (GC-MS). Additionally liquid chromatography and mass spectrometry (LC/MS) can be used for substances which show poor gas chromatographic properties or are sensitive to temperature.

The main advantage of chromatographic techniques such as GC-MS is the possibility of analyzing a high number of substances within one run. This minimizes costs and allows a high throughput of samples. In fact it is possible to run a maximum of 50 samples per day and per GC-MS instrument.

24.5.2.1 Metabolism

To a large extent anabolic androgenic steroids are metabolized by phase I and phase II reactions and only a few AAS are excreted unchanged in urine for a short period of time after administration. To detect the misuse of AAS which are not excreted in urine or only to a small extent, the analytical method cannot rely on monitoring only the parent steroid but must identify its metabolites. Detection of an AAS metabolite in urine is proof for the misuse of a banned anabolic androgenic doping substance. This presumes that the metabolite cannot be generated from endogenous steroids in the body compartment.

Metabolites of the most frequently misused anabolic steroids have been investigated by different working groups in recent years (Schänzer 1996; Schänzer and Donike 1993). Basically the metabolism of AAS follows the metabolic pathways of the principal androgen testosterone. This includes reduction of the double bond at C4-C5 to form 5α- and 5ß-isomers, the reduction of the 3-keto group to a 3α-hydroxy function and, in case of 17ß-hydroxy steroids with a secondary hydroxy group, the oxidation yielding a 17-keto function. Additionally many AAS are metabolized by cytochrom P-450 hydroxylation reactions, and steroids with hydroxy groups mainly at C-6ß, C-16α and C-16ß are produced. In the metabolism of stanozolol, a synthetic steroid with a condensed pyrazol ring on the steroid A-ring, further hydroxylation occurs at C-4ß and C-3′ of the heterocyclic ring (Fig. 24.5). In general in the course of phase I metabolism steroids are enzymatically transformed

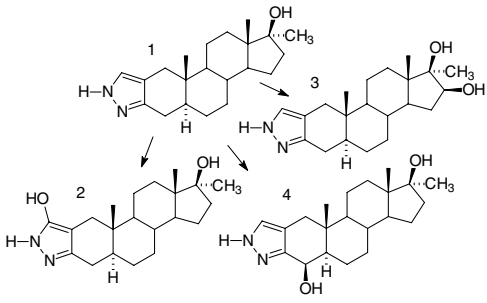


Fig. 24.5 The main metabolites in the metabolism of stanozolol (1), 3′-hydroxy-stanozolol (2), 16ß-hydroxystanozolol (3), and 4ß-hydroxystanozolol (4).

to more polar but pharmacologically inactive compounds. Phase I reactions are often followed by phase II processes, also known as phase II conjugation. In the case of AAS and their metabolites the reaction creates steroid conjugates with sulfate or glucuronic acid (Thevis *et al.* 2001). These highly polar compounds are then rapidly eliminated in the urine.

In the last ten years excretion studies performed with 17α-methyl steroids demonstrated that the metabolism of AAS is highly complex and the detection of more than 20 metabolites after administration of one single AAS is not unusual. Similar results regarding the high number of metabolites are already known for the metabolism of testosterone (Kochakian 1990).

24.5.2.2 Pharmacokinetics

A further important factor which has to be considered for detection of AAS is the pharmacokinetics of the parent compound and its excreted metabolites. As AAS are misused during training and the number of checks are limited, it is desirable to detect AAS as long as possible after their last administration. Analysis of the parent steroids and/or their metabolites, which are excreted very rapidly, is less effective for screening analysis than the detection of metabolites excreted long-term: these are steroids detectable for the longest possible period of time after administration (Schänzer 1996). The main differences between the pharmacokinetics of AAS are caused by their pharmaceutical preparation and the kind of application. Depot preparations, e.g. 19-nortestosterone injected intramuscularly as its undecanoate ester (Deca-Duraboline®), are detectable in urine for several weeks, whereas most oral preparations are completely eliminated within a few days after intake. Once they became aware of these scientific data, athletes switched their doping activities to AAS with short elimination times and to steroids which were believed to be undetectable.

24.5.2.3 Sample preparation

For sample preparation of anabolic steroids it has to be considered that most of the AAS and their metabolites are excreted in conjugated form. Following sample preparation unconjugated steroids can be separated by extracting an aliquot of urine (e.g. 2 ml) with a polar organic non-water miscible solvent. Based on their polar and acidic character, conjugated steroids are not extractable and remain in the aqueous layer. These conjugates (mainly glucuronides) can be liberated by enzymatic hydrolysis of the urine specimen. The enzyme used can be added directly to the urine or to an isolate obtained via an adsorber resin. Enzymatic hydrolysis is achieved completely using enzyme preparations with ß-glucuronidase from E.coli or ß-glucuronidase/arylsulfatase from Helix pomatia. The "free" steroids (conjugated fraction) are then extracted from the aqueous phase via a simple liquid extraction with tert-butyl methyl ether, or in case of less polar steroids, with an alkane (e.g. n-pentane).

The first analysis is a screening procedure by which all banned AAS are detected in one single analytical run. Suspicious samples are confirmed by a second aliquot of the same urine specimen, which is isolated using a substance-specific isolation technique.

24.5.2.4 Derivatization

Based on the polar groups of AAS (hydroxy and keto groups) high interactions with polar functions of the GC-column phase reduce the detectability of AAS at low concentrations. Derivatization of polar functions of AAS can lead to a distinct improvement in peak intensity and detection limit of the analytical method. The most frequently used derivatization methods are acylation (e.g. trifluoroacetylation) and silylation (e.g. trimethylsilylation). For doping analysis of AAS silylation is the method of choice and the introduction of a trimethylsilyl group to an AAS is the most common derivatization reaction, converting polar groups such as hydroxy and keto functions to less polar trimethylsilyl ethers with excellent GC behaviour. For this kind of derivatization a respectable reagent MSTFA (N-methyl-N-trimethylsilyltrifluoro-acetamide) was developed (Donike 1969). Additionally, the mass spectrum is generally changed to higher and more abundant molecular and fragment ions, which also improves the signal-to-noise ratio of the substance to be identified compared to the analytical and biological background. Therefore derivatization for GC-MS detection of substances isolated from biological fluids unequivocally yields a more accurate analytical result, which is an absolute requirement in view of the complex matrix and large number of possible interferences.

24.5.3 Detection of synthetic anabolic androgenic steroids

In some instances AAS are differentiated into endogenous and exogenous AAS according to their route of administration. The term "synthetic" should amplify the fact that these AAS are not produced in the body, they are chemically synthesized and can only enter the circulating blood system by exogenous application. AAS which are naturally synthesized in the glands of mammalian cells are called endogenous steroids, even though their application can be exogenous. As synthetic AAS and/or their metabolites are not present in the human organism, their identification in a urine sample of an athlete constitutes the misuse of a banned steroid. The criteria for identification of a substance are based on the analytical method applied.

In GC-MS identification of synthetic AAS obtained from a urine specimen it is mandatory to register a full mass spectrum or a selected ion monitoring (SIM) profile of the main abundant fragment ions. The mass spectrometrical data (MS spectrum or SIM profile) of the isolated substance should be in accordance with an authentic synthesized reference substance or, in the event that a synthesized reference metabolite is not available, with a well-characterized metabolite from an excretion study with the corresponding AAS. In addition to the MS data, the GC retention time of the isolated steroid has to agree with the GC retention time of the reference substance. For this purpose reference metabolites of frequently *misused* AAS but not commercially available were synthesized (Schänzer and Donike, 1993).

As an example Fig. 24.6 shows the criteria for a positive sample for a long-term excreted metabolite of metandienone:

- registration of a full mass spectrum which can be compared with the reference spectrum or
- in case of low concentrations, a selected ion monitoring (SIM) profile with the main intense fragment ions of the metandienone metabolites 17,17-dimethyl-18-nor-5ß-androst-1,13-dien-3α-ol.

To increase the efficiency of AAS misuse testing and to detect AAS for a longer period of time after administration more selective and sensitive MS techniques were used during the last decade. The main improvements were first, installation of more sensitive and selective mass spectrometers, and secondly, by substance-specific sample preparation (Schänzer *et al.* 1996). The use of high resolution mass spectrometry (HRMS) was announced to the public at the Olympic Games 1996 in Atlanta. This technique was established after 1992 in a few IOC-accredited laboratories. The advantage of HRMS became apparent before Atlanta when, during doping testing by the International Weightlifting Federation, more than forty athletes were confirmed positive only by HRMS and not by the conventional MS technique. Following these results the IOC decided that it was neccessary that accredited laboratories use more sophisticated equipment, such as HRMS or MS/MS.

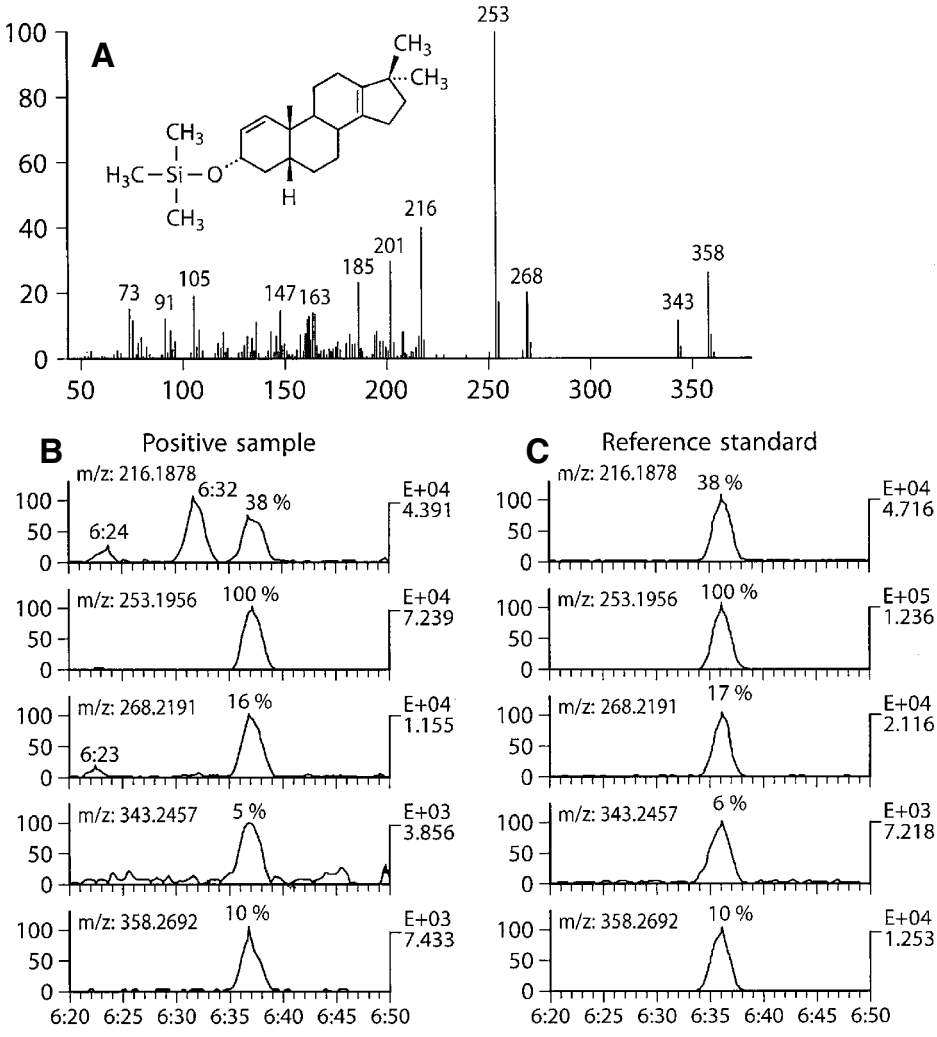


Fig. 24.6 Criteria for a positive confirmation: 1. The registrated EI mass spectrum (e.g. mass spectrum of an isolated metabolite of metandienone: 17,17-dimethyl-18-nor-5ß-androsta-1,13-dien-3α-ol TMS (A) has to be in accordance with the mass spectrum of an authentic reference substance, or 2. The main abundant fragment ions of the isolated substance show similar intensities (B) when selected ion monitoring (SIM) registration is applied in comparison to the intensities of the same fragments of the reference compound (C).

The basic principle of HRMS is that elements do not have an integral number of atomic weight but a decimal form. Only carbon, as the reference element, has an integral number of 12 as its atomic weight. Thus hydrogen does not have an atomic weight of 1 but 1.00783, nitrogen the weight of 14.00307, and oxygen the weight of 15.99491. Molecular fragments with the same integral number of mass, e.g. the

fragment ions $C_3H_6O^+$ and $C_3H_8N^+$ both have the rounded mass 58 but the exact calculated mass of 58.04186 for $C_3H_6O^+$ and 58.06567 for $C_3H_8N^+$. Neither mass fragments can be separated by conventional (low resolution) mass spectrometry but only by using high resolution MS with a resolution of 2500. Thus in practical terms, in this example the instrument (HRMS) can be set to detect only the signal of the mass of 58.04186 for $C_3H_6O^+$, and all masses differing by more than 0.0024 masses, such as 58.06567 for $C_3H_8N^+$, will be discriminated. Based on this fundamental physical principle HRMS analysis of AAS steroids and their metabolites isolated from urine reduces the biological background and increases the signal-to-noise ratio, yielding a much higher selectivity in screening and confirmation.

24.5.4 Detection of endogenous anabolic androgenic steroids

24.5.4.1 Indirect detection methods

The misuse of testosterone by athletes is also tested by GC-MS analysis of urinary extracts. However, the method reveals only the presence of testosterone and its ratio to epitestosterone. The mass spectrometrical data alone indicate whether testosterone originates exogenously (doping) or whether it was produced endogenously. In 1983 Donike *et al.* developed a method to calculate urinary excreted testosterone by a ratio to 17-epitestos-terone. Both isomeric steroid hormones are excreted mainly as glucuronides which are enzymatically hydrolyzed before GC-MS analysis. The urinary testosterone/epitestosterone ratio (T/E ratio) represents a relatively constant factor within an individual and alterations under physical excercise have not been noted. Exogenous application of testosterone results in an increase in the urinary concentration of testosterone glucuronide, whereas epitestosterone glucuronide is not influenced. Based on measurements of large reference groups Donike proposed a T/E ratio of 6:1 as a marker to handle a urine specimen suspicious for testosterone misuse. An increased T/E value ($T/E > 6$) is not immediately considered as a positive sample. Following the WADA rule the athlete has to be further investigated and it has to be determined that the increased value is not caused by physical or pathological conditions. In practice this requires several test samples of the athlete and evaluation of previous tests in order to establish the athlete's individual T/E reference values (subject-based reference values). The test sample is considered positive when the tested T/E ratio clearly exceeds the subject-based reference values ($>$ mean $+$ 3 standard deviations) of the athlete. In addition to the T/E ratio the testosterone and epitestosterone concentrations as well as the concentrations of the main testosterone metabolites are assessed.

Doping with dihydrotestoserone (DHT) became public knowledge after the Asian Games in 1994 when seven athletes were tested positive for DHT misuse. The criteria for DHT doping are also based on statistical methods and population-based

reference values with limits for the ratios of DHT/epitestosterone, epitestosterone, DHT/etiocholanolone, 5α-androstane-3α, 17ß-diol/5ß-androstane-3α,17ß-diol, and androsterone/etiocholanolone established (Kicman *et al.* 1995; Donike *et al.* 1995).

The main weakness of all the methods confirming doping with endogenous AAS is the application of statistical parameters. These methods are therefore indirect methods and they only confirm that an increased value varies from the normal values of the athlete. These methods do not identify any physical characteristics of the exogenous steroid differing from the steroid produced endogenously as direct proof of doping.

24.5.4.2 Direct detection method: gas chromatography – combustion – isotope ratio mass spectrometry (GC-C-IRMS)

The T/E ratio results can be supported by gas chromatography-combustion-isotope ratio mass spectropmetry (GC-C-IRMS). This method was first introduced by Becchi *et al.* in 1994 and has been adopted by other research groups (Aguilera *et al.* 1996; Horning *et al.* 1997; Shackleton *et al.* 1997) with distinct modifications. The principle of IRMS is the precise measurement of the $^{13}C/^{12}C$ isotope ratio of organic compounds. This method became practical for trace analysis in doping control when instruments combining gas chromatography and isotope ratio mass spectrometry were developed. Isotopes are elements with the same number of protons but different numbers of neutrons. Carbon occurs in three kinds of isotopes: ^{12}C (6 protons and 6 neutrons) with a frequncy of approximately 98.9%, ^{13}C (6 protons and 7 neutrons) at a rate of 1.1% and ^{14}C (6 protons and 8 neutrons), a radioactive isotope with a half-life of 5760 years (used in determination of age), in traces. In the course of synthesizing organic compounds ^{12}C atoms react slightly faster than ^{13}C atoms. This effect results in a reduction of the ^{13}C amount compared to ^{12}C. The $^{13}C/^{12}C$ ratio is calculated in promill [$\delta^{13}C(‰)$] relative to a reference gas with a standardized $^{13}C/^{12}C$ ratio. The δ-value becomes more negative when the ^{13}C portion is reduced, as was explained during synthetic pathways. For isotope measurement urinary excreted steroids have to be isolated to high purity. Most research groups use HPLC separation of steroids, or isolation of steroidal diols is performed via the Girard reagent (Shackleton *et al.* 1997). For gas chromatographic separation derivatization is applied using acetylation of steroids with the aim to improve GC peak shape or analysis refers to the underivatized steroids.

Steroids are separated by gas chromatography followed by complete oxidation to carbon dioxide in a combustion chamber. The carbon dioxide is then introduced to the mass spectrometer where the exact masses m/e 44 for $^{12}CO_2$ and m/e 45 for $^{13}CO_2$ are independently registered. For this kind of isotope ratio measurement a minimum of 5–10 ng of a steroid has to be used to obtain precise data. The $^{13}C/^{12}C$

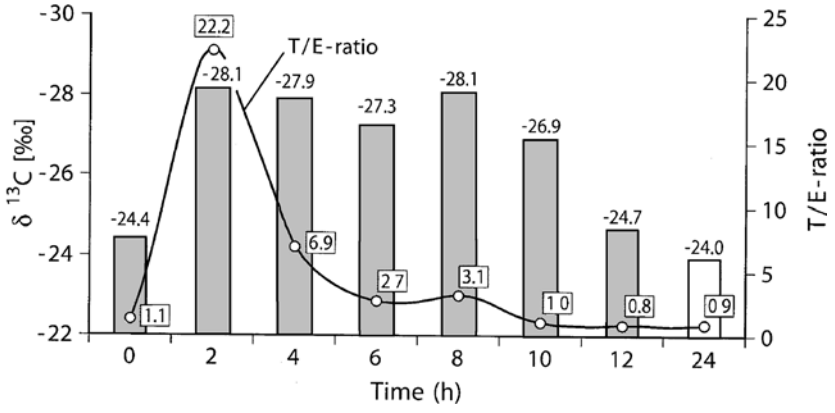


Fig. 24.7 Testosterone determined in urine after oral application of 40 mg of Andriol® (testosterone undecanoate): T/E ratio (line) and carbon isotope ratio mass spectrometry (column).

ratio can be estimated with an accuracy of ± 0.0002% (± 0.2 permill to the $^{13}C/^{12}C$ ratio of the reference gas).

Fig. 24.7 presents data of GC/C/IRMS and T/E ratio analysis after oral administration of 40 mg of testosterone undecanoate (Andriol®) to a single male volunteer. A direct proof of exogenous testosterone application is possible as the δ-values are decreased to −28 ppm after administration, in comparison to −24 ppm before intake and at the end of the elimination curve. It is also obvious that the δ-values are still decreased when the T/E ratio drops below six and is close to the normal value. This method can therefore also be used when ethnic differences influence testosterone metabolism, e.g. in Asians who have low T/E ratios and when a testosterone application will not necessarily exceed the T/E ratio of six (de la Torre *et al.* 1997). Exogenous testosterone also influences the $^{13}C/^{12}C$ ratio of the metabolites of testosterone. Based on these data it was proved that precursors within the synthetic pathway of testosterone, such as pregnanediol, pregnanetriol (metabolites of progesterone and 17α-hydroxyprogesterone) and cholesterol are not influenced by exogenous testosterone, whereas testosterone and its metabolites have decreased δ-values indicating exogenous application. The results of a positive testosterone finding are presented in Fig. 24.8. The T/E-ratio of the positive urine sample was 14.7. Following the rules, the athlete was further investigated and 10 urine samples were collected over a period of two days and analyzed. The T/E ratio during this study was 1.0 ± 0.1 and confirmed that the sample with a T/E ratio of 14.7 was not in accordance with endogenous production of testosterone and was considered as an offence against the doping regulations. The IRMS data of the corresponding positive urine sample (Fig. 24.8) show the decreased values for testosterone and the metabolites androsterone and etiocholanolone, whereas the higher δ-values of

		Positive sample	Endocrinological study
Indirect method	T/E-ratio	14.7	1.0*
	$^{13}C/^{12}C$ Isotope ratio in δ [‰]		
Direct method	Cholesterol	-24.0	-24.4
	Pregnanediol	-24.0	-24.2
	Testosterone	-29.1	-25.1
	Androsterone	-29.2	-25.0
	Etiocholanolone	-29.8	-25.9

*T/E-ratio 1.0 ± 0.10, n = 10 samples

Fig. 24.8 T/E-ratio and IRMS results of a testosterone positive urine sample and urine sample of the same athlete obtained during an endocrinological study.

the precursors and the values obtained from the endocrinological study were in the same range.

It was also suggested to detect testosterone misuse by analysis of testosterone esters in blood (de la Torre 1995), but this method is limited to the application of testosterone esters in the form of injectable preparations and is not applicable to the analysis of urine samples.

The isotope ratio mass spectrometry method can additionally be applied to detect and identify doping with other endogenous AAS such as dihydrotestosterone and dehydroepiandrosterone, where reliable methods are less efficient or not available.

24.6 Key messages

- Misuse of androgens in competitive sport has been banned since 1974 and is tested by IOC and WADA accredited laboratories.
- Androgens are used by athletes during training to improve muscle strength. For this reason doping tests have been extended to out-of-competition tests.
- Non-therapeutical hormones such as prohormones of testosterone and nortestosterone have been marketed as nutritional supplements since 1999 in many countries e.g. United States.
- Positive doping cases have been proved to originate from the use of nutritional supplements "contaminated" with prohormones of nortestosterone.
- Testosterone, nortestosterone, stanozolol and metandienone represent the most frequently misused AAS in controlled sports.
- Androgens are detected and identified by gas chromatographic / mass spectrometric analysis of urinary extracts.

- Derivatization methods for steroid analysis improve detection limits for anabolic steroids.
- Synthetic androgens are extensively metabolized and doping tests are focused on urinary excreted metabolites.
- Doping with endogenous steroids is controlled by indirect methods, e.g. testosterone misuse is tested by a ratio of testosterone to epitestosterone (6:1). Positive findings are followed by additional studies to exclude physiological and pathological influences.
- Recently direct methods, such as gas chromatography-combustion-carbon isotope ratio mass spectrometry have become available to identify doping with endogenous steroids unambiguously.

24.7 REFERENCES

Aguilera R, Becchi M, Casabianca H, Hatton CK, Catlin DH, Starcevic B, Pope HG Jr (1996) Improved method of detection of testosterone abuse by gas chromatography/combustion/isotope ratio mass spectrometry analysis of urinary steroids. J Mass Spectrom 31:169–176

Becchi M, Aguilera R, Farizon Y, Flament MM, Casabianca H, James P (1994) Gas chromatography/combustion/isotope-ratio mass spectrometry analysis of urinary steroids to detect misuse of testosterone in sport. Rapid Commun Mass Spectrom 8:304–308

Beckett AH, Cowan DA (1979) Misuse of drugs in Sport. Brit J Sports Med 2:185–194

Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunell TJ, Tricker R, Shirazi A, Casaburi R (1996) The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. J Med 335:1–7

Brand WA (1996) High precision isotope ratio monitoring techniques in mass spectrometry. J Mass Spectrom 31:225–235

Brooks RV, Firth R, Summer NA (1975) Detection of anabolic steroids by radio immunoassay. Br J Sports Med 9:89–92

Canadian Centre for Drug-Free Sport (1993) News Release – Over 80,000 young Canadians using anabolic steroids, Montreal

Clasing D (1992) Doping – verbotene Arzneistoffe im Sport. Gustav Fischer Verlag, Stuttgart – Jena – New York

Delahaut PH (1997) Immunoassay and immunoaffinity chromatography for the detection of drug residues. In Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds) Proceedings of the 14th Cologne Workshop on Dope Analysis 1996, Sport und Buch Strauß, Köln, 275–283

de la Torre X, Segura J, Polettini A, Montagna M (1995) Detection of testosterone esters in human plasma. J Mass Spectrom 30:1393–1404

de la Torre X, Segura J, Yang Z, Li Y, Wu M (1997) Testosterone detection in different ethnic groups. In Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds) Proceedings of the 14th Cologne Workshop on Dope Analysis 1996, Sport und Buch Strauß, Köln, 71–89

Donike M (1969) N-Methyl-N-trimethylsilyl-trifluoracetamid, ein neues Silylierungsmittel aus der Reihe der silylierten Amide. J Chromatogr 42:103–104

Donike M, Bärwald KR, Klostermann K, Schänzer W, Zimmermann J (1983) Nachweis von exogenem Testosteron. In Heck H, Hollmann W, Liesen W, Rost R (eds) Sport: Leistung und Gesundheit, Deutscher Ärzte Verlag, Köln, 293–298

Donike M, Zimmermann J, Bärwald KR, Schänzer W, Christ V, Klostermann K, Opfermann G (1984) Routine Bestimmung von Anabolika im Harn. Deutsch Z Sportmed 1:14–23

Donike M, Ueki M, Kuroda Y, Geyer H, Nolteernsting E, Rauth S, Schänzer W, Schindler U, Völker E, Fujisaki M (1995) Detection of dihydrotestosterone (DHT) doping: alteration in the steroid profile and reference ranges for DHT and its 5α-metabolites. J Sports Med Phys Fitness 35:235–250

Duchaine D (1989) Underground steroid handbook II. Technical Books, Venice, USA

Franke WW, Berendonk B (1997) Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. Clin Chem 43:1262–1279

Geyer H, Parr MK, Mareck U, Reinhart U, Schrader Y, Schänzer W (2003) Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroid – An international study. Submitted for publication

Grundig P, Bachmann M (1995) World anabolic review 1996. Sport Verlag Ingenohl, Heilbronn

Handelsman DJ, Gupta L (1997) Prevalence and risk factors for anabolic-androgenic steroid abuse in Australian high school students. Int J Androl 20:159–164

Haupt HA, Rovere GD (1984) Anabolic steroids: A review of the literature. Am J Sports Med 12:469–484

Horning S, Geyer H, Machnik M, Schänzer W, Hilkert A, Oeßelmann J (1997) Detection of exogenous testosterone by $^{13}C/^{12}C$ analysis. In Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U (eds) Proceedings of the 14th Cologne Workshop on Dope Analysis 1996, Sport und Buch Strauß, Köln, 275–283

Kamber M (1995) Mitteilung in Doping – Information und Prävention. Magglingen 7:4–7

Kicman AT, Coutts SB, Walker CJ, Cowan DA (1995) Proposed confirmatory procedure for detecting 5α-dihydrotestosterone doping in male athletes. Clin Chem 41:1617–1627

Kicman AT, Bassindale T, Cowan DA, Dale S, Hutt AJ, Leeds AR (2003) Effect of androstenedione ingestion on plasma testosterone in young women; a dietary supplement with potential health risks. Clin Chem 49:167–9

Kochakian CD (1976) Anabolic-androgenic steroids. Springer Verlag, Berlin Heidelberg New York

Kochakian CD (1990) A steroid review: Metabolite of testosterone; significance in the vital economy. Steroids 55:92–97

Leder BZ, Longcope C, Catlin DC, Ahrens B, Schoenfeld DA, Finkelstein JS (2000) Oral androstenedione administration and serum testosterone concentrations in young men. JAMA 283:779–782

Massé R, Ayotte C, Dugal R (1989) Integrated methodological approach to the gas chromatographic mass spectrometric analysis of anabolic steroid metabolites in urine. J Chromatogr 489:23–50

Schänzer W, Donike M (1993) Metabolism of anabolic steroids in man: Synthesis and use of reference substances for identification of anabolic steroid metabolites. Anal Chim Acta 275:23–48

Schänzer W (1996) Review – Metabolism of anabolic androgenic steroids. Clin Chem 42:1001–1020

Schänzer W, Delahaut P, Geyer H, Machnik M, Horning S (1996) Longterm detection and identification of metandienone and stanozolol abuse in athletes by gas chromatography/high resolution mass spectrometry (GC/HRMS). J Chormatogr B 687:93–108

Shackleton CH, Phillips A, Chang T, Li Y (1997) Confirming testosterone administration by isotope ratio mass spectrometric analysis of urinary androstanediols. Steroids 62:379–387

Taylor WN (1982) Steroids and the athlete. McFarland & Company, London

Thevis M, Opfermann G, Schmickler H, Schänzer W (2001) Mass spectrometry of steroid glucuronide conjugates. I. Electron impact fragmentations of 5a-/5ß-androstan-3a-ol-17-one glucuronides, 5a-estran-3a-ol-17-one glucuronide and deuterium-labelled analogues. J Mass Spectrom 36:159–168

Wade N (1972) Anabolic steroids: Doctors denounce them, but athletes aren't listening. Science 176:1399–1403

Ward RJ, Lawson AM, Shackleton CHL (1975) Screening by gas chromatography-mass spectrometry for metabolites of five commonly used anabolic steroid drugs. Br J Sports Med 9:93–97

Wilson JD (1988) Androgen abuse by athletes. Endocr Rev 9:191–199

Wilson JD (1996) Androgens. In Hardmann JG, Limbird IE, Molinoff PB, Ruddon RW, Goodman Gilman A (eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th edition, McGraw Hill, New York, 1441–1457

Yesalis CE, Kennedy NJ, Kopstein AN, Bahrke MS (1993) Anabolic-androgenic steroid use in the United States. JAMA 270:1217–21

Zimmermann J (1986) Untersuchungen zum Nachweis von exogenen Gaben von Testosterone. Thesis, German Sports University, Cologne, Hartung-Gorre Verlag, Konstanz

Subject index