Nutrition

in Thalassaemia & Pyruvate Kinase Deficiency

A GUIDELINE FOR CLINICIANS

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THALASSAEMIA INTERNATIONAL FEDERATION

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**“The good physician treats the disease;
the great physician treats
the patient who has the disease.”**

Sir William Osler (1849-1919)

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FOREWORD

On behalf of the Board of Directors of the Thalassaemia International Federation (TIF), it is my great privilege to write the foreword to the 'Nutrition in Thalassaemia & Pyruvate Kinase Deficiency: A Guideline for Clinicians'.

There is much scattered literature concerning nutrition in the congenital anaemias. These conditions have a much broader field of nutritional and metabolic effects compared to the acquired deficiency anaemias which specifically refer to correcting the deficiency. In congenital anaemias there are influences both from possible nutritional deficiencies, the influence of medication (especially chelating agents), possible nutrient toxicities and /or benefits, and the emergence of complications which possibly have nutritional factors in their causation and may, through organ damage, such as liver damage, influence nutritional factors and further metabolic effects.

These complex influences may have consequences on the daily food intake. Both patients and their physicians request dietary guidance, hoping both to avoid harm and to increase benefit from foodstuffs. Through the various research publications it is difficult for physicians to have a clear guideline leading to precise dietary advice to individual patients.

The congenital anaemias, especially in their most severe clinical phenotypes, require a clear understanding of the underlying mechanisms of how diet influences disease. Moreover, since individual patients have different degrees of organ damage in the course of their lifelong and continually changing exposure to toxic effects such as caused by anaemia or iron oxidative stress, the assessment and advice must be individualised. 'One size does not fit all'.

Patients, in their quest for guidance, have two possible sources of reliable information: a dietician who knows well the haemolytic anaemias and those caused by ineffective erythropoiesis, or a physician/haematologist who knows well both the anaemias and the dietary influences. Both these specialists are sometimes hard to come by!

The Thalassaemia International Federation, true to its long tradition of producing guidelines and educational material for healthcare professionals, has requested experts in the field of nutrition to bring into a single publication a synopsis of the long list of research and clinical facts concerning nutrition in two important haematological syndromes: the beta thalassaemias and pyruvate kinase deficiency. These two conditions are representative of the many rare hereditary anaemias for which there is scant literature or advice available.

The objective is to produce a critical review of what is known about nutrition in these conditions to be used as a reference guide, mainly for the haematologist who is not experienced in the issues of diet yet has to answer questions often without the benefit of a dietitian. On the other hand such a review may also be of assistance to dietitians who must answer questions concerning diet in hereditary complex conditions which may be rare and seldom met in their daily practice.

In this publication the authors have brought together current knowledge and have attempted a clear guide which will enhance clinical practice in which patient wellbeing is the sole objective.

TIF wishes to thank the two scientists-nutritionists Dr Ellen Fung and Mrs Tamara Schryver, who are the lead authors/editors as well as the long list of physicians led by Dr Michael Angastiniotis, TIF Medical Advisor, who have contributed to this publication. It is only through their hard work and undivided dedication that this valuable resource reached to a completion. Deep appreciation and sincere respect go to Drs Anne Yardumian and Androulla Eleftheriou, TIF Executive Director for their overall support, coordination and invaluable contribution in the completion of this publication.

On behalf of the Board of Directors of the Thalassaemia International Federation (TIF)

Panos Englezos
President

CHAPTER 1
Introduction to this book

Most patients with thalassaemia and pyruvate kinase deficiency [PKD] require significant clinical intervention to promote or maintain optimal health. This usually involves frequent clinical tests and monitoring, repeated red blood cell transfusions and chelation therapy for some, and management of complications. The frequency of medical tests and procedures, the timing of red blood cell transfusions, when to initiate chelator medication, as well as the type, dose and regimen are typically decided upon by their physician in consultation with the patient. The management is well described in international Guidelines (Cappellini et al, 2021; Taher et al, 2017) adapted to each individual's needs, in consultation with the patients themselves.

The close relationships built between patients with these disorders and treating physicians and other relevant health care professionals across medical disciplines allow for management and lifestyle discussions, well beyond the basic concerns, including nutrition and physical activity. However, the training most clinicians receive in nutrition is basic, and this text aims to build on that to enable them better to engage in discussion with their patients about it, and to help decide when referral for specialist dietetic assessment and management is required.

Caregivers and most patients with these conditions are highly motivated to change their dietary patterns to optimise outcomes related to their loved ones' or own health, but many face obstacles that clinicians should acknowledge. Barriers to healthy eating are embedded within the socioecological model – see figure 1.1 (Zorbas et al, 2018).

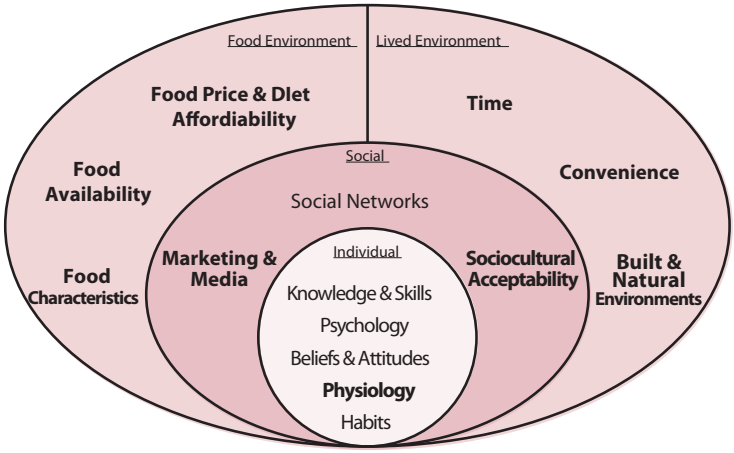

Figure 1.1. *Summary of themes derived from data synthesis, represented according to the socioecological model.* Size indicates the relative importance of a factor; boldface text indicates that a factor is more commonly identified as a barrier, rather than a facilitator or neutral factor, to healthy eating.

Understanding these barriers enables the clinician to have a more thorough discussion with the patient and to make appropriate referrals, as necessary, to a registered dietitian, social worker, or to offer other appropriate resources to enhance the likeliness of successful dietary changes. Lifestyle changes, even with motivated patients, can be difficult to make and maintain so every effort is needed to support people through these changes.

There is an abundance of nutrition information available to patients online from numerous sources. Not all the information available is reputable, some of it contradictory, and – in a few instances - potentially dangerous for patients with these blood conditions. On the other hand, literature focused on nutrition specifically for patients with thalassaemia and PKD is sparse. The purpose of this guideline is to provide the scientific basis for optimal "nutritional health" in patients with these conditions. Over the past few decades, as many basic essential clinical concerns have been addressed , healthcare professionals have focused more attention to quality of life concerns for patients, including nutritional needs, and numerous small independent studies have been published (Goldberg et al, 2022). For this book, the literature has been extensively reviewed and summarised, and where there is little information available specifically relating to these conditions, expert opinion is provided.

It is recognised that there are unique dietary practices around the world, and country-specific food fortification programs. This guideline therefore does not aim to provide one prescription for optimal nutrition, but to give a framework from which to develop personalised nutritional plans. For patients with thalassaemia, few studies explicitly compare the nutritional status of transfusion dependent vs non-transfusion dependent patients; where available, these data will be reviewed and summarised. In some cases, inferences will be drawn from our understanding of the nutritional requirements of individuals without thalassaemia and PKD.

Given the scant nutritional recommendations available in published literature for these at-risk patient populations, this text is intended to serve multiple purposes. First, it will be a resource to paediatricians and haematologists who regularly care for patients with thalassaemia and PKD. For clinicians who rarely care for patients with thalassaemia or PKD, and also for dietitians and nutritionists - who may be well versed on how to address nutritional issues in the general populations and across some medical conditions, but might be unfamiliar with these conditions including their frequent complications and the medications frequently prescribed – it will provide background and understanding of aspects of these conditions which place additional stressors on nutrition and metabolism.

The text is organised by first providing a brief overview of the thalassaemias and PKD. Nutritional status in relation to iron overload and chelation will be reviewed, followed by a discussion of the impact nutrition has on the development of, or treatment for, complications frequently observed in thalassaemia including diabetes, low bone mass, poor growth, delayed pubertal development, cardiomyopathy, nephrolithiasis and dyslipidemia. For PKD, common complications that result from iron overload and chronic anaemia include endocrinopathies such as diabetes, low bone mass, pulmonary hypertension, cholelithiasis, and thrombosis. The steps involved in comprehensive nutrition assessment are provided followed by an in-depth discussion of our current understanding of both macro and micro-nutrient nutrition. Nutraceuticals, botanicals and nutritional supplements report-

edly consumed by patients will also be discussed. Given the symbiotic relationship between nutrition and physical activity, studies that have explored the importance of exercise in these conditions will also be summarised. The final chapter will focus on recommendations for nutritional monitoring and, where there is sufficient evidence to support the practice, suggestions for supplementation will be made.

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CHAPTER 2

Thalassaemias and Pyruvate Kinase Deficiency – overview of the conditions

This book deals with two serious congenital conditions which, if not diagnosed early and appropriately managed, lead to severe anaemias affecting and damaging vital organs and every aspect of the patients' lives. The lifelong nature of the conditions, and the need for continued medical intervention for many, result in a considerable burden to patients, families and health services. The thalassaemia syndromes are more common (Weatherall, 2010) while conditions like pyruvate kinase deficiency (PKD) are relatively rare (Secrest et al, 2020). These are autosomal recessive disorders with a wide global distribution.

In these conditions, increased destruction of red cells, and/or premature release of immature forms, result in anaemia. Clinical complications arise from this and from the management of the conditions, including red blood cell transfusions which can lead to iron overload and tissue damage. Iron chelation used to combat this can, among other adverse effects, lead to the loss of micronutrients. For some, intake of low nutrient dense foods, and insufficient or excessive dietary intake, coupled with higher energy needs and altered hormone sensitivity complicate the condition (Fung et al, 2012).

Overview of the thalassaemias

Thalassaemia refers to a group of inherited conditions whose basis is reduced or absent production of globin chains, components of the haemoglobin molecule, because of issues in the respective genes coding for them. Haemoglobin is the vital oxygen-carrying molecule contained within the red blood cells, consisting of proteins surrounding a heme molecule with a central iron. The protein part is composed of paired globin chains, the main ones being α and the β globin chains, while minor components in post-natal life are γ and δ globin chains. Thalassaemias are named after the globin which is underproduced or lacking – β globin in the case of β thalassaemias and α globin in the α thalassaemias.

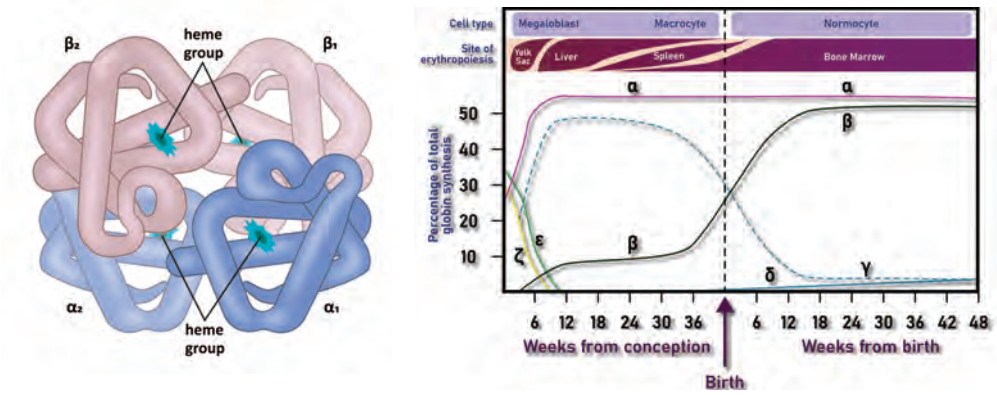

Figure 2.1

HbA, adult haemoglobin, is made up of a balance of two α and two β chains (α2β2). Balanced numbers of α and β globin chains are necessary to form a complete oxygen-carrying molecule. Any significant reduction in the synthesis of either leads to an accumulation of the other, normally-produced globin type. This has serious consequences to the production of functional haemoglobin, and the production of healthy durable red cells. The main damage is to the young red cells in the haemopoietic tissue, as the accumulated protein (α globin chains in the case of β thalassaemia, or β globin chains in the case of α thalassaemia) causes varying degrees of damage to the developing red cell.

β thalassaemias

The prevalence of β thalassaemia asymptomatic heterozygotes, or healthy carriers, varies from 0.1% to around 20% of some populations, with over 40,000 affected births – infants who inherit a thalassaemic β globin gene from both parents - annually worldwide. HbE, a thalassaemic variant haemoglobin which is very common in some Asian populations, contributes to this high birth frequency (Modell and Darlison, 2008; Colah et al, 2010) as if co-inherited with a β thalassaemia gene it can give rise to a similar clinical condition. Alpha [α] thalassaemia is more widespread geographically, but most carry the single gene deletion (α+) with little clinical consequence, whereas the less frequent α^0 mutations which may cause HbH disease and hydrops fetalis are more confined (Hockham 2019). High carrier rates of the various thalassaemia-causing genes are in a global belt of the tropical and subtropical regions coinciding with the regions where malaria is, or was previously, endemic. In some localised populations, clinically affected individuals may be as many as 1 in 1000 - 2000 of the population, examples being Cyprus, Maldives and tribal areas of India (figures extracted from TIF database).

Pathophysiology

In β thalassaemia the excess α chains accumulate as insoluble complexes in the red cell precursors, causing oxidative damage to the cell membrane and early cell death (apoptosis): this is termed ineffective erythropoiesis.

Ineffective erythropoiesis causes:

- Anaemia, due to reduction in mature red blood cells following death of erythroid precursors.
- Compensatory increase in red cell production, through erythropoietin-induced stress erythropoiesis. The bone marrow of patients with β thalassaemia major contains 5-6 times more erythroid precursors than that of healthy controls. Unless corrected by transfusion, this leads to medullary expansion with bone deformities and destruction of bone micro-architecture, and can cause masses of extramedullary haemopoietic tissue.
- Increased destruction of circulating abnormal red cells by macrophages, mainly in the spleen, causing splenomegaly which may then 'trap' other blood components, platelets and white cells and reducing their numbers - this is called hypersplenism.
- Hepatomegaly due initially to extramedullary haemopoiesis, but which can also later be caused by heart failure or liver disease.
- Increased production of erythroid progenitor cells requires an increased iron supply, so there is suppression of hepcidin and increased absorption of iron
- Excessive metabolic burden which can result in poor growth.

Genotype/phenotype relationships

The severity of these pathophysiological effects, and the clinical severity of the condition for each individual, varies mainly according to the degree of globin chain imbalance as expressed by the ratio of α / non-α globin chains. A main factor determining the degree of chain imbalance is the genotype of each individual patient:

- There are over 350 reported mutations in beta globin genes, and novel mutations are continuously emerging through DNA sequencing [Ithanet database; Thein, 2018]. According to the amount of beta globin chain production produced, mutations are described as β^0, β^+, β^{++}. The combination of these inherited by an individual contributes largely to the clinical severity of the resulting condition – from mild microcytic anaemia needing little or no red cell transfusion, through to fully transfusion dependent thalassaemia [TDT].
- In addition, modifier genes may be co-inherited which can alter the α/ non-α ratio; a common example of this is the co-inheritance of an α thalassaemia gene in people with β thalassaemias, which reduces the excess α globin chains and ameliorates the condition.
- Co-inheritance of variants which increase the synthesis of fetal haemoglobin (HbF, α2γ2) will also modify the chain imbalance since γ globin chains partially compensate for the β chain deficit. The main examples of genes involved in γ gene expression include hereditary persistence of fetal Hb (HPFH) and some loci outside the β-globin cluster.

Predicting phenotype from genotype is not always accurate, and this may complicate genetic counselling. However, knowledge of the genotype along with possible modifiers may help the planning of clinical management and potentially direct the choice of new therapies such as gene replacement and editing.

Gene variants causing thalassaemia syndrome

1. HbE is a common structural variant in Asian populations. It is produced in reduced amounts, and homozygotes show mild globin-chain imbalance (Fucharoen and Weatherall, 2012). Clinically significant forms occur when there is double heterozygosity with β thalassaemia mutations. In populations where HbE is common, thalassaemia mutations are also common. HbE β thalassaemia gives a condition of highly variable clinical severity, ranging from mild anaemia to full transfusion dependency. HbE β^0 thalassaemia is the most severe genotype (Suriyun et al, 2022). However, individuals with the same genotype may experience different clinical severities, so as yet unknown factors may be involved.
2. Hb Lepore is a fusion of δ and β globin genes resulting in δ-β globin fusion chains. These variants are significantly under-produced and thus behave like β-thalassaemias (Goncalves et al, 2002). They can cause clinically significant thalassaemia syndromes, both in the homozygous state and in compound heterozygosity with β thalassaemia.
3. Other rare variants e.g. Hb Knossos

Metabolic issues resulting from the pathophysiology

- Anaemia – tissue hypoxia with long-term effects on energy level, growth and exercise tolerance. Limited activity affects bone health, and untreated severe anaemia will result in heart failure.
- Cell destruction – folic acid deficiency is a consequence of increased folate requirement to support red cell metabolism, caused by increased erythropoiesis and apoptosis.
- Red cell breakdown results in increased free haemoglobin and heme in the circulation, causing activation of immune cells. There are detoxification mechanisms (haptoglobin binding haemoglobin, and haemopexin binding heme porphyrin) but these may be overwhelmed, and unbound heme can cause inflammation which may affect the vascular epithelium and cause vascular dysfunction (Vinchi et al, 2021).
- Increased absorption of iron - iron absorption is largely controlled by a hormone produced in the liver, hepcidin, which reduces iron absorption and bioavailability. Erythroid factors secreted by apoptotic erythroid cells are increased in hyperplastic states such as thalassaemia, and these inappropriately suppress hepcidin, so facilitating iron absorption. Iron overload overwhelms the capacity of transferrin, the main transport protein, to bind and detoxify iron. Non-transferrin-bound fractions of plasma iron (NTBI) promote the generation of malonyldialdehyde and free hydroxyl radicals, propagators of oxygen-related damage (Ribeil 2013). Highly reactive Fe2+ subspecies of NTBI, labile plasma iron (LPI), can enter cells through calcium channels. Reactive oxygen species (ROS) are produced, causing oxidant damage to mitochondria, membrane lipids and nucleic acids and leading to cellular dysfunction and death, fibrosis, and necrosis in tissues such as the myocardium, the liver, and endocrine glands.
- Immune system - iron homeostasis also influences the immune system including altered function of macrophages.
- Disruption of bone micro-architecture – the expanded erythropoietic tissue causes mechanical damage to bone tissue, with destruction of medullary trabecular and cortical thinning, and also a disturbance of bone metabolism with increased bone resorption and bone disease, manifesting as increased propensity to fracture, deformities and bone pain (Charoenngam et al, 2021).

- **Pigment gallstones** are prominent in all the congenital anaemias where haemolysis is a prominent feature.

The effects of treatment

While in non-transfusion dependent β thalassaemia [NTDT] these problems continue and increase over time, in transfusion-dependent conditions they are modified by treatment interventions (Kamperidis et al, 2021; Chapin et al, 2022). The basic treatment consists of red cell transfusions, and iron chelation to control the effects of iron overload. Effective treatment has been well defined by clinical investigation and experience, and guidelines are published aiming to ensure optimal outcomes for each individual (Cappellini et al, 2021; Taher et al, 2023). However, these standards are variably adhered to in practice; this may be due to limited resources, poor public health and medical infrastructure, clinical inexperience, poor patient adherence to therapy and other social determinants of health (Eleftheriou and Angastiniotis, 2021). This results in widely differing clinical outcomes and quality of life of patients within and across countries, within and across different regions of the world.

a) Blood transfusion. The aim of red cell transfusions in thalassaemia major is to suppress endogenous, ineffective erythropoiesis as well as to increase the level of Hb to optimise the person's wellbeing and function. The recommendation is to maintain the pre-transfusion Hb above 90-100g/dl. This is usually sufficient to prevent bone deformities, allow normal growth and activity levels, and support a good quality of life (QoL). In addition, absorption of iron and iron loading from the gut is reduced (Cazzola et al, 1997).

Maintaining this standard is a major challenge to many health services, since blood donation is inadequate in many countries where thalassaemia is prevalent. Under-transfusion results in continued anaemia, marrow expansion, and bone disease. Blood safety is a further issue, and the transmission of infected blood will add to complications, especially where infection with hepatitis viruses contributes to chronic liver disease and adds to the morbidity of hepatic iron overload (WHO 2017; Lai et al, 2013; Shah et al, 2019; Javed et al, 2022).

In NTDT and under-transfused TDT patients, the severity and timing of complications varies by individual. Lower Hb levels are associated with more frequent complications in both groups (Wanchaitanawong et al, 2021).

b) Iron chelation. Iron loading in NTDT and other chronic anaemias that do not depend on regular blood transfusions is a slow process, whereas in TDT iron accumulation is more rapid. This rapidly overwhelms the iron binding capacity of proteins and NTBI is released. To counter the toxic effects of free iron, iron chelating agents are given with the aim of binding and excreting excess iron. The challenges to such treatment include resource, since they are the most costly aspect of routine management, and the difficulties patients have in adhering to daily, lifelong regimes.

The main iron chelators used for iron overload patients are desferrioxamine [deferoxamine in US] (DFO), deferiprone (DFP), and deferasirox (DFX) (Cappellini et al, 2021). These may be used

singly or in various combinations (Origa et al, 2022); the choice of regime is tailored to each patient's needs as determined by the current level of iron load, their previous chelation history, and any objective or subjective adverse effects. Chelation is necessary even in the 'milder' syndromes (NTDT) if iron overload is demonstrated.

Monitoring iron levels and for organ damage, and tailoring chelation accordingly, is a central part of management of the conditions.

Multi-disciplinary care including psychosocial support

Given the potential for complications in almost all organ systems, patients require attention from physicians across medical and scientific disciplines including, but not confined to, haepatologists, cardiologists, endocrinologists and psychologists (Radke et al, 2019). Care is ideally managed by a co-ordinated multi-professional and multidisciplinary team while the core members will include paediatricians or paediatric haematologists, and adult haematologists.

α thalassaemias

Pathophysiology

In α thalassaemia, there is a reduced production of α globin chains, which are regulated by a cluster of two genes, located on chromosome 16; in health there are thus four genes regulating α globin chain production (Harteveld and Higgs, 2010; Farashi and Harteveld, 2018). Deletions or other mutations on one or two of the genes produce an asymptomatic carrier state with microcytosis as the main haematological manifestation. When three or all four of the genes are affected then clinically significant syndromes arise – HbH Disease and Hb Bart's Hydrops fetalis respectively. 'Hydrops' describes the excessive accumulation of serous fluid in the tissues and cavities of the body.

HbH disease

HbH disease is a condition in which, due to inactivation of three α globin genes, a globin chain imbalance arises with an excess of β-globin chains accumulating in erythroblasts; these form tetramers (β4) known as HbH. They have high oxygen affinity, do not deliver significant amounts of oxygen to tissues, and are unstable. They form intracellular precipitates, which causes apoptosis of erythroblasts, and ineffective erythropoiesis, while in circulating red cells they cause oxidative damage to cell membranes and haemolysis (Chui et al, 2003). The degree of anaemia and the severity of the clinical picture depends, among other factors, on the genotype.

Most common causes of α thalassaemia are deletions of one (α+) or both (α0) α-globin genes on a chromosome 16. Deletional mutations are very common throughout all tropical and subtropical regions, including Eastern Asia, the Mediterranean area, the Indian subcontinent, the Middle East, and Africa (Piel and Weatherall, 2014). Less common are mutations that affect transcriptional or translational processes which are labelled non-deletional mutations, and include those which produce elongated chains such as Hb Constant Spring [HbCS] or unstable variants (Chui et al 2003, Higgs and Weatherall, 2009). These non-deletional mutations result in a greater reduction of α globin chain synthesis and so a more severe clinical picture than the deletional types.

The condition is usually a mild to moderate form of α thalassaemia. Anaemia is variable. In general, deletional HbH disease is associated with higher levels of Hb, and more normal red blood cell indices, than non-deletional HbH disease, and transfusion is not usually needed in the deletional forms. Haemolytic crisis can occur, usually triggered by infection, and may necessitate blood transfusion, as may aplastic crises usually caused by erythrovirus B19.

Growth rate is not affected in most children. Iron overload is expected from increased iron absorption, plus that from any blood transfusions, and is greater in those with non-deletional forms; it can be significant and can lead to hepatic fibrosis (Chan et al, 2021). Pulmonary hypertension is uncommon, and is not related to splenectomy, in contrast to people with β thalassaemia [Yin et al, 2013] Other complications include hepatosplenomegaly and gall stones. Bone disease may manifest in older age groups (Zarei et al, 2016).

Monitoring and treatment of people with HbH

The primary objective of clinical management for people with this condition is careful monitoring to allow early detection of any complications, and correction of any tissue damage. Monitoring should include haemoglobin checks; most people run at levels of 8 – 11 g/dl with little variation, and annual monitoring is usually sufficient. Haemolytic or aplastic episodes can occur, and patients should be invited to attend for a check at any time if they become more symptomatic. In a minority of patients, mainly those who have non-deletional mutations, regular transfusions may be necessary at some stage of their lives, when they should be followed and managed like TDT patients, aiming for a pre-transfusion Hb of 9-10g/dl. Growth monitoring should be undertaken, even though the majority have normal growth with no deformities.

Since there is considerable variability in iron loading, it is wise to measure serum ferritin at least annually from childhood, with MRI LIC measurements every two years from adolescence and according to transfusion history [Ang et al, 2017].

Haemoglobin (Hb) Bart's hydrops fetalis

Haemoglobin (Hb) Bart's hydrops fetalis is caused by deletion or inactivation of all four α globin genes; it usually causes such severe anaemia in the fetus that intra-uterine death results. Effective screening for couples at risk of having a pregnancy with this condition can allow early pre-natal diagnosis and offer of termination. Intra-uterine blood transfusions have sometimes been successful (Horvei et al, 2021). In utero stem cell transplantation, using maternal stem cells, is currently in early clinical trials (Sagar et al, 2020), paving the way for future in-utero gene therapy (Almeida-Porada et al, 2019).

Pyruvate kinase deficiency (PKD)

Pyruvate kinase deficiency is a rare condition estimated to occur in between 3 and 8 per million of the population, at least in western populations (Secrest et al, 2020). However, clusters have been described, for example among the Pennsylvania Amish (Rider et al, 2011). PKD is also found in the Middle East and sub-Saharan Africa and is thought to have been an adaptation to malaria (van Bruggen et al, 2015). Cases may be mild, with similar haematological findings to other

haemolytic anaemias, and it is likely that cases are missed so that the real prevalence may be much higher than current estimates suggest. As for all these blood disorders, the scarcity of national registries leads to inaccuracy in epidemiological estimates.

Pathophysiology

PKD is the most common cause of hereditary non-spherocytic haemolytic anemia (Al-Samkari et al, 2020). Pyruvate is a key substrate that serves as an intermediate for the breakdown, building, or recycling of molecules into high energy compounds in anaerobic conditions such as glycolysis and fermentation, and in aerobic conditions including mitochondrial oxidative phosphorylation (Melkonian and Schury, 2022).

In mature red blood cells, which lack mitochondria, glucose catabolism is completed through glycolysis via two competitive branches: the Embden-Meyerhof pathway (EMP), which converts 90% of glucose to adenosine triphosphate (ATP), and the hexose monophosphate pathway (HMP), responsible for recycling nicotinamide adenine dinucleotide phosphate (NADPH) to reduced glutathione; the latter is favoured when RBCs are oxygenated (van Wijk and van Solinge 2005; Grace et al, 2019; McMahon et al, 2021). At the terminal end of glycolysis, the enzyme pyruvate kinase catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate through the transfer of a phosphate group resulting in the production of pyruvate, ATP, 2,3-diphosphoglycerate (2,3-DPG), and NADH. Overall, there is much less energy produced from glycolysis relative to oxidative phosphorylation (2 molecules of ATP per 1 molecule of glucose compared to 32 molecules of ATP per 1 molecule), but it occurs 100 times faster (Melkonian et al, 2022).

In people with PK deficiency, in vitro enzyme rates vary from <25% of normal in homozygotes, to 40-60% in heterozygotes (Grace and Barcellini, 2020). Blocked from being able to convert PEP to pyruvate: PEP, 2,3-DPG and other metabolites flood the cell destabilising Na+/K+ pumps. RBC cell membrane integrity is compromised, and potassium and water leak out, resulting in cell dehydration, contraction, crenation and premature cell death (Yaish, 2022).

PK enzyme activity decreases with erythrocyte age, but reticulocytes, young red cells, increase from the normal 0.5-2.5% to 4-11% allowing for the higher energy yielding process of oxidative phosphorylation (Grace and Barcellini, 2020; Roy et al, 2021). Unfortunately, PKD-reticulocytes in the spleen are disadvantaged by the low oxygen environment and are destroyed. Splenectomy increases reticulocyte activity in people with the condition up to 20-70% (Al-Samkari et al, 2020; Grace and Barcellini, 2020).

PKD is caused by mutations in the PKLR gene which encodes PK in erythrocytes from its locus on band 1q21 (van Wijk and van Solinge, 2005), with over 300 mutations identified to cause enzyme deficiency (Bianchi et al, 2020a; Bianchi et al, 2020b). Affected individuals may be homozygote or compound heterozygote for different alleles. There is much clinical variability, which is not directly dependent on the PK enzyme activity but on complex metabolic factors within the red cell (Roy et al, 2021) and other factors such as splenic activity.

Clinical manifestations

Red cell destruction may start in intrauterine life and cases of hydrops have been described requiring intrauterine transfusions (Grace and Barcellini, 2020). Early onset of severe anaemia is associated with a more severe clinical course. More usual manifestations are intrauterine growth retardation, premature birth and neonatal anaemia with jaundice requiring treatment. Ineffective erythropoiesis and extramedullary haemopoiesis have been described (Boscoe et al, 2021). These are the more severe manifestations of PKD; most patients have a much milder course.

Anaemia ranges from steady state 6.5 to 11 g/dL, and those more severely affected may require regular transfusions, particularly in childhood, or periodic blood transfusions for episodes of acute haemolysis or aplastic crises. Traditionally, the need for regular transfusions has been more frequent in children under the age of 12 years, especially if their growth is poor and Hb <7g/dl. An estimated ten percent of adults with PKD receive regular transfusions (McMahon et al, 2021). Subjective symptoms of low Hb, such as fatigue and low energy levels, are commonly experienced by larger numbers of affected people.

Jaundice with indirect hyperbilirubinaemia is almost universal. Increase in direct bilirubin may indicate the presence of gallstones which occur in up to 50% of patients. They can occur at any age and are more common in patients who have had a splenectomy (Boscoe et al, 2021). Cholecystectomy is frequently needed.

Splenomegaly is common (around 80%) and splenectomy is frequent since it may improve the anaemia and reduce transfusion requirement.

Iron overload is expected due to increased iron absorption, decreased hepcidin levels, and any blood transfusions. In a PKD Natural History Study, 82% of non-regularly transfused patients had iron overload defined by LIC >3mg/g dry wt, with onset at a young age (van Beers et al, 2019). Liver fibrosis and cirrhosis due to iron overload affect up to 6% of adults. Endocrine dysfunction is less common than in the thalassaemia syndromes; thyroid disease affecting up to 9% of the adult population seems to be the most prevalent (Boscoe et al, 2021), while data on hypogonadism and delayed puberty are not well defined. Cardiac complications also are less prominent.

Osteopenia, bone pain and bone fragility are common, affecting up to 1/3 adults (Grace and Barcellini, 2020). Contributory factors include ineffective erythropoiesis with marrow expansion, anaemia and low vitamin D levels. Bone pain was reported by 38% of patients over 18 years (Grace et al, 2018b).

Pulmonary hypertension occurs in about 5% of patients and incidence increases with age. Leg ulcers are also seen in 5% of adults.

The psychosocial impact of the condition, affecting various aspects of life and reducing the overall quality of life, has been well described (Barcellini et al, 2021; NORD 2020). PKD can heavily impact quality of life, varying between patients and at various stages of life. The most common symptoms include tiredness/exhaustion/fatigue, and difficulty concentrating. People with PKD report a high level of anxiety, low self-esteem, social isolation, depression, judgement from others, and bullying related to jaundice. While the majority of patients and caregivers affected by PKD report their haematologist manages their condition "well" (82%) and regards PKD a serious condition (78%), less than half (44%) report that their haematologist searches for and finds solutions to optimise the management of their condition. Patients often benefit from referrals to psychosocial services.

Diagnosis and monitoring

The haematological findings are those of a non-specific anaemia with low haemoglobin, low red cell indices, some aniso-poikilocytosis, reticulocytosis, and increased erythroblasts in peripheral blood in severe cases (Chonat et al, 2021). Haptoglobin is reduced. After splenectomy, reticulocytes are greatly increased and spiculated irregularly contracted red cells are often seen. Elevated lactic dehydrogenase (LDH) and serum bilirubin levels reflect haemolysis. Serum ferritin and transferrin saturation are expected to increase, depending on age and transfusion history.

Specific tests include measuring PK enzyme activity in red cells, using a standardised spectrophotometric assay, although there are pitfalls in the interpretation of results, for example if there is a marked increase in the reticulocyte count, and reference ranges are different between centres. The degree of PK enzymatic activity reduction does not predict the clinical severity. DNA sequence analysis of PKLR gene avoids the pitfalls of enzyme testing, although time consuming and relatively expensive. However, not every mutation detected by DNA analysis is a disease-causing variant, so the pathogenic nature needs to be confirmed by functional analysis. It is recommended that such tests are referred to specialised laboratories for a definitive diagnosis. A timely and accurate diagnosis allows for appropriate patient education and management. Recommended monitoring is summarised in Appendix 12.3

Approaches to treatment

Phototherapy and exchange transfusion in jaundiced newborns may be required depending on the degree of hyperbilirubinaemia.

Blood transfusion is required by some patients: in a cohort of 250 patients 48% required regular transfusions under the age of 5 years (Grace et al, 2018a). The proportion transfused decreases in older age groups, with ~10% adults receiving regular transfusion. Many others require occasional transfusion, for haemolytic or aplastic crises, during pregnancy, and with aging. Folic acid supplementation is typically prescribed as in other haemolytic anaemias.

Splenectomy reduces transfusion requirement for most; increasing the Hb level by a median of 1.6g/dl, and up to 5 g/dl (Grace et al, 2018a) and a high proportion of patients undergo splenectomy, many under the age of 5 years; thereafter they are at higher risk of infection or sepsis (12%) and

thrombosis (1.3%) even during childhood (Grace 2018a). Cholecystectomy is considered at the time of splenectomy since the formation of gallstones is very frequent.

In the long-term, thrombotic complications, such as deep vein thrombosis, pulmonary embolism, stroke and portal vein thrombosis, have been described. The usual post-splenectomy monitoring and preventative treatment are recommended. However, routine splenectomy in paediatric patients with PKD may change if mitapivat, a pyruvate kinase activator, is approved for use in children as recent FDA approval in adults has been beneficial in increasing hemoglobin. Currently ACTIVATE-Kids and ACTIVATE-KidsT clinical trials (ClinicalTrials.gov NCT05175105, ClinicalTrials.gov NCT05144256) are underway with the aim of increasing hemoglobin $\geq$1.5 g/dL and measuring any change in iron concentration, height, weight, bone mass, bone mineral density, and several other key indicators of growth. Because of the risk of infection and thrombosis in patients with PKD, splenectomy should be considered on an individual basis and considered with the advice of a paediatrician or haematologist expert in PKD.

Mitapivat (trade name Pyrukynd®) is available for adults in the U.S., E.U., and U.K. Compared to placebo, 40% of adults with PKD who had not received regular blood transfusions participated in a phase 3, randomized, controlled trial and sustained an increase of $\geq$ 1.5 g/dl in hemoglobin level compared to no change in the placebo group (Al-Samkari et al, 2022). Adults with PKD in these countries are in various stages of accessing access to the medication and adding this to their treatment plan.

For those with iron overload, iron chelation is required, preferably using an oral agent (deferasirox or deferiprone).

Any complications such as endocrinopathies, pulmonary hypertension and leg ulcers should be managed by appropriately experienced specialists in a multidisciplinary setting.

Pregnancy should be preceded by pre-pregnancy assessment for any complications, and counselling. Multidisciplinary care throughout pregnancy – including the haematologist, obstetrician, cardiologist and others according to needs – is recommended for more severe cases. The need for partner testing in a pregnant woman known to have PKD should be discussed.

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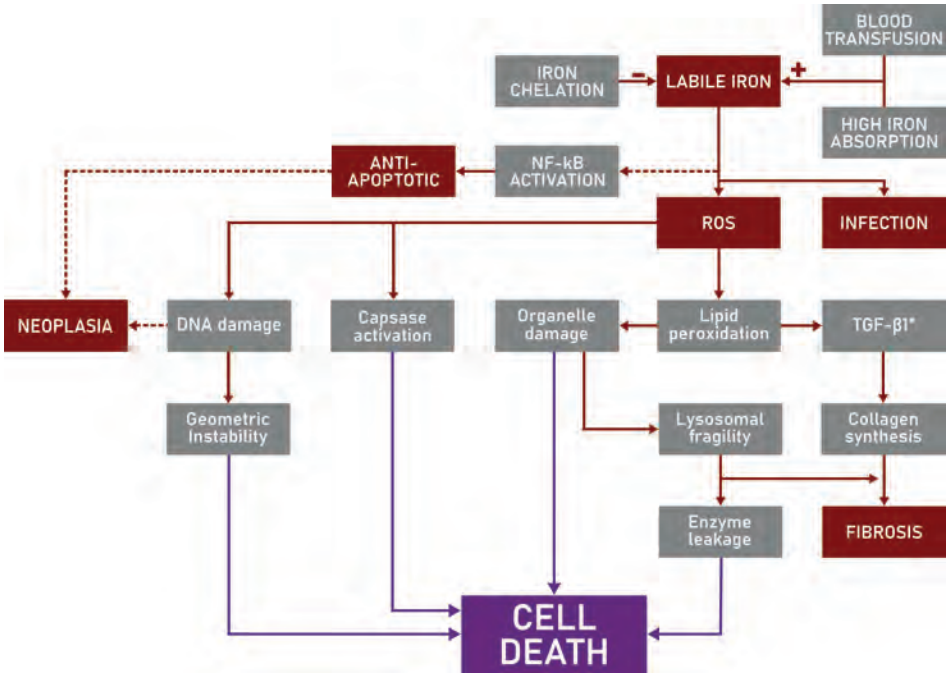
CHAPTER 3
Iron overload and chelation

Iron overload occurs when iron intake is increased over a sustained period of time, either as a result of repeated red blood cell transfusions or increased absorption of iron through the gastrointestinal (GI) tract. Both of these may occur in the thalassaemias and in other congenital anaemias including PKD. In addition, there is primary iron overload which is the direct result of ineffective erythropoiesis affecting the survival of red cell progenitors as well as the survival of erythrocytes. Blood transfusion therapy is the major cause of iron overload in thalassaemia major (transfusion-dependent thalassaemia, TDT), whereas increased GI absorption is more important in non-transfusion dependent thalassaemia (NTDT, sometimes also referred to as intermedia), albeit at about one tenth of the iron loading rate seen in transfusion dependent patients.

Fig 3.1: *A summary of the mechanisms for toxic effects of iron overload*



Prescribing and monitoring iron chelation in people with iron overload is a whole subject in itself, and this text does not attempt to cover it in any detail. Readers needing more information are referred to TIF's Guidelines for the Management of Transfusion Dependent Thalassaemia (Cappellini et al, 2021) and for Non-Transfusion Dependent Thalassaemia (Taher et al, 2017) where comprehensive details can be found.

The three licensed chelators, together with some of their adverse effects, are only briefly outlined here, including their capacity to deplete other micronutrients as well as iron, and the gastro-intestinal side effects many users experience, which can impact on nutrition.

Desferrioxamine, (DFO) given by subcutaneous infusion over 8 – 12 hours, 5-6 times per week at a dose of 20-40 mg/kg/day, can be effective and there are decades of experience with its use. However, children and adult patients often find it difficult to tolerate, so adherence is often poor. Much support and encouragement may be needed if they are to continue on it. Unless used by continuous infusions, such as in the treatment of iron-induced cardiac failure, there are long periods between infusions when there is no chelator circulating, so there is no continual protection against labile iron. Iron excretion is improved when an oral dose of ascorbic acid is taken at the start of each infusion.

Its main side effects are: local reactions at the infusion site; bone effects – damage to growth plates of long bones and especially spine in growing children; optic nerve damage and – more commonly – high tone hearing loss; and increased risk of infection with Yersinia enterocolitica; patients receiving this medication should be advised to stop it and seek urgent medical assessment if they develop diffuse abdominal pain with fever and gastro-intestinal upset. It can also cause zinc deficiency, which may need supplementation.

The adverse effects on bone growth, hearing and sight are minimised if the dose is kept within the 'therapeutic index', where the mean daily dose of desferrioxamine (mg/kg) / serum ferritin (ug/l) is under 0.025 (Porter et al, 1989). Audiometric and retinal toxicities especially are more likely with desferrioxamine when storage iron levels are low, and close monitoring is particularly important under these circumstances.

Deferiprone (DFP) is taken by mouth. The affinity for iron is relatively low compared to DFO and DFX. DFP is a small, non-charged molecule, and crosses the cell membrane readily, suggesting suitability as an intracellular chelator. It has a relatively short plasma half-life and consequently t.d.s. or even q.d.s. dosing is needed to optimise drug levels over the 24-hour period. Drug and iron complexes are predominantly excreted in the urine, giving the urine a red colour. A liquid preparation is available for young children and appears to be well tolerated and effective.

Liver iron may not be adequately controlled over the longer term with DFP monotherapy at the standard dose of 75mg/kg/day; and the dose may be increased to a maximum dose, if appropriately monitored, of 100 mg/kg /day. A consensus view, based on published trials and clinical experience, is that DFP therapy produces a reliable and rapid reduction in myocardial iron loading compared with other chelating drugs. Adverse effects include agranulocytosis, neutropenia, and arthropathy. Gastro-intestinal disturbance, intermittent elevation in ALT, zinc deficiency, and increased appetite are often reported. Arthropathy is usually reversible and in some cases, DFP can be re-introduced once symptoms have subsided. Agranulocytosis (absolute neutrophil count of < 0.5 x109 /l) is a severe and potentially fatal adverse effect of DFP, occurring in ~ 1.5% of patients, at a median of 5-6 months after starting therapy. There is a high risk of recurrence of agranulocytosis with re-challenge. Neutropenia (neutrophil count in the range 0.5 – 1.5 ×109 /l) occurs in ~ 7% of patients; it is more common in splenectomised patients, and is usually self-limiting, but can be recurrent and

lead into agranulocytosis. The drug also chelates zinc, and deficiency requiring supplementation can occur.

Adherence to chelation therapy is generally improved in patients switched from DFO to DFP, and those who find DFO infusions intolerable may have better long- term control of iron stores with DFP.

Deferasirox (DFX) is also orally active, and is available as film-coated tablets, which can be crushed for children [aged two years or older] too young to swallow it whole. It is taken once a day at a starting dose of 14 mg/kg/day escalating if tolerated and iron load necessitates, to 28 mg/kg/day; 21 mg/kg/day is the usual maximum.

Careful monitoring of renal function, liver function and ferritin is necessary. Abnormalities of renal function with deferasirox (DFX) may be more likely when ferritin values are below 500 ug/l so that monitoring creatinine and urinary protein are particularly important at these levels, and the drug dose should usually be lowered. Tubular dysfunction and glomerular filtration rate abnormalities with DFX, are mostly non-progressive but in rare cases it can cause a generalised dysfunction of proximal tubular cells, Fanconi syndrome, characterised by hypokalaemia, hypophosphatemia, hypercalciuria, metabolic acidosis, hyperaminoaciduria, and hyperuricosuria. In these cases, the drug should be withdrawn immediately. Other symptoms include skin rash and gastro-intestinal side effects, with nausea, diarrhoea, abdominal pain and occasionally upper GI ulceration, which can limit dose escalation to levels required to maintain negative iron balance. Zinc deficiency can probably result from using DFX and this should be monitored (Erdoğan et al, 2013).

Unless severe, most symptoms usually reduce with continued medication. Because of its convenience and once-daily dosing, it is the chelator most often chosen for children and adults who have no contra-indication. It should not be used in patients with renal impairment and eGFR of < 60 mls/min/1.73m2 or in those with severe hepatic impairment.

The chelators can be used together in various **combination regimens**, which can be useful in minimising side effects and maximising efficacy, but these regimens should be prescribed and supervised only by professional teams with high levels of specialty expertise.
Alcohol should be avoided by people with iron overload (Porter and Rawla, 2023).

Issues to remember concerning chelation

- Response to chelation is affected by the rate of blood transfusion, the dose used and the duration of exposure to the chelating agent.
- The treatment will not be effective if it is not taken regularly so that good management includes support for patient adherence.
- In frequently transfused patients, adequate chelation is mandatory for managing iron overload as dietary restriction would have insufficient impact.

Basic recommendations (Pinto and Forni, 2020) for effective iron chelation include:

1. Encourage long-term patient adherence through patient education, careful discussion of any subjective side-effects and how they can be managed, shared decision making, pharmacist support, and motivational interviewing.
2. Ensure daily treatment with constant chelator presence in the circulation.
3. Conduct adequate assessment and monitoring of chelation therapy with the aim of removing iron toxicity and maintaining body iron levels near normal and safe ranges.
4. Avoid the risk of over-chelation.
5. Monitor cancer risk in adults, especially hepatocellular and thyroid malignancies.

Absolute change in total body iron in response to chelation can be estimated from change in liver iron concentraion. Chelation therapy can reverse iron-mediated cardiac dysfunction within weeks by rapid chelation of labile iron, if 24 hour chelation cover is achieved (Pennell et al, 2013). Over- chelation increases the possibility of side effects from the chelating agents, the dose should therefore be decreased as serum ferritin or liver iron conentration fall. Optimal chelation must be tailored for each individual patient, and will vary according to their current clinical situation.

Dietary considerations

In irregularly transfused or non-transfused patients with milder forms of anaemia, absorption of iron from food is increased and so:

a) avoidance of dietary supplements containing iron

b) restriction of iron rich foods is to be considered, including heme-iron contained in red meat from mammals, processed meat, and blood containing foods (Milman 2021)

c) food and drink that may delay or reduce non-heme iron absorption is to be considered. One example is taking tea with meals, though coffee, red wine (polyphenols), soya bean proteins, milk and egg proteins may have similar inhibitory effects on non-heme iron absorption. (Milman 2020; Zijp et al,2000)

d) substances that possibly enhance iron chelation include silymarin and turmeric. Evidence is limited so that there cannot be any firm recommendation.

e) iron chelation may increase the excretion of some essential minerals, mainly zinc. Zinc supplementation is recommended if deficiency is detected by monitoring.

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CHAPTER 4

Nutrition & Relationship to Common Complications

Growth deficiency

In untreated or poorly treated anaemias, growth may be poor because reduced oxygen delivery, due to low haemoglobin levels, may result in significant tissue hypoxia. This has been well documented in thalassaemia (Saxena, 2003; Skordis and Kyriakou, 2011; De Sanctis et al, 2013). Data on growth are not reported in PKD and other congenital anaemias even though severe anaemia may occur from infancy. Growth disturbance is a major problem for untreated patients with thalassaemia, along with bone deformities related to ineffective erythropoiesis and bone marrow expansion. However, correction of the anaemia does not fully correct this observed deficit in all patients (Skordis and Kyriakou, 2011), often related to iron overload. The pathogenesis of growth failure is multifactorial (Arab-Zozani et al, 2021) and key contributing factors in people with thalassaemia can include:

- Chronic anemia
- Transfusion-related iron overload
- Chelation toxicity: mainly high dose desferrioxamine (DFX) - induced bone dysplasia (De Sanctis et al, 2000) now rarely encountered; also zinc deficiency, which may affect growth and bone health, and also affect the immune system.
- Endocrine deficiencies caused by iron overload: hypothyroidism, hypogonadism, GH deficiency/insufficiency, dysregulation of the GH-IGF-1 axis.
- Chronic liver disease
- Under-nutrition: inadequate macronutrient intake - kilocalories, protein, and micronutrient intake - zinc, folic acid, vitamin D, carotenoids, and retinol binding proteins (Fung et al, 2012)
- Psychosocial stress, emotional disturbance

Three phases of growth disturbances according to age of presentation are well recognised and have different causes (Skordis and Kyriakou, 2011):

- **Phase 1:** in the first phase growth disturbance is mainly due to hypoxia, anaemia, ineffective erythropoiesis and poor nutrition.
- **Phase 2:** During late childhood, growth retardation is mainly due to iron overload affecting GH-IGF-1 axis and other potential endocrine complications. Although appropriate iron chelation therapy can improve growth and development, children and adolescents with transfusion-dependent thalassaemia (TDT) treated intensively with desferrioxamine remain short as well, showing body disproportion between the upper and lower body segment.

- **Phase 3:** After the age of 10-11 years, delayed or arrested puberty is an important contributing factor to growth failure in adolescent patients, who do not exhibit a normal growth spurt.

It is recommended that assessment of growth is made in the paediatric clinic every 6 months (De Sanctis et al, 2013) including:

- Recording parental heights at the first visit (and calculating mid-parental height).
- Accurate measurement of standing and sitting height, and weight at each visit, and head circumference especially during the first two years of life. Stature measured using a Harpenden stadiometer.
- Plotting growth data on growth charts or international growth charts (WHO) (see Appendix 12.1 for examples)
- Calculating annual growth velocity (GV), body mass index (BMI) and upper/lower segment ratio. (Note: The average height velocity varies at different developmental phases, normal ranges: include: Infancy: 23-28 cm/year, Childhood: 5.0-6.5 cm/year, Puberty: 8.3 cm/year (girls), 9.5 cm/year (boys)).
- Consideration of additional factors such as the genotype, the haemoglobin level, the adequacy of iron chelation and the degree of iron overload, and associated complications (endocrines, heart and liver, chronic infections)

A child is considered short if

- the height is less than the 3rd percentile or 2 SD below the mean height for age and sex.
- the height is within normal ranges, but GV is consistently <25th percentile over 6-12 months or
- he/she is excessively short for his/her mid-parental height, though absolute height may be within the normal percentiles.

Despite the improved use of iron chelation over the years resulting in a significantly lower prevalence of endocrinopathies, and improvement of iron levels, the final adult height of patients with TDT remains significantly shorter compared to their peers. Growth hormone deficiency may be the cause but there are no guidelines for assessment of GH in adults at present. GH deficiency in adults is associated with a lack of positive wellbeing, depression, feeling of social isolation, as well as reduced bone and muscle mass. GH deficiency is also associated with altered lipid metabolism and increased adiposity (Yuen et al, 2022).

Recommendation
For details of endocrine assessments which should be undertaken from age 9 years, or earlier if there are any concerns about growth, see Appendix 12.2

Delayed puberty and hypogonadism

This is the most frequently encountered effect of endocrine damage initiated by damage to the hypothalamic-pituitary gonadal axis, causing hypogonadotrophic hypogonadism in up to 50% of adolescents and to a lesser extent adult-onset hypogonadism (de Sanctis et al, 2004).

Delayed puberty is defined as the lack of pubertal development in girls by the age of 13 years, and by the age of 14 in boys. Clinically puberty is evaluated by evaluation of Tanner stage and the growth rate; indicators include absence of testicular development (less than 4 ml) after 14 years, and lack of breast development in girls by the age of 16 years, including a lack of pubertal progression in a period of 6–12 months, after a spontaneous beginning of puberty (de Sanctis et al, 2013; Casale et al, 2022).

If pubertal delay is suspected, detailed endocrinological investigations by a specialist will be required (see Cappellini et al TIF 2021 guidelines, ch 8; and Casale et al 2022). Psychological and emotional issues may also influence pubertal maturation and such factors should be considered especially in girls. Delayed puberty can cause significant psychological distress and low self-esteem.

Treatment depends on age, degree of iron overload, damage to the hypothalamic-pituitary gonadal axis and whether chronic liver disease is involved. It is recommended to start replacement therapy with gonadal hormones in patients affected by hypogonadism in pre-menopausal age, after ruling out contraindications.

Recommendations: interventions to prevent and manage growth and stature deficits in both thalassaemia and other rare anaemias

Early Prevention:

- The anaemia should be corrected by blood transfusion early in life, both to increase tissue oxygenation, and to suppress endogenous ineffective erythropoiesis (IE). IE and erythroid expansion result in a hypermetabolic state, and pressure on bone architecture leading to bone disease and deformities which can affect growth. (Longo et al, 2021)
- Ensuring adequate caloric intake which has been shown to increase significantly IGF-I levels in thalassaemic children, and partially correct growth impairment in those without endocrinopathy or cardiomyopathy. (Soliman et al, 2004)
- Monitoring iron overload and ensuring adequate iron chelation, aiming to prevent endocrinopathies and other organ damage.
- Ensuring family and patient support: psychological support to help adherence to a difficult daily routine, and social support to minimise 'out of pocket' expenses, searching for donors and other stress factors. Overall emotional support favours normal growth.

Managing detected growth discrepancies, usually detected in later childhood:

- Maintain an appropriate pre-transfusion level of Hb.

- Strict adherence to iron chelation, tailored and guided by regular monitoring, aiming for serum ferritin of <1000 ug/l. High dose desferrioxamine may cause platylospondyly and metaphyseal cartilaginous dysplasia, affecting skeletal growth and final height (De Virgiliis et al, 1988) and doses in childhood, and until growth is complete, should not exceed 40 mg/kg/day dose range, and unless there is concern about iron levels is usually nearer 20 mg/kg/day.
- Identifying and correcting specific nutritional deficiencies. The most common are zinc, vitamin D and selenium. Deficiencies as high as 72% have been reported (Napoli N 2006; Bulgurcu et al, 2021; Goldberg EK 2022).
- Correcting endocrinopathies, especially GH-IGF-1 deficiency, but also hypothyroidism and glucose homeostasis
- Timely induction of puberty if there is delay in order to gain a normal pubertal growth spurt and bone accretion.
- Monitoring and early management of organ damage, especially heart and liver disease

Hypothyroidism

The cause of damage to the thyroid gland is mainly iron toxicity, and so its occurrence is related to the level of effectiveness of chelation and adherence of patients to effective chelation treatment. Iron deposition in the thyroid has also been described in pyruvate kinase deficiency (Nagai et al, 1994). There are clinical stages or grades of poor thyroid function which can be described by the results of diagnostic tests (TSH & Free T4):

- Preclinical hypothyroidism (Type A), characterised by TSH (5-10μu/ml) but a normal FT4.
- Preclinical hypothyroidism (Type B), characterised by a raised TSH (>10μu/ml) but normal FT4.
- Primary, overt hypothyroidism, characterised by a high TSH and a low FT4.
- Secondary hypothyroidism, characterised by a normal TSH but a low FT4.

Screening for hypothyroidism should start no later than 9 years of age. If it is detected, other tests include the detection of thyroid autoantibodies, and ultrasound scan of the thyroid.

Hypothyroidism is characterised by a retardation of the metabolic process and by dislipdaemia, which may lead to serious cardiovascular disease (Szczepanek-Parulska et al, 2022). Overt hypothyroidism is associated by increased total cholesterol and low-density lipoprotein cholesterol, increased triglycerides and lipoprotein-a, including increased homocysteine, which may result in the progression of atherosclerosis and increase cardiovascular risk. This is a risk which may be increasing with age in thalassaemia patients (Farmakis et al, 2020) and the role of these factors is further discussed in a later section.

Recommendation: It is recommended to start hormone replacement therapy if overt primary hypothyroidism is found (Casale et al, 2022), using L-thyroxine. The subclinical forms of hypothyroidism, which are more frequent, can progress over time to overt forms or can regress, with a normalisation of thyroid function; they must be closely monitored. (See Appendix 12.2 for monitoring guidelines)

Hypoparathyroidism

Reduced parathormone secretion results in hypocalcaemia and hyperphosphataemia. Hypoparathyroidism is not expected to occur before the second decade. Once detected, hypocalcaemia will trigger a more detailed investigation including phosphate, magnesium and parathormone measurement. If patients develop hypocalcaemia symptoms and/or low serum calcium levels with high phosphate levels, then treatment should be initiated. Symptoms include paraesthesia, muscle pain, cramps, convulsions, tetany, and heart involvement (ECG changes, prolonged QTC interval, and heart failure).

Recommendations:

- In order to maintain calcium and phosphate levels in the normal range and prevent or control symptoms, it is recommended to use calcium carbonate or calcium citrate, which is preferable in case of side effects (Casale et al, 2022). Vitamin D or its analogues (calitriol on average 0.25 – 1.0 µg/day in 2 doses) will normalise calcium and phosphate levels. Treatment should also be monitored with 24-hour urinary excretion of calcium in order to avoid nephrocalcinosis, soft tissue calcification and renal stone development. In severe cases with tetany or heart failure, intravenous calcium is required.
- Dietary recommendations include a diet rich in calcium mainly from natural sources, such as milk and other dairy products, fish, vegetables, seeds (e.g. chia), and fortified cereals. On the other hand, intake of phosphorus rich foods should be limited; these include eggs, meat, yogurt, beans, lentils, nuts and carbonated soft drinks.

Impaired glucose tolerance and diabetes mellitus

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism due to impaired insulin secretion or action, or a combination of both, resulting in absolute or relative insulin deficiency and hyperglycaemia. It is a common complication of transfusion-dependent thalassaemia, affecting 20-30% of individuals (Chatterjee and Bajoria, 2009).

The aetiology includes iron overload causing pancreatic β cell destruction which is the predominant cause in thalassaemia and PKD, but also autoimmunity, insulin resistance secondary to liver disease and therefore development of type 1 or 2 diabetes.

The recognition of glucose dysregulation in people with thalassaemia is important because early diagnosis and treatment with an intensive chelation regimen (monotherapy or combined) can improve insulin secretion and glucose metabolism (de Sanctis et al, 2022).

Diagnostic criteria for diabetes mellitus

Normal blood glucose is < 5.5 mmol/l (<100 mg/dL)

Diabetes mellitus can be diagnosed by:

- Fasting blood sugar >126 mg/dl or 7 mmol/l (2 measurements) after an 8 hour fast
- Symptoms of hyperglycaemia and random plasma glucose value ≥ 200 mg/dl or 12.2 mmol/l. The classic symptoms of hyperglycaemia are polyuria, polydipsia and unexplained weight loss.
- Glucose >200 mg/dl or 11.1 mmol/l on Glucose Tolerance Test at 2 hours after ingestion of 75g of glucose

Prediabetes is an intermediate state of glycaemia control with a high risk of developing diabetes in the following years. It is defined as the presence of blood glucose levels above normal but below diabetes thresholds (100-126mg/dl or 5.6-7mmol/l). A glucose tolerance test gives a result of 140 – 199 mg/dl or 7.9 mmol/l – 11 mmol/l.

Pathophysiology

Iron overload leads to cytotoxicity of the insulin-producing beta cells in the pancreas. A new hypothesis suggests that exhaustion of beta pancreatic cells subsequent to chronic hyperinsulinemia may also be involved (Li et al, 2014). This is supported by studies reporting higher fasting insulin level and beta cell functionality in these patients (Bhat and Periasamy, 2014).

Age at Initial presentation

DM is more frequent in patients with thalassaemia over the age of 25 (Li et al, 2014) and is associated with higher incidence of cardiac complications and heart failure (Chatterjee and Bajoria, 2009; Pepe et al, 2013; Ang et al, 2014,). This may lead to a progressive organ deterioration despite using iron chelator treatment.

Evidence of the role of iron in the development of DM

In a study conducted by Mehrvar A et al (2008), TDT patients with DM were found to have been transfused more total blood per occasion (1.8 blood units) than non diabetic patients (1.6 blood units). Bazi et al (2017) reported a significant difference for mean five-years ferritin level between the patients with either DM or impaired fasting glucose and those with normal fasting glucose, with a seven times higher risk of DM in the patients with mean five-years ferritin of >6000 ng/ml. At lower levels, an average of ten-years ferritin levels of >1500 ug/l and >1250 ug/l were shown to increase the risk of DM 3.4 and 4.9 times respectively (Ang et al, 2014).

T2* Magnetic Resonance Imaging (MRI) provides a sensitive and non-invasive method for assessment of iron deposition to different organs, and T2* MRI value of pancreas was reported to be inversely associated with fasting blood glucose level in TDT patients (de Sanctis et al, 2022).

Other related factors

Genetic susceptibility, liver dysfunction, liver fibrosis or cirrhosis, and viral infections have also been implicated as contributing factors. Prevalence of DM was significantly higher in patients who were seropositive for anti-HCV antibodies (Mowla et al, 2004). Other factors which may also play a role are chronic anaemia, zinc deficiency, vitamin D deficiency, and disturbed micro-circulation in the pancreas.

Prevention of diabetes mellitus

People with pre-diabetes should follow a healthy lifestyle which should include a good diet and regular physical activity. At the same time, an aggressive approach should be taken to managing any co-existing factors such as smoking, hypertension, obesity and dyslipidemia. There is currently no medication of demonstrated value to prevent DM.

Diabetes mellitus targets

Fructosamine is more reliable than HbA1c in people with thalassaemia and PKD receiving transfusions as, depending on how recently they received a transfusion, some of the HbA1c will be that of the donor. The target is to maintain fructosamine < 400 umol/L.

Pre-prandial glucose values (capillary blood) should be <130 mg/dl or 7.2 mmol/L.

Post-prandial glucose values (capillary blood) – taken 2 hours after starting a meal - should not exceed 180 mg/d or 10 mmol/l.

The goals of glycemic control should be individualised in relation to age, life expectancy, duration of diabetes, living conditions (elderly people or people who live alone), the ability to perceive hypoglycaemia and the existence of cardiovascular disease.

In addition to glycaemic control, because adults with DM have an increased risk of developing atherosclerosis and microvascular complications, good blood pressure and lipid control, antiplatelet therapy and smoking cessation are equally important. Blood pressure should be ≤140/90 mmHg, or <130/80 mmHg in people with type 1 DM of younger age, at increased risk of complications or in the presence of renal disease. In patients with dyslipidaemia, especially low levels of HDL, control of cholesterol level is important. People with DM and cardiovascular disease of atherosclerotic aetiology should be considered for administration of SGLTII inhibitors such as anagliflozin, dapagliflozin, and empagliflozin, or GLP-1 agonists such as dulaglutide, exenatide, or semaglutide for type 2 diabetes aiming also to reduce the risk of cardiovascular disease.

Lifestyle recommendations

People with DM should be encouraged to make lifestyle changes at each therapeutic step. Regular self-monitoring is recommended as well as checking fructosamine every 3 months. When the target glucose in a few days, or fructosamine after 3 months, is not achieved a change in treatment should be made.

Diet and exercise are essential components in prevention and treatment of DM. Education on both their implementation and the benefits should form part of standard care for people with thalassaemia and PKD. Recommendations include:

- Small, regular meals, spread over the day
- high-fibre, starchy foods, such as wholegrain breads and cereals, beans, peas, lentils and oats

- fruit and vegetables every day, including salad with each meal
- small amounts of mono-unsaturated fats and oils, such as olive oil and rapeseed oil (vegetable oil)
- fewer fried foods and cut fat off all meat; preferring less fatty meats, such as chicken or turkey
- artificial sweeteners instead of sugar, honey, glucose or fructose
- avoid large portions of starchy carbohydrates, such as potatoes, bread, rice and pasta.
- avoid large amounts of saturated fats, such as butter, lard, ghee, red palm oil, pastry, cheese, crisps, mayonnaise and cakes
- avoid too much salt; food may be flavoured with pepper, garlic, herbs and spices
- no more than 14 units of alcohol a week for patients with thalassaemia and PKD with normal (<1.8 mg/g or 32 mmol/g), borderline (1.8 mg/g-3.2 mg/g (57 mmol/g)), or mild (3.2-7.0 mg/g (125 mmol/g)) liver iron concentration values (LIC). Alcohol consumption is not recommended for patients with moderate to elevated liver iron levels. 14 units are about 6 pints of average strength beer or 6 medium (175ml) glasses of average strength wine

Adrenal insufficiency

Compared to the endocrinopathies outlined above, adrenal insufficiency has only quite recently been fully recognised as a complication (Scacchi et al, 2010). It is often a subtle impairment of adrenocortical function, but it may become clinically relevant in case of major stressful events. There may be elements of both iron-related pituitary underproduction of ACTH, and direct iron damage to the adrenal glands. The basal morning cortisol level is normal in most adults with thalassaemia, but up to 60% have a subnormal response to a glucagon stimulation test, using a peak cortisol level of < 18 ug/dl as abnormal (Huang et al, 2015). Males are more often affected than females. It is recommended that as well as a morning cortisol level, a dynamic test of adrenal sufficiency such as a Synacthen stimulation test should be considered as part of these patients' specialist endocrine reviews, to identify when appropriate replacement therapy might be required (Baldini et al, 2017; Motta et al, 2020).

Bone disease

Bone and blood forming haemopoietic tissues are in close proximity, and osteoblasts play an important role in haematopoiesis, forming a local 'niche' microenvironment in which haematopoietic stem cells develop (Shiozawa and Taichman, 2012; Wu et al, 2021). Osteocytes, the major cells in bone matrix, are important regulators both of bone metabolism and hematopoiesis. The interaction between the two tissues becomes more evident in pathological processes (Pajevic and Krause, 2019). For example primary bone disease, such as osteopetrosis or osteogenesis imperfecta, affects bone marrow and haemopoietic function, and conditions affecting haemopoietic tissue, such as the anaemias (Crippa et al, 2019), multiple myeloma, or leukaemia, will impact bone health.

The objective of this chapter is to explore the role of nutrition and lifestyle in the management of these issues.

Bone disease in congenital anaemias

Congenital anaemias cause an imbalance in bone mineral turnover by suppression of osteoblast activity and increasing bone resorption (Perisano et al, 2012). The pathological sequence is initiated by ineffective erythropoiesis - early death of developing red blood cells - which is a feature of congenital anaemias which are not due to hypoplasia or decreased erythroid progenitor cells. Beta thalassaemia is the major example in which bone marrow expansion is the most prominent feature (Piga, 2017; Yavropoulou et al, 2022)

Ineffective erythropoiesis results in anaemia, which causes an increased release of erythropoietin and so erythropoietin-driven expansion of erythroid precursors. The result is hyperplasia of the marrow (Cazzola, 2021) which causes disruption of the bone architecture and cortical thinning, and may lead to deformities of skull, facial bones and long bones with a propensity to fractures in early life. In adult life osteoporosis and bone pain are frequent complications. In untreated or undertreated cases, there may also be red cell production in extramedullary sites.

Conditions in which ineffective erythropoiesis is a major pathogenetic mechanism include, as well as TDT and NDTD (Kaewsakulthong et al, 2022), pyruvate kinase deficiency in which bone deformities are found in 9% of cases (Fattizzo et al, 2022) and osteoporosis in 15.6% (Boscoe et al, 2021). In the ACTIVATE-T trial studying mitavpivat in people with PKD, over 50 % of adults with a median age of 34 years had osteopenia, and almost 20% had osteoporosis (Al-Samkari et al, 2022). Other conditions causing ineffective erythropoiesis are sickle cell anaemia, inherited sideroblastic anaemias, and congenital dyserthyropoietic anaemias, as well as some acquired conditions such as megaloblastic anaemias and myelodysplastic syndromes.

Bone microarchitecture is disrupted at an early age in these conditions and there is reduced bone mass leading to increased bone fragility and susceptibility to fractures. These are features of osteoporosis, a condition clinically defined by Dual Energy X-ray Absorptiometry (DXA). This non- invasive technique measures Bone Mineral Density (BMD) in the hip, lumbar spine and - in children - the whole body.

Vitamin D deficiency and other nutritional deficiencies are an important consideration in increasing bone resorption. In a study from the USA over 30% of patients with thalassaemia consumed less than the estimated average requirement of vitamin A, D, E, folate, calcium and magnesium (Fung et al, 2012). In a recent study from Greece 92.2% of patients were 25-OHD deficient (Lidoriki et al, 2022), confirming many other studies in which vitamin D deficiency was found to be common in thalassaemia. Since vitamin D is active in both bone metabolism and calcium absorption, any deficiency is expected to affect bone health; low levels of 25-OHD have been found in TDT and are associated with low BMD. Deficiency has also been described in other congenital anaemias, including Diamond Blackfan Anaemia (Lahoti et al, 2016). The effect of vitamin D is linked to calcium - an essential mineral in bone, which in turn can be affected by hypercalciuria which can be caused by hypoparathyroidism.

Zinc is a microelement which under conditions of health is found mostly in bone and muscle and plays a role in the formation of these tissues (Rondanelli et al, 2021a). It is commonly found to be deficient in people with thalassaemia, but supplementation has not been demonstrated to significantly impact BMD according to a Cohrane review (Bhardwaj et al, 2016). This is in contrast to the findings of Fung et al (2013) who reported that 25 mg/day zinc taken for 18 months increased whole body BMD in adolescent and young adults with low bone mass after controlling for pubertal stage, and zinc supplementation stabilised spine and hip BMD Z-score. Low values of magnesium are also related to the presence of osteoporosis (Rondanelli et al, 2021b).

In addition to nutritional and lifestyle factors, genetic factors are also involved in the genesis of osteopathy in these conditions, especially involvement of genes affecting collagen, which is the major protein of bone, such as the COLIA1 gene (Wonke et al, 1998). Vitamin D receptor polymorphisms possibly also have a role (Dresner Pollack et al, 2000).

Low bone mass and the risk of fractures increase with advancing age. The prevalence of osteoporosis is variable, ranging from 13 - 50% (De Sanctis et al, 2013), even in patients who are adequately transfused and well iron chelated. Bone disease can severely affect quality of life, not only with fractures and deformities, but also with pain and spinal deformities which may result in nerve compression. Preventative measures and early intervention are necessary to minimise these problems.

Nutritional and life-style recommendations concerning bone health.

- Physical activity must be encouraged. Patients with chronic anaemias often limit their daily exercise either due to symptoms such as fatigue or because of complications or because they believe that exercise can be harmful. Encouragement of a free level of activity in childhood, provided adequate level haemoglobin levels are maintained, is beneficial. For older patients, any complications must be taken into consideration, especially if heart disease is present, but some exercise - particularly aerobic exercise and walking - are essential to maintain bone health. Mechanical strain, such as during exercise, is an important physiological factor that regulates bone formation. There are no firm data to set minimal or maximal limits; the generally accepted minimum for healthy individuals is 150 minutes per week of moderate intensity activity (See Chapter 10 Physical Activity).
- Ensure calcium homeostasis is in balance. This can be disturbed by vitamin D deficiency, hypoparathyroidism and hypercalciuria.
  - Calcium intake is also important to bone health. Given calcium absorption is influenced by vitamin D, maintaining adequate 25-OHD is important. Dietary sources of calcium are preferred to supplements, however if supplementation is necessary no more than 500 mg/day is recommended.
  - Hypoparathyroidism occurs in up to 19% of patients, and should be screened for by monitoring serum calcium and phosphate; followed by measurement of parathyroid hormone if it is suspected. Deficiency is managed using either vitamin D or calcitriol (a vitamin D analogue) to normalise plasma calcium and phosphate levels.

- Hypercalciuria may develop as a result of parathormone treatment, excessive doses of vitamin D, or a renal calcium leak which from an inherent kidney problem.

▶ Zinc supplementation may be necessary to treat bone disease especially if serum zinc is found to be low. However, adequate food sources are generally recommended, including meat, eggs, fish, cheeses and cereals.

Liver complications

The liver is the first site of iron deposition in all iron loading anaemias, since hepatocytes are the main site of iron storage, the site of synthesis of hepcidin and the production of iron-storage proteins. In these anaemias, the capacity of iron binding proteins to store iron is overwhelmed, especially in transfusion dependent anaemias; this allows unbound iron to accumulate in hepatocytes and trigger oxidative stress leading to cell death. In addition viral hepatitis is a risk in even occasionally transfused patients, since both hepatitis B and C are blood born infections, although adequate screening of donor units reduces this risk greatly. The inflammation caused by any viral hepatitis, as well as iron toxicity, are the main causes of liver damage in the thalassaemias and other anaemias. Hepatic cell death triggers an increase in fibrotic tissue, and there may be progression from inflammation to fibrosis and eventually cirrhosis. Further, in some patients, steatosis of the liver appeared to increase the risk of liver fibrosis (Ricchi et al, 2018; Padeniya et al, 2022).

In anaemias where haemolysis is a major feature, gallstones with intrahepatic cholestasis are common and can contribute to liver disease. In pyruvate kinase deficiency, haemolysis gives rise to indirect hyperbilirubinaemia, with severe disease manifesting in the perinatal period where the majority (207 (90%) of 230) of newborns required phototherapy and/or exchange transfusion. Overall, 3% of PKD patients develop liver cirrhosis (Grace RF 2018).

Progression to hepatocellular carcinoma (HCC) has been reported in β-thalassaemia, especially in NTDT (Fragatou et al, 2010; Finianos A 2018; Ricchi et al, 2021) and is now a leading cause of premature death in the thalassaemia syndromes.

Timely identification of risk factors for the progression of liver disease, including iron overload and viral hepatitis, is essential so that preventive interventions can be offered. Regular monitoring of patients is a key factor in early detection of risk factors.

Recommendations for prevention and management of liver disease

- Adequate iron chelation.
- Hepatitis B vaccination.
- Attention to blood transfusion safety practices.
- Direct antiviral therapy (DAAs) where hepatitis C infection is identified.
- Anti-inflammatory/hepatoprotective agents with anti-HCV effects form a promising therapeutic regimen for advanced HCV-infected patients during or after treatment

with DAAs (Li et al, 2018). Possible agents include silymarin (Polyak et al, 2013), metformin (Abdel Monem et al, 2021), andrographolide, curcumin, oxymatrine and bicyclol. These act mainly through anti-oxidation, anti-inflammation, anti-proliferation and immunomodulation and they can serve as an adjunct to DAAs. However, additional randomised clinical trials are required before their general use is recommended.

- Avoid consumption of alcohol.
- General dietary factors: Energy intake through the diet should balance energy expenditure in order to avoid deposition of fat in cells other than adipocytes; any imbalance of energy intake will affect liver disease. However, micro - or macronutrient deficiencies may occur in chronic liver disease, and these must be recognised. There is an increased risk of fat-soluble vitamin deficits and patients with hepatocellular disease are more susceptible to protein deficiency. Cirrhotic patients have a higher risk of micronutrient deficiency. Vitamin K deficiency is caused by decreased liver storage levels. Patients with severe parenchymal or obstructive hepatic disease may have reduced production of vitamin D. All patients with liver pathology require full nutritional status assessment regularly and individualised nutritional advice offered by professionals, with supplements addition as necessary. (Silva et al, 2015).

Nutrition and the heart

Cardiac disease remains a major concern in thalassaemia. Although the availability and use of flexible chelation regimens has greatly decreased early deaths from cardiac failure, increased inflammation due to iron-overload can accelerate the atherosclerotic process, while juvenile diabetes and endocrine alterations may set in earlier than in non-thalassemic people. Data on 159 TDT patients from Torino, age 40.9 ± 8.4 years, revealed the following cardiovascular risk factors: hypertension 12%, and low serum lipid levels with low HDL levels: 49.6% of patients had HDL < 40 mg/dL. The low levels of total cholesterol were countered by a high prevalence of dyslipidemia with low values of HDL. 28% of patients had diabetes mellitus, 3.8% had at least one episode of heart failure, 56 (35.9%) showed early signs of heart failure, 34 (22%) had a diagnosis of diastolic dysfunction, 60 (38%) developed a left ventricular ejection fraction <55%, and 33 (21.4%) had supraventricular arrhythmias (Barbero et al,2021). Interestingly, patients with hypogonadism showed an increased rate of cardiovascular events. Hypogonadotropic hypogonadism may have a strong influence on the development of metabolic syndrome, altering hormonal levels, and increasing cardiac siderosis. Gonadal dysfunction may interfere with cholesterol levels, and in this study hypogonadic patients had higher levels of total cholesterol and LDL.

Common manifestations of cardiac involvement are:

- Atrial fibrillation, which is the commonest arrhythmia encountered in people with thalassaemia, affecting as many as one third of patients, is also common in PKD. One of the most important causes of AF is cardiac iron overload and the harmful effects of increased oxidative stress. Iron-induced AF can be reversed by using an intensive iron chelation regimen (Malagù et al, 2022)

- Heart failure (HF) is the end results of all conditions severely affecting cardiac structure and function and is amongst the main causes of death in patients with thalassaemia, particularly in those with suboptimal blood transfusion or iron chelation therapy (Farmakis et al, 2017). Optimising chelation therapy is crucial (Pennell et al, 2013), and continuing restriction of salt consumption and abstinence from alcohol are essential in patients with heart failure, along with a general healthy diet and lifestyle, also including smoking cessation and regular exercise tailored to each patient's health status.

Nutritional factors affecting heart complications

Vitamin D deficiency has been associated with cardiac function and cardiac iron load in patients with thalassaemia: serum 25-hydroxy-vitamin D (25-OHD) concentration has been shown to positively correlate with left ventricular ejection fraction and inversely with N-terminal B-type natriuretic pro-peptide (NT-proBNP) (Ambarwati et al, 2016). In addition, serum 25-OHD and 25-OHD/1,25-OHD ratio have both been associated with cardiac T2*, a magnetic resonance imaging (MRI) marker of cardiac iron load (Wood et al, 2008; Dejkhamron et al, 2018). As a result, vitamin D supplementation has been suggested to maintain a 25-OHD concentration at 30-40 ng/mL in order to promote cardiac heath, along with preventing osteoporosis, hypercalciuria and nephrolithiasis (Goldberg et al, 2022).

Zinc deficiency may affect the heart and vessels through modulation of oxidative stress (Rosenblum et al, 2020). Zinc deficiency has been associated with heart disease in the general population, but no evidence on this association exists in patients with thalassaemia. In a meta-analysis of 7 studies, a decrease in serum zinc ion concentration in non-thalassaemic patients was associated with the incidence of coronary heart disease (Meng et al, 2022). In addition, clinical studies – again in non-thalassaemic groups - have shown that zinc supplementation in patients with heart failure was followed by improvement in echocardiographic indices of cardiac function (Rosenblum et al, 2020). In thalassaemia, zinc deficiency has been associated with impaired glucose metabolism, which is, in turn, related to heart disease. Zinc supplementation is suggested in thalassaemia, particularly those with diabetes or impaired glucose metabolism, and is often required in patients on desferrioxamine and deferiprone therapy (Goldberg et al, 2022).

Selenium deficiency has been associated with coronary artery disease in a meta-analysis of 30 studies, mostly observational ones (Flores-Mateo et al, 2006). In a meta-analysis of 16 randomised clinical trials (Ju et al, 2017), selenium supplementation was followed by improvement in markers of inflammation and oxidative stress in patients with coronary artery disease, but did not improve lipid profile or mortality.

Iron overload, resulting from repetitive blood transfusions and increased intestinal iron absorption, represent a major complication in patients with thalassaemia, leading to cardiac dysfunction and heart failure. Restriction of nutritional iron is important in patients with non-transfusion-dependent thalassaemia, in whom iron overload results from increased nutritional iron absorption; these patients should be advised to maintain a low-iron diet. In contrast, in

patients with transfusion-dependent thalassaemia, the impact of nutritional iron is low compared to that of transfusional iron, and a low-iron diet may affect patients' quality of life and create a false sense of security that may prevent optimal adherence with iron chelation therapy and so is not generally recommended (Fung, 2010).

Metabolic and other disorders related to nutrition

Disorders of glucose metabolism. The pathophysiology of abnormal glucose metabolism in thalassaemia is complex and multifactorial, but the main factor is iron overload leading to pancreatic and hepatic injury with subsequent decreased insulin sensitivity, impaired glucose tolerance, impaired fasting glucose or diabetes mellitus (De Sanctis 2016). Disorders of glucose metabolism are strongly associated with heart disease. They constitute a risk factor for the development of atherosclerotic cardiovascular disease, including coronary artery disease, cerebrovascular disease and peripheral artery disease as well as heart failure (Seferovic et al, 2018; Visseren et al, 2021). Individuals with diabetes have high cardiovascular risk, regardless of other risk factors. As a result, frequent monitoring of markers of glucose metabolism and the endorsement of a healthy lifestyle - with maintenance of normal body weight, healthy eating and frequent exercise - are of utmost importance for patients with thalassaemia, for prevention and early diagnosis and treatment (Seferovic et al, 2018, Cosentino et al, 2020; Cappellini et al, 2021).

Body composition: In a small clinical study of 67 adult thalassaemia patients, the majority had a normal body mass index, but 21% were overweight, and altered body composition was observed, characterised of by increased adiposity, low levels of lean body mass and high rates of sarcopenic obesity – that is, obesity in combination with loss of muscle mass and strength (Lidoriki et al, 2022). Increased visceral adiposity has been associated with cardiovascular disease in patients with normal weight or overweight in individuals in the general population (Darroudi et al, 2022). In addition, sarcopenic obesity also seems to increase the risk of cardiovascular disease in older subjects in the general population (Evans et al, 2021).

Dyslipidaemia. Lipids are hydrophobic or amphipathic small molecules which include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides and phospholipids. The crucial role of lipids in cell, tissue and organ physiology is evident by their unique membrane-organising properties, providing cells with functionally distinct subcellular membrane compartments. Biological functions of lipids include components of cellular membranes, modulating immunological responses, and endocrine actions - for example steroid hormones.

The observed changes in circulating lipid subclasses in thalassaemia patients present a pattern reminiscent of patients with cardiometabolic or mitochondrial diseases, reflecting dysfunction in mitochondria and peroxisomes. These changes may have a potential in both predictive diagnostics and therapeutics (Botta et al, 2021). Hypercholesterolaemia is uncommon in patients with thalassaemia, who usually have low serum levels of total cholesterol and low-density (LDL) cholesterol. Dyslipoproteinemia in thalassaemia is a consequence of lipid peroxidation associated with iron overload (Haghpanah et al, 2010). The low cholesterol levels are related to depletion of vitamin E and the severity of thalassaemia. These are findings from Thailand in a group of patients

with TDT and HbE/β thalassaemia. The changes in lipid profile were related to iron overload, associated with increased oxidative stress, lipid peroxidation and liver damage. These appear to be a major cause of future morbidity and mortality in TDT patients. Hypocholesterolemia may be protective against cardiovascular disease, but many TDT patients with low levels of HDL-C also have hypertriglyceridemia levels, and elevated concentrations of highly atherogenic triglyceride-rich lipoprotein remnants (derived from chylomicrons and VLDL-C). These may contribute to age related morbidity and mortality (Sengsuk et al, 2014).

In accordance with these findings, it seems that subclinical atherosclerosis is common among thalassaemia patients and may be evident even from childhood, with studies revealing increased carotid artery intima-media thickness, an early marker of atherosclerosis in children with thalassaemia (Hahalis et al, 2016; Sherief et al, 2017; Ibrahim et al, 2022,). In addition, endothelial dysfunction has been shown in patients with thalassaemia (Aggeli C 2005).

In conclusion, the frequently observed metabolic changes in patients with thalassaemia, including dysglycaemia, impaired body composition and dyslipidaemia, in combination with the ageing of the thalassaemia population (Farmakis et al, 2020), all point to an increased risk for future cardiovascular events and highlight the importance of a healthy lifestyle, including proper eating.

Recommendations for specific dietary interventions

- ***Antioxidants.*** Free heme resulting from erythrocyte destruction, and excess iron derived from blood transfusions and increased intestinal absorption, are highly oxidising agents and sources of reactive oxygen species in patients with thalassaemia. Oxidative stress is known to play a role in the pathophysiology of thalassaemia and its complications including cardiovascular disease, and many agents with antioxidant properties have been tested as potential therapies, including vitamins C and E, flavonoids (like silymarin), curcuminoids, zinc supplementation, alpha lipoic acid, N-acetylcysteine (NAC), l-carnidine, arginine, fermented papaya, Omega-3 fatty acids and gum arabic. (Bou-Fakhredin et al, 2020). Small studies have shown promising results with some of these, in terms of cardiovascular complications, but no solid evidence exists to recommend any of them for routine clinical use.

  In animal studies, the addition of antioxidants, such as vitamin C, N-acetylcysteine, or acetaminophen to chelation therapy has been shown to reduce the risk of arrhythmias such as atrial fibrillation, which constitutes the most common sustained arrhythmia in patients with thalassaemia and which is associated with an increased risk of stroke and predisposition to cardiac dysfunction and heart failure (Nomani H 2019).

- ***Macronutrients to limit.*** Several nutritional elements or dietary patterns have been associated with an increased risk of atherosclerosis, arrhythmogenesis and cardiovascular disease. These include saturated and trans fats, ketogenic and liquid

protein diets, the Southern and other diets, energy drinks and excessive caffeine intake, as well as heavy alcohol drinking, all of which should therefore be avoided.

- ***Nutrients with protective effects.*** In contrast, other nutrients and dietary patterns have cardioprotective and antiarrhythmic properties. These include fish, nuts and other foods enriched in omega-3 polyunsaturated fatty acids, the Mediterranean and other healthy diets, vitamins E, A and D and certain minerals (magnesium, potassium, selenium). Antiarrhythmic mechanisms include modification of cell membrane structure by n-3 polyunsaturated fatty acids, their direct effect on calcium channels and cardiomyocytes and their important role in eicosanoid metabolism, enhancing myocyte electric stability, lowering heart rate, and improving heart rate variability (Manolis et al, 2022).

Pulmonary hypertension

Pulmonary arterial hypertension is an age-related complication in the thalassaemias, affecting mostly adults with NTDT although reports of the condition in children have been reported. Patients with β-thalassaemia major are less frequently affected and even those with NTDT the frequency in well treated patients is around 2% (Derchi et al, 2014). The condition is associated with the risk of right-sided heart failure and carries a risk of mortality (Pinto et al, 2022). It also affects exercise tolerance and quality of life. Risk factors increasing the risk of pulmonary hypertension include chronic anemia and ineffective erythropoiesis, haemolysis, iron overload, vasculopathy, splenectomy and hypercoagulability. The diagnosis is made by echocardiography measuring tricuspid valve regurgitant velocity, TRV, and cardiac catheterisation. Prevention by measures to ameliorate ineffective erythropoiesis are most important while drug therapy such as sildenafil and bosentan are used mainly based on data from other conditions (Taher et al, 2023).

Pulmonary arterial hypertension is also reported in pyruvate kinase deficiency affecting 3% of children 12-18 years and 5% of adults over 18 years (Grace and Barcellini, 2020).

Recommendations for specific dietary interventions

- a low-sodium diet (2000 mg/day) can help manage fluid retention and oedema.
- additional consideration for fluid management is to limit intake to 2 litres/day (Pulmonary Hypertension Association 2021).

Nephrolithiasis in thalassaemia

As the life expectancy of individuals with thalassaemia increases, age-related conditions such as kidney stones - nephrolithiasis - are increasingly common (Motta et al, 2020). While the incidence of nephrolithiasis in the general population is 9%, in those with thalassaemia is estimated to be at least double that, ranging between 18 to 59% based on different reports (Ricchi et al, 2012; Scales et al, 2016; Wong et al, 2013, 2017). Kidney stones are more common in adults with thalassaemia than children (Wong et al, 2013) but the precise prevalence and age at which the risk of stones increases is not known.

Kidney stones are a result of biomineralisisation at the interface of the renal papilla or collecting duct with the urine, or within the urine itself. Stone formation may be triggered by changes in saturation, solubility, hydrodynamics, or an imbalance between stone promoting and inhibiting constituents (Ratkalkar and Kleinman, 2011). Stones can have a highly complex composition, including ordered matrix proteins, trace metals, organic acids, lipids, and polysaccharides (Ramaswamy et al, 2015; Witzmann et al, 2016). Most stones are calcium-based, specifically calcium oxalate. It is unclear if patients with thalassaemia have a different profile of stone type. One report suggests both struvite and calcium oxalate type stones can occur in this patient group (Wong et al, 2017), but further study is required.

In non-thalassaemic populations, renal stones commonly cause episodes of acute renal colic with severe pain, nausea and vomiting. They can also be associated with end-stage renal disease, decreased bone density, and increased fracture risk. The recurrence rate for kidney stones is as high as 50%, so it is important to identify risk factors and implement strategies to reduce their incidence and recurrence.

In the general population, ~ 50% of the risk of stones is due to genetic factors but behavioural, environmental, metabolic, and disease-associated factors also contribute significantly. Limited information is known about the risk factors for nephrolithiasis in people with thalassaemia, and whether they differ. General guidelines have been proposed for managing nephrolithiasis in thalassaemia (Sayani et al, 2022). Here, we focus specifically on nutritional risk factors, but will also briefly highlight the various disease-associated and metabolic abnormalities in thalassaemia that may contribute to the increased incidence of kidney stones.

Nutritional risk factors for nephrolithiasis (see Table 4.1)

Diet. In the general population, a diet rich in vegetables and low in animal protein reduces stone formation due to higher intake of citrate and phytate, which inhibit stone formation in the urine by competing for binding of calcium and other minerals (Ratkalkar and Kleinman, 2011). Conversely, a diet rich in animal protein increases stone risk by increasing calciuria, uricosuria and oxaluria, while reducing citrate excretion (Taylor et al). A high fructose diet also increases the risk of kidney stones by increasing calciuria, oxaluria and uricosuria (Taylor and Curhan, 2008). High sodium intake increases stone formation by increasing calciuria and reducing citrate excretion (Curhan et al, 1993). Diets containing average amounts of calcium, moderate protein, and low sodium help to reduce recurrence of kidney stones and are to be encouraged (Borghi et al, 2002). The dietary risk factors for stone formation in thalassaemia have not been specifically studied, but children and adults with thalassaemia generally tend to have diets with less meat and sodium, and fewer iron and zinc rich foods (Fung et al, 2012).

Hydration. In general, increased fluid intake decreases stone formation due to dilution of stone-forming minerals (Curhan et al, 1996), and tea consumption is associated with 10% fewer stones. How these factors contribute to stone risk in thalassaemia is unknown. Tea is encouraged in thalassaemia since its bioactive compounds including epicatechins have been shown to decrease absorption of dietary non-heme iron (de Alarcon et al, 1979; Fung et al, 2012).

Table 4.1: *Proposed Risk Factors for Nephrolithiasis in Patients with Thalassaemia*

Dietary and Lifestyle Factors	Environmental Factors	Biochemical Factors	Disease Associated Factors
- Low dietary calcium intake - Calcium supplementation - High dose vitamin C supplementation - High fat mass per body mass index - Low physical activity	- Warm climates	- Hypercalciuria - Hyperuricosuria - Hyperoxaluria - Hypocitraturia	- Splenectomy - Ineffective erythropoiesis - Iron overload - Iron chelation - Diabetes - Hypoparathyroidism

Calcium. Supplemental calcium intake is associated with increased risk of stones in the general population (Curhan et al, 1997). However, increasing dietary calcium reduces the risk of incident nephrolithiasis, likely due to calcium binding of oxalate in the gut, reducing intestinal oxalate absorption and eventual urinary excretion (Curhan et al, 1993). Many thalassaemia patients have a lower intake of dairy foods rich in calcium due to lactose intolerance, personal choice or cultural beliefs (Fung et al, 2012). Since decreased bone mineral density is a concern in people with thalassaemia, calcium supplements are often prescribed; their role in nephrolithiasis has not been fully studied. In a small case-control study evaluating renal calculi in patients on desferrioxamine or deferasirox, vitamin D and calcium supplementation did not appear to increase the risk of stone formation (Ricchi et al, 2014).

Vitamin D. Vitamin D deficiency is common in patients with thalassaemia, who are often prescribed daily or high-dose intermittent vitamin D supplementation. In a meta-analysis, vitamin D supplementation was not associated with a higher risk of kidney stones in the general population (Malihi et al, 2016). Thalassaemia patients with serum 24-OH vitamin D levels > 30 ng/ml have been shown to have a higher incidence of hypercalciuria, but the impact on stone incidence and recurrence is unknown (Quinn et al, 2011). Further study of the role of vitamin D supplementation in stone formation in thalassaemia is thus needed.

Vitamin C. Doses of vitamin C greater than 1000 mg/day are associated with increased risk of nephrolithiasis in healthy men, thought to be due to metabolism of vitamin C to oxalate (Ferraro et al, 2016). It is thus generally recommended for those with high risk for kidney stones to avoid high doses of vitamin C. Individuals with thalassaemia are often vitamin C deficient, since iron overload exhausts antioxidants including vitamin C (Elalfy et al, 2016). Moderate doses of vitamin C, 2 – 3 mg/kg/day, are recommended to improve the efficacy of desferrioxamine; but high doses of vitamin C should be avoided.

Zinc. Both low and high dietary zinc have been associated with a higher risk for stone formation in the general population (Tasian et al, 2017). In thalassaemia, urinary zinc loss is nearly four times

higher than in to controls (Erdoğan et al, 2013), which is further increased with diabetes (Fung et al, 2015) and by the use of chelators such as desferrioxamine and deferiprone (al-Refaie et al, 1995). The role of zinc deficiency on risk of nephrolithiasis in thalassaemia, however, has not been evaluated.

Obesity. In the general population, individuals with a history of nephrolithiasis have been shown to have a higher body mass index (BMI) (Wong et al, 2013, 2017). Patients with thalassaemia generally have a normal BMI, however their fat content may be high for body weight (Fung et al, 2010), possibly increasing their risk for kidney stones.

Environmental risk factors

Chronic exposure to extreme temperatures due to climate and occupation increase stone risk (Borghi et al, 1993; Tasian et al, 2014). The high temperatures may increase risk of stones due to low urine volume and increased concentration of stone-forming minerals (eg. calcium, oxalate). Increased fluid intake of > 2.5L/day can help counteract this (Pearle et al, 2014). Although the environmental risk factors for kidney stone formation in thalassaemia have not been evaluated, most individuals with thalassaemia live in climates with high temperatures which may contribute to their increased risk.

Metabolic risk factors

Metabolic risk factors for stone formation include hypercalciuria, hypocitraturia, hyperuricosuria, hyperoxaluria, alkaline urine pH and low urine volume (Sayani et al, 2022).

Hypercalciuria is commonly seen in individuals with TDT, with a prevalence ranging between 30 – 69 % (Aliberti et al, 2022; Quinn et al, 2011). In thalassaemia, a higher rate of kidney stones has been reported in those with hypercalciuria, with a recent study of 176 adults noting stones in 14.8% of hypercalciuric patients versus 3.7% of non-hypercalciuric individuals (Aliberti et al, 2022). In thalassaemia, the etiology of hypercalciuria is likely multifactorial including renal disease, hypoparathyroidism, iron chelation, diabetes and high bone turnover. Transfusions and deferasirox may exacerbate hypercalciuria.

Hyperuricemia and hyperuricosuria have been reported in 40% and 80% of over 100 thalassaemia patients respectively (Chaloemwong et al, 2019). Risk factors for hyperuricemia were intact spleen and a lower fractional excretion of uric acid. However, their relationship to nephrolithiasis risk was not clear since there was only one case with nephrolithiasis.

Disease-associated risk factors

In thalassaemia, splenectomy, increased erythropoiesis, iron overload and chelation therapy, and iron-associated complications, are additional disease-specific risk factors for nephrolithiasis.

Splenectomy. One study noted 91% of patients with kidney stones were splenectomised (Ricchi et al, 2012). High circulating erythroblasts post-splenectomy may increase urinary uric acid.

Iron load and iron chelation. Serum ferritin has been shown to be lower in thalassaemia patients with kidney stones, raising a possible role of iron chelation (Wong et al, 2013). Renal and tubular dysfunction may result from anaemia, hypoxia, iron overload and iron chelation (Mahmoud et al, 2021; Ponticelli et al, 2010; Quinn et al, 2011). Baseline renal abnormalities and hypercalciuria are seen with both desferrioxamine and deferasirox, however, urine calcium excretion is greater with deferasirox. The association of deferasirox with hypercalciuria and stone risk warrants further study to guide management.

Hypoparathyroidism. Low parathyroid hormone reduces renal calcium re-absorption, resulting in risk for hypercalciuria and kidney stones in those on calcium supplementation.

Diabetes. In the general population, diabetes is an independent risk factor for the development of kidney stones (Prochaska et al, 2016) may contribute to the risk of nephrolithiasis in people with thalassaemia.

High bone turnover. A relationship between osteoporosis, hypercalciuria, kidney stone and risk of fractures has been reported in the general population but has not been adequately studied in thalassaemia (Lauderdale et al, 2001; Melton et al, 1998; Wong et al, 2013). Bisphosphonates, specifically alendronate, have been shown to reduce bone turnover and hypercalciuria in the general population (Giusti et al, 2009). However, studies that showed bisphosphonates' ability to reduce bone mineral density and bone turnover markers in thalassaemia did not report on urinary calcium excretion (Dede et al, 2016). Thiazide diuretics and bisphosphonates, as in the general population, may have the potential to reduce hypercalciuria in thalassaemia.

Summary and recommendations

- Patients with thalassaemia have an increased risk for nephrolithiasis.
- There are no reports of increased incidence compared to the standard population of nephrolithiasis in people with PKD; however, people experiencing abdominal pain should seek the advice of a trained medical professional.
- Though there are few dedicated large studies of nephrolithiasis in thalassaemia, risk factors have been proposed based on understanding from the general population.
- There is insufficient thalassaemia-specific evidence to provide evidence-based recommendations for monitoring, prevention and management of kidney stones.
- Guidelines for monitoring have been adapted from the general population to the known or proposed risk factors for stone formation in thalassaemia (Pearle et al, 2014). See Table 4.2.
- Approaches to management are summarised in Table 4.3

Table 4.2: *Prevention of Nephrolithiasis*

Factor Category	Guidance
Hydration	Encourage adequate hydration. If prior history of nephrolithiasis, encourage fluid intake >2.5 L/day
Sodium intake	Encourage less than 2300 mg sodium/day
Fruit and vegetable intake	Encourage 5 to 7 servings a day
Body weight	Encourage BMI between 18.5- 22.9 kg/m2 (Asian) or 18.5-24.9 kg/m2 (all other groups)
Non-contact weight bearing physical activity	Encourage minimum of 150 min/week of moderate intensity activity (adults); 60 min/day (children and adolescents)
Calcium intake	1000 mg/day (adults < 50 years) with focus on dietary sources. Limit supplemental calcium to 500 mg Ca (elemental) per day
25OH Vitamin D	Maintain between 30 - 50 ng/mL (75 -125 nmol/L)
Manage Diabetes	Maintain serum fructosamine 400 umol/l
Transfusion therapy	Optimise therapy to reduce ineffective erythropoiesis
Vitamin C supplements	Avoid supplements >1000 mg/day.
Hypogonadism	Focus on prevention and management of hypogonadism
Chelator therapy	Monitor adverse effects of chelators on zinc, phosphate and calcium excretion

Table 4.3: *Management of Nephrolithiasis*

Factor Category	Guidance
Comprehensive care	Encourage clinical care management from team of specialists: urologist, nephrologist, endocrinologist, and dietitian
Treat hypercalciuria	Low salt diet, protein restriction (adults only) and consider a thiazide diuretic. In adults with hypercalciuria and osteoporosis, bisphosphonate therapy may be considered
Treat hyperuricosuria	Dietary purine restriction, increased fluid intake and urine alkalinisation, improve transfusion regimen if evidence of increased ineffective erythropoiesis, and consider allopurinol
Treat hypocitraturia	potassium citrate
Hydration	Maintain urine output greater than 2-2.5 L/day
Chelation therapy	Reducing chelation dose or switching chelation therapy is unclear. May not be possible for many patients to change chelation due to concerns over iron overload and tolerance

Ca: calcium; PTH: parathyroid hormone; 25-OHD: 25 hydroxy vitamin D; BMI: body mass index
(Adapted from Pearle MS et al, J. Urol., 2014;192:316; Sayani et al, Open J. Urol., 2022;12:209-227).

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CHAPTER 5

Overview of risk factors for nutritional deficiencies and approach to nutritional assessment

Risk factors for nutritional deficiency in thalassaemia and PKD

The aetiology of nutritional inadequacy in patients with thalassaemia and PKD is multi-factorial. In general, the environment within which nutritional deficiencies develop is different for affected children and adults - although for all age groups, cultural food restrictions and inaccurate information regarding foods to avoid, as well as individual food intolerances, may contribute. Inadequate intake in combination with increased excretion and elevated requirements of nutrients leads to increased risk for nutritional deficiencies.

Inadequate dietary intake is the most obvious reason for nutritional inadequacy. A number of factors may play a role in reducing food intake in thalassaemia including stress, depression, adrenal insufficiency, fatigue, physical inactivity and various cultural influences: (Fung, 2012; Fung et al, 2015; Nanas et al, 2009; Huang et al 2015). In general, severe anaemia is known to contribute to poor appetite, a biological determinant of food intake along with hunger and taste. For some patients, nausea or other gastrointestinal complaints accompany the use of oral chelators and may lead to some missed meals, food intolerance and decreased or altered food intake. Reports suggest that caloric intake appears to be balanced with expenditure in many adults with thalassaemia, yet nutrient density is lacking leading to poor intake of sufficient micro-nutrients and trace elements (Fung, 2012).

Anecdotal food diaries from people with PKD often include the exclusion of otherwise healthy, iron-containing plant-based foods like spinach, beans, legumes, lentils, whole grains and tomatoes in favor of higher-calorie, nutrient poor, filler foods. Additionally, there is a nutritional cost of the accelerated red blood cell production in both conditions, increasing requirements for folic acid, B12, B6, protein, zinc and other nutrients required for erythropoiesis.

In thalassaemia, the increased need for anti-oxidants, including vitamins C and E, β-carotene, copper and zinc, has been shown to be related to protection against the oxidative stress and inflammation resulting from iron toxicity (Walter et al, 2006; Fibach et al, 2019). People with PKD experience a vicious cycle of chronic haemolysis, inflammatory response, and iron overload but no specific nutrients have been studied to protect against the damage or slow the cycle (Zaninoni et al, 2020).

Though few published studies have tested specific nutrient absorption in these conditions, there is little evidence to suggest that absorption is disturbed or that there is an alteration to gastrointestinal function. Tea, however, is frequently consumed by adults with thalassaemia due to its inhibitory properties on dietary non-heme iron absorption. However, when tea is consumed as the replacement beverage for milk at meals for young children, it will result in the displacement of calories, protein, calcium, zinc and other essential nutrients found in abundance in milk – Table 5.1

Table 5.1: *Nutrient Composition of 'Milk Tea' compared to Whole Milk*

8 oz cup of Black Tea with 1 Tbsp Milk + 1 tsp sugar	8 oz cup of Whole Cow's Milk Fortified with Vitamin D
25 Kcal 0.5 g Protein 0.5 g Fat 5.0 g Carbohydrate 17 mg Calcium 1.5 mg Magnesium 12 mg Phosphorus 20 mg Potassium 6 IU Vitamin D 7 µg Vitamin A 0.1 mg Zinc	150 Kcal 8 g Protein 8 g Fat 12 g Carbohydrate 280 mg Calcium 24 mg Magnesium 200 mg Phosphorus 320 mg Potassium 100 IU Vitamin D 110 µg Vitamin A 1 mg Zinc

8 oz = ~ 230 mls.

Essential trace minerals may be lost along with iron during chelation therapy, thus increasing the requirement for these minerals. When hepatic iron concentration is significantly elevated, zinc and copper losses are negligible. However, when hepatic iron levels near the normal range, chelator binding capacity is greater than the amount of circulating free iron and this can lead to chelation of both zinc and copper. Though chelation adherence is critical to overall health of the patient, time spent focused chelation therapy may borrow from time spent in planning for meals, food shopping and meal preparation, and fatigue from anemia or gastrointestinal stress from the medication may lower energy and motivation for these tasks. Factors which may contribute to nutritional deficiencies in these conditions are outlined in Table 5.2.

Table 5.2: *Summary of factors that may lead to nutritional deficiencies in patients with thalassaemia and PKD*

Dietary & Nutrient Intake	Energy Expenditure & Nutrient Losses
• Inadequate or inappropriate intake due to feeding behavior issues in toddlers and young children • Poor appetite due to anaemia, adrenal insufficiency, zinc deficiency or inactivity • Inactivity and inadequate fluid intake may lead to chronic constipation, lowering appetite and dietary intake • Insufficient nutrient density (foods high in calories yet limited nutritional value)	• Increased mineral losses due to non-specific chelation effects • Essential trace mineral sequestration (Zn, Cu) in the liver due to iron-overload • Increased non-transferrin bound iron leading to increased oxidative stress and antioxidant consumption • Ineffective erythropoiesis and increased cardiac output leading to elevated energy expenditure

• Lactose intolerance leading to avoidance of dairy products rich in protein, calcium, vitamin D and potassium • Avoidance of foods rich in heme-iron leading to limited protein and zinc intake; avoidance of nonheme-iron leading to limited intake of key vitamins and phytonutrients • Avoidance of tomato-based and other vitamin-C products, which enhance iron absorption, leading to vitamin C and lycopene shortage • Replacement of nutrient dense beverages such as whole milk with tea • Nausea, cramping abdominal pain from use of oral chelators leading to missed meals, specific food intolerances and decreased food consumption • Increased consumption of low nutrient dense, filler foods for satiety that contain sodium, trans fat, and sugars	

Approach to nutrition assessment

In health, optimal nutritional status is essential to support growth and pubertal development from childhood through adolescence (Inzaghi et al, 2022; Nijjar and Stafford, 2019), and is integral to bone health (Prentice et al, 2006), optimal immune function (Venter et al, 2020), and academic performance (Rampersaud et al, 2005). Though fewer studies have been conducted in thalassaemia and none in PKD, nutritional status has been shown to improve growth (Fuchs et al, 1997; Soliman et al, 2004), and specific nutrients have been shown to improve bone density (Fung et al, 2013; Ozdemir et al, 2013) and decrease risk factors for the development of diabetes (Fung et al, 2020; Matter et al, 2020).

Developing an individualised patient care plan must begin with a complete nutritional assessment. This relies on the 'ABCD's': Anthropometric, Biochemical, Clinical and Dietary assessment tools. Taking accurate **anthropometric measures** is the first step for both paediatric and adult patients. At the most basic level this includes height and weight and the calculation of body mass index (kg/m2). For children and adolescents, calculation of height and weight percentiles from appropriate reference data is helpful. Height and weight Z-scores (standard deviation scores) are particularly useful in patients whose growth values fall significantly below the 3rd percentile on the growth chart. Calculation of growth velocity normalised per year is informative to determine if the child is growing at a rate consistent with their age and gender. Examples of growth charts can be found in Appendix 12.1. Though country specific growth charts are available, they are not recommended, as optimal growth should be independent of race or ethnicity (Natale et al, 2014).

Historically, sitting height has also been assessed in patients receiving desferrioxamine, because of frequent vertebral height deformity and overall truncal shortening in young patients prescribed > 50 mg/kg/day (Haidar et al, 2012; Levin et al, 1995,). As high dose desferrioxamine is now not recommended, and seldom used, the need for regular sitting height assessments may be unnecessary.

For patients at risk for malnutrition, more advanced anthropometric assessment may include body circumferences and skinfold measures to assess waist to hip ratio, subcutaneous fat stores and mid-arm muscle circumference (MAMC). More recently mid-upper arm circumference (MUAC) has been used as a simple alternative measure of nutritional status as there is less error involved in the measurement, given it does not require the combined assessment of a triceps skinfold (Mramba et al, 2017). Online tools are available to the clinician for MUAC Z-score calculation based on data from NHANES (peditools.org Chou et al, 2020). Lean mass, MAMC and skinfold thicknesses have been reported to be low in some (Fung et al, 2010; Elalfy et al, 2020) but not all patients with thalassaemia (Goldberg et al, 2018). How far a patient deviates from normal will provide an indication of under or over-nutrition as well as muscle and fat reserves.

Total body fat and lean mass may be useful for the clinician when counselling patients to participate in more physical activity, and when they observe changes in these measures they may be more motivated to continue to exercise. Total body fat mass may also provide a better understanding of underlying insulin resistance or glucose intolerance in a patient with a normal BMI but high fat mass. Body composition can be assessed most easily by a whole-body Dual Energy X-ray Absorptiometry (DXA) scan. A whole body DXA scan is recommended for patients > 10 years with thalassaemia (Cappellini et al, 2021). The procedure is non-invasive, has minimal radiation exposure and takes less than 2 minutes, and is anyway recommended to assess for bone density. Guidelines for DXA examination for adults with PKD vary based on baseline findings; typically, annual assessments are recommended for those identified with osteopenia or osteoporosis. Children with PKD should receive their first DXA scan between ages 16-18 years. Alternative commonly used non-invasive tools to assess body composition include bioelectrical impedance, under-water weighing or air displacement plethysmography. A discussion of these techniques is beyond the scope of this book; however the reader may refer to the summary of body composition techniques by Ceniccola et al (2019) for more information. Dietitians and other nutritional professionals are well versed in these assessments, and can help interpret the anthropometric information.

Biochemical information can be obtained by monitoring a patient's blood, urine or stool. Nutrient levels in these materials provide useful information regarding specific nutrient deficiencies or toxicities, as well as information about possible cause. Each nutrient has its own unique metabolism, regulated typically by the intestine or kidney. Up or down regulation of nutrient intestinal absorption or re-absorption at the distal tubule in the kidney maintains homeostasis in the face of variable dietary intake. Exhaustion of these mechanisms may result in increased risk for nutritional deficiency. As an example, increased urinary calcium excretion can be an indication of negative calcium balance, while low circulating levels of 25 hydroxy vitamin D [25-OHD] is sign of poor vitamin D intake or storage and risk for vitamin D deficiency. Details regarding how nutritional status is assessed for essential nutrients of interest in thalassaemia is provided in Table 5.3.

Table 5.3: *Biochemical tools to assess essential nutrients most commonly deficient in patients with thalassaemia and PK Deficiency*

Essential Nutrient	Preferred Sampling	Example Adult Reference Range*	Causes of Derangements
Folate	Serum folate, protect from light (reflects acute status) Red blood cell folate (reflects long term status)	> 5.9 ng/mL > 366 ng/mL	Increased: vitamin B-12 deficiency Decreased: Liver disease, malabsorption syndromes, HIV infection
Vitamin B12	Serum vitamin B12, fasting When low, follow up test recommended urinary methylmalonic acid (MMA)	200 – 900 pg/mL	Increased: chronic renal failure, congestive heart failure, diabetes Decreased: lack of intrinsic factor – 'pernicious anaemia', celiac disease, malabsorption syndromes, folate supplementation can mask a B-12 deficiency, if suspected test urinary MMA
Vitamin C	Plasma, serum or white blood cell ascorbate	Serum: 0.4 – 1.5 mg/dL	Decreased: Alcoholism, hyperthyroidism, cancer
Vitamin A	Plasma or serum retinol, fasting	30 – 80 ug/dL	Increased: glucocorticoid therapy, oral contraceptives Decreased: zinc deficiency, hypothyroidism
Vitamin D	Serum 25OH Vitamin D significant seasonal variability – lowest in winter, highest in summer	30 – 50 ng/dL	Decreased: Malabsorptive syndromes, pancreatic insufficiency, very limited sun exposure Note: 1,25-OHD is a poor indicator of vitamin D status though may be useful in differentiating deficiency
Vitamin E	Plasma or serum alpha tocopherol, fasting, protect from light Plasma gamma tocopherol	0.5 – 1.8 mg/dL	Increased: Hyperlipidemia, Decreased: malabsorptive syndromes

Vitamin K	Serum or plasma phylloquinone, fasting Follow up: coagulation screen (PT) or PIVIKA	0.13 – 1.19 ng/mL	Decreased: hepatic disease, malabsorptive syndromes
Calcium	Serum calcium Ionized serum calcium Total body bone mineral content by DXA	8.5 – 10.5 g/dL 4.8 to 5.6 mg/dL BMC Z-score >-2.0	Serum calcium is tightly regulated and not indicative of nutritional status. Serum Ca should be corrected for low albumin when necessary [Corrected Ca= (4-albumin in g/dL x 0.8) + serum Ca in mg/dL] Deficiency is difficult to assess in clinical situations, calcium balance is the gold standard in research studies, BMC by DXA useful though not specific to calcium homeostasis
Copper	Serum Copper, fasting morning When low, follow up: Ceruloplasmin	70 – 120 ug/dL	Increased: Wilsons disease, birth control medication Decreased: Menkes disease, prolonged high dose zinc supplementation
Iron	Serum ferritin Transferrin saturation or % TIBC not informative in TDT	<200 ug/L	Iron deficiency is rare in Thal/PKD. Serum ferritin is a poor indicator of iron loading, especially in NTDT.
Magnesium	Serum magnesium, fasting	1.6 – 2.6 mg/dL	Increased: Dehydration, renal insufficiency, diabetes, hypothyroidism Decreased: Hypoparathyroidism, malabsorptive syndromes

Selenium	Urinary selenium, spot morning urine (assesses deficiency or toxicity) Plasma selenium (reflects dietary intake)	10 – 35 ug/g creatinine 23 – 190 ug/L	Decreased: Hepatitis, cardiomyopathy
Zinc	Serum or Plasma Zinc, fasting, morning sampling	70 – 120 ug/dL Lower values in non-fasting samples	Increased: estrogen Decreased: food intake, infection, hypoalbuminemia, with time of day

Blood should be drawn pre-transfusion for any blood test in a transfusion dependent patient. Consult local lab guidelines for specimen handling (e.g. fasting, time of day) which may have significant effect on results. Avoid haemolysis.

PIVIKA: protein induced by vitamin K absence

**Reference ranges obtained from: Tietz NW. Clinical Guide to Laboratory Tests. 3rd Edition, 1995 and Kleinman, Ronald E., and Frank R. Greer. Pediatric Nutrition. Ed. Ronald E. Kleinman and Frank R. Greer. 8th edition. Itasca, IL: American Academy of Pediatrics, 2020.*

Reference ranges may vary substantially by reference laboratory, consult local laboratory for age specific reference ranges.

This table includes those nutrients most commonly found to be deficient in patients with thalassaemia and PKD. In theory, very poor dietary intake and increased metabolic stress could lead to deficiency in any of the essential vitamins and minerals.

Clinical assessment includes checking for symptoms or visible signs of possible nutritional deficiency such as hair loss, changes in hair color, edema, skin lesions, bleeding gums, fatigue, poor concentration or decreased appetite, referred to as a 'nutritional physical'. Many of these signs are non-specific, and only appear in cases of severe malnutrition. Examples of some of the signs and symptoms typically observed with severe nutritional deficiencies are provided in Table 5.4, while pictorial images are provided in the extensive color atlas reference by Baran R (1991). In patients with thalassaemia and PKD, anaemia-related fatigue can result of the condition itself, and in most instances is not primarily dependent on nutritional adequacy.

Table 5.4: *Examples of typical signs and symptoms of severe nutritional deficiencies*

Nutrient Name & Function	Food Sources	Signs / Symptoms of Deficiency	Signs / Symptoms of Toxicity	Drug Interactions / Comments
Folate *Function: Coenzyme in single carbon transfer in RNA & DNA, needed for proper cell division*	Dark green leafy, beans, fresh fruit, Peanuts, yeast, liver, kidney	Nausea, diarrhea, megaloblastic anemia, impaired cellular immunity, neural tube defects, cleft lip/palate in infants of women with folate deficiency	Masks B-12 deficiency	Interactions with: methotrexate, seizure medications, oral contraceptives
Vitamin B-12 *(cyanocobalamin)* *Function: Myelination and neuron function, red blood cell synthesis*	Foods of animal origin only: Fish, liver, eggs, cheese	Pernicious! macrocytic / megaloblastic anemia, ataxia, muscle weakness, (takes years to develop)	Diarrhoea, Very rare	Interactions with: Proton pump inhibitors Deficiency is masked by folate supplementation
Vitamin C *(ascorbic acid)* *Function: collagen biosynthesis, non-heme iron absorption, bone formation, antioxidant properties*	Fresh citrus fruits, tomatoes, potatoes, strawberries (destroyed by cooking)	Fatigue, bleeding gums, perifollicular hemorrhage, scurvy	Nausea, vomiting, kidney stones, rebound scurvy when high dose supplementation is abruptly stopped	Interactions with: iron, acetaminophen, aspirin, statins, warfarin
Vitamin A *(retinol)* *Function: Immune function, growth and development, male and female reproduction , proper vision*	Retinyl esters: Liver, kidney, eggs, dairy, Pro-vitamin A carotenoids: yellow, orange, red colored vegetables	Night blindness, xeropthalmia, blindness, keratomalacia, poor bone growth, increased infection	Anorexia, hepatotoxicity, alopecia	Interactions with: hepatotoxic drugs, tetracycline, warfarin
Vitamin D *(calciferol)* *Function: calcium absorption, maintains serum calcium, bone growth and remodeling, immune and neuromuscular function*	Fatty fish, egg yolk, fortified foods	Rickets, osteomalacia, muscle fatigue, hypophosphatemia, cardiomyopathy	Hypercalcemia, poor growth, vomiting, nephrocalcinosis	Interactions with: digoxin, thiazides, verapamil Anti-seizure medications interfere with vitamin D metabolism

Vitamin E (tocopherol) *Function: Antioxidant, protects against reactive oxygen species*	Wheat germ oil, sunflower, safflower, soybean and oils, peanuts, peanut butter	Hemolytic anemia, increased oxidative stress, ataxia, neuropathy	Impaired leukocyte function	Interactions with: Aspirin, iron, ibuprofen, naproxen, anticoagulants, some statins and chemotherapy medications Fat malabsorption associated with vitamin E deficiency
Vitamin K (phylloquinone, K1 Menaquinone K2) *Function: co-enzyme for a carboxylase in hemostasis and bone metabolism*	Green vegetables, soy oil, seeds, fruits	Bruising, bleeding/poor blood poor bone mineralization	hyperbilirubinemia	Interactions with warfarin Primary deficiency quite rare
Calcium *Function: bone structure, mediates blood vessel contraction & dilation, muscle function, blood clotting, nerve transmission, hormonal secretion*	Milk, cheese, yogurt, dark green leafy vegetables, fortified milk alternatives	Osteopenia, tetany	Hypercalciuria, kidney stones	Interactions with: some blood pressure medications and calcium channel blockers
Copper *Function: Intestinal iron absorption, free radical scavenging*	Sshellfish, meat, legumes, nuts, cheese	Menkes Disease (genetic Cu deficiency), hypochromic anemia, neutropenia, osteoporosis, depigmentation of skin and hair	Wilson Disease (genetic Cu toxicity) Nausea, vomiting, liver damage, zinc deficiency	Interactions: None
Iron *Function: essential to hemoglobin for oxygen transfer to tissues, growth, neurological development*	Red meat (heme), shellfish, legumes, fortified cereals (non-heme)	Microcytic anemia, fatigue, delayed cognitive development, poor growth, pica	Liver toxicity, cardiomyopathy, endocrinopathy, Increased risk of infection	Interactions with: tetracycline, ciprofloxacin, some seizure medications Vitamin C increases non-heme iron absorption.

Clinical assessment also includes consideration of medical history to identify co-morbidities with nutritional implications (e.g. coeliac disease), opportunistic infections, complications such as diabetes, usage of medications with nutrition implications including chelation therapy, food and drug interactions for example proton pump inhibitors, and behavioral risk factors like cigarette smoking, alcohol abuse, and pica that affect or are affected by diet and nutritional status. In infants and children, these assessments should also include weaning and feeding practices as well as dietary intolerances. All these factors should be considered when conducting an introductory nutritional interview.

In determining total caloric needs for a patient, there are published validated calculations that have been developed for non-thalassemic populations which are based on weight, height, gender and age of the individual patient (for example https://www.ncbi.nlm.nih.gov/books/NBK591034/ and also Schofield, 1985; Mifflin et al, 1990); these are complex and should only be undertaken and interpreted by nutrition specialists with a thorough understanding of them. Although patients with thalassaemia may have increased energy expenditure and therefore increased caloric needs, unfortunately, there are no thalassaemia derived calculations. Calculations for non-transfusion dependent patients with sickle cell disease have been published (Buchowski et al, 2002; Williams 2002), though the utility of these calculations in patients with thalassaemia and PKD has not been studied.

Assessment of **dietary intake** is the final essential step in the comprehensive nutritional status assessment. Dietary assessment is a science in itself and entire careers have been dedicated to creating and validating tools for clinical use. The results can provide useful information not only on dietary quantity and quality, but also on appetite change, food allergies and intolerances. Obtaining accurate dietary intake of school-aged children and teenagers can be especially difficult as they may consume the majority of their meals and snacks outside the home. Considering the population of interest, questions regarding cultural food restrictions, food insecurity and/or access to fresh fruits and vegetables should also be considered. Understanding an individual's food restrictions and preferences will be integral to personalising their nutrition recommendations.

Most commonly used methods to quantify intake in a clinical situation include the 24-hr food recall method, 3 or 7-Day Food Record and Food Frequency Questionnaires. Each method has benefits and limitations, as discussed in detail in previous reviews (Block, 1982; Tabacchi, 2014; Foster and Bradley, 2018; Bailey, 2021). Once dietary information has been obtained, it is quantified using a dietary analysis tool (e.g. NDSR Food Processor® https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC2662624/) and compared to reference data from healthy individuals. The dietary assessment is only as robust as the nutrient data within the analysis tool. If the tool does not contain culturally specific food dishes, the dietary analysis may not accurately assess a patients' usual intake, an important consideration for individuals with chronic disease who consume foods that are not reflected in the database. Further, nutrient requirements may exceed those of the healthy individual reference data, therefore comparisons made may give a false impression of nutritional adequacy or inadequacy. A summary of the dietary assessment tools used by nutritional professionals along with their limitations is provided in Table 5.5.

Table 5.5: *Advantages and Limitations of Dietary Assessment Methods*

Dietary Assessment Methods	Description of Method	Advantages	Limitations
24-Hour Recall	Patient is asked to report on all food and fluids consumed in previous 24-hour period	- Patient will not change intake, reports on actual consumption - Patients typically able to remember what they ate yesterday with prompting questions - Better at assessment of macronutrients vs. micronutrients - Patient literacy not required	- Patient may be a poor historian of food intake with regard to portion size and exact ingredients of mixed dishes - Only considers one day of intake, which may not be representative of usual intake or seasonal intake - Requires a trained nutrition professional to gather most accurate information
Food Record	Patient is asked to write down all food and fluids that are consumed in a certain time period, typically 3 to 7 days	- Can be an accurate representation of intake especially if measuring instruments or scales are provided - If record takes place over a 7-day period may be a more accurate representation of intake variability across weekdays and weekends	- Patient may change intake, eat healthier or consume fewer calories as a result of reporting fatigue - Patient may alter intake or choose to make simpler meals to ease the recording process - Significant time for the patient - Patient literacy is required
Food Frequency Questionnaire	Patient is asked to recall how much and how often a specific food is consumed. Validated questionnaires may reflect intake over 1 week period to 12 months	- Can be an accurate representation of dietary intake over a longer period of time - For certain nutrients found in a small number of foods or food groups (e.g. calcium), food frequency assessment can be a more accurate estimation of intake - Can be quantitative, semi-quantitative or qualitative	- Questionnaires may not reflect cultural food groups - Patient may be a poor historian and unable to accurately assess portion size - Not as precise of a tool to assess intake - Patient literacy is required
Screener or History	- Patients are asked about usual consumption of foods that contain a specific nutrient (calcium) or specific food groups (e.g. fruits and vegetables) - Typically reflective of intake over the previous month or year	- Can be an accurate representation of a specific nutrient or group of nutrients - More be a more accurate representation of seasonal changes in dietary intake	- Patient may be a poor historian and unable to accurately assess portion size - Limited to assessment of just one nutrient or group of nutrients, not reflective of total dietary intake - Patient literacy is required

Adapted from Bailey R. Curr Opin Biotechnol 2021;70:90-96.

Summary and recommendations

- The environment within which nutritional deficiencies develop is somewhat different between paediatric and adult patients with thalassaemia and PKD, though for all age groups, food intolerances, cultural food restrictions, and inaccurate information regarding foods to avoid, may all contribute to poor nutrient intake.
- Patients with thalassaemia exhibit multiple risk factors that may lead to nutritional deficiencies including inadequate intake, insufficient nutrient density, avoidance of certain food groups, increased nutrient loss, increased nutrient requirements and increased energy expenditure. Patients with PKD are similar, though it has not been established with certainty that they have increased energy needs or may compensate in food-dense environments.
- Developing an individualised patient care plan must begin with a comprehensive nutritional assessment.
- The comprehensive nutrition assessment is composed of ABCDs: anthropometric, biochemical, clinical and dietary assessment tools.
- Anthropometry, at a minimum, should include height and weight, and calculation of growth velocity as well as sitting height and body composition as needed.
- Nutrient specific biochemistries should be conducted on an annual basis.
- A review of signs and symptoms of nutritional deficiencies should be included as part of the annual comprehensive thalassaemia and PKD physical exam with the assistance of an experienced dietitian.
- Dietary intake assessment by a registered dietitian is valuable to uncover significant deviations from food group and nutrient recommendations.

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CHAPTER 6
Macronutrients

Young patients with thalassaemia have historically presented with poor growth and delayed pubertal development (De Sanctis et al, 2013). Growth failure has been a common occurrence regardless of phenotype or country of origin (Raiola et al, 2003; Hamidah et al, 2008; Gomber and Dewan, 2006; Karamifar et al, 2005; Taher et al, 2015; Arab-Zozani et al 2021). Though the growth rate in very young children may be normal, it typically starts to falter at the beginning of puberty, and both transfusion dependent (TDT) and non-transfusion dependent (NTDT) patients have rarely achieved their genetic potential for height (Arab-Zozani 2021). Even recent reports collected from contemporary patients who are regularly transfused and chelated have reported significant short stature and malnutrition (Elalfy et al, 2020; Raghuwanshi et al, 2020; Bulgurcu et al, 2021). For the general paediatrician, poor rate of growth is a hallmark of undernutrition in children and a 'red flag' that something is amiss. The necessity to address growth concerns before they worsen is of interest for all patients.

Growth failure in thalassaemia is multifactorial and may be related to chronic anaemia in those not fully transfused, but also to chronic liver disease, intensive use of chelating agents, endocrinopathies (hypogonadism, delayed puberty, hypothyroidism, disturbed calcium homeostasis and bone disease), dysregulation of the growth hormone/insulin like growth factor-1 axis as well as nutritional deficiencies. A more detailed discussion of growth failure can be found in Chapter 4. The focus of this chapter is the relationship between macronutrient intake and growth failure and delayed pubertal development in children and adolescents with thalassaemia.

Prior reports have shown that not all of the growth failure observed is related to hypogonadism and growth hormone deficiency (Skordis and Kyriakou, 2011). Therefore, if nutritional status is partly to blame, the general assumption holds that total calories and protein intake are inadequate to meet the specific needs of the individual. In these patients, the nutrition-related growth failure may be related to increased requirements, decreased dietary intake, or both.

Energy expenditure

A simple equation defines the energy relationship in the human body. During steady state when a patient is in balance, energy or caloric intake equals demand or expenditure. For adults, when the equation is balanced, body weight is maintained, though it is feasible that body composition can be altered. When intake is greater than expenditure, energy is stored and body weight increases; individuals lose weight when expenditure exceeds dietary intake. In children, if expenditure exceeds intake, growth and pubertal development may be compromised. Total energy expenditure (TEE) is comprised of energy expended when the body is at rest (REE), the thermogenic response to food (TEF) and the energy required for physical activity, growth and or the disease/healing processes.

$$TEE = REE + TEF + [activity + growth + disease]$$

For most individuals REE is the largest component of TEE, though the contribution will vary based on the activity of the individual. REE is primarily the energy required to maintain cell membrane pumps, muscle function, metabolic processes of the liver, kidney and brain as well as cardiac output and respiration. The strongest determinant of REE is lean mass as fat mass has minimal metabolic activity. This is relevant to the estimation of REE in patients with thalassaemia given that most equations used to determine REE are based on total body weight and not lean mass. For examples of equations used to estimate energy expenditure see Mifflin et al (1990) and the recent systematic review of equations used in paediatric populations by Fuentes-Servin et al (2021). Patients with thalassaemia have reportedly low lean mass for body weight (Fung, 2010), therefore the REE may be overestimated in thalassaemia adults.

Other components of TEE include TEF, activity, growth and disease processes. TEF comprises energy required for the digestion, absorption and storage of food. On average, TEF is 8 to 10% of TEE though will vary with composition of the diet: dietary protein and carbohydrates require more energy to assimilate compared to dietary fat (Fung, 2000). Although there are no studies which have assessed TEF in thalassaemia, dietary composition is similar to the general population so the expectation is that the contribution to TEE would be similar (Fung et al, 2012). Various disease processes may result in elevated caloric requirements secondary to increased protein turnover, inflammatory processes, or alterations in cardiac or respiratory function. For children and adolescents, the energy and protein needed for growth must also be considered as part of TEE. For healthy individuals, voluntary activity is the most variable component of TEE. Physical activity is reportedly reduced in adolescents and adults with thalassaemia compared to the general population (Fung et al, 2015). This will be explored in Chapter 10.

Indirect calorimetry has been used to measure REE, or the number of calories consumed when an individual is at rest, in 7 adult transfusion dependent thalassaemia patients aged 22-30 years with average BMI: 18.2 kg/m2 (Vaisman et al, 1995). Measurements were taken on the morning prior to transfusion when Hb was < 9g/dL and again 3 days following a transfusion when Hb > 12 g/dL. They found that energy expenditure decreased by 9% following a red cell transfusion ($p=0.02$). Pre-transfusion REE was elevated when compared to predicted amounts based on weight, height and age (range 98 – 132% predicted). REE returned to near normal amounts post transfusion, which was negatively related to haemoglobin concentration but not related to heart rate. Given this is the only direct report of energy expenditure in the condition, it is difficult to draw conclusions to the broader population of patients with thalassaemia. However, similar calorimetry studies combined with whole body protein turnover have been conducted in sickle cell disease, another hemoglobinopathy with increased red-cell turnover (Buchowski et al, 2002; Williams et al, 2002; Hibbert et al, 2006). Findings from these studies indicate that increased erythropoiesis combined with cardiac energy consumption account for most of the increased energy and protein requirements. Others have calculated that adults with thalassaemia who have poorly controlled diabetes may have even greater energy requirements due to metabolic demand of glycosylating unstable haemoglobin (Wiwanitkit et al, 2007). Overall, the potentially increased need for total kilocalories in thalassaemia is thought to be related to increased protein requirements for red cell turnover, as well as increased cardiac output. What has yet to be clarified, however, is how much

higher are caloric needs for patients with thalassaemia, and if these needs change throughout the transfusion cycle for those with TDT.

Dietary intake

Few studies have explored dietary intake in large samples of contemporary patients with thalassaemia (Fung, 2010; Fung et al, 2012; Goldberg 2018 et al; Elalfy et al, 2020). The North American Thalassemia Clinical Research Network conducted a cross-sectional analysis of dietary intake in 221 adult and pediatric patients with a variety of phenotypes though 90% were transfusion dependent (Fung et al, 2012). Subjects were asked to complete a validated food frequency questionnaire (The Block Questionnaire) which included the intake of nutritional supplements. Average kilocalorie intake was between 107 to 163% of estimated caloric requirement based on age, gender, weight, height and assumed sedentary lifestyle. Average macronutrient distribution was similar across age groups, though adult subjects tended on average to consume more kilocalories as fat than is considered acceptable (38% vs. 20 to 35%). Results suggested that although patients with thalassaemia generally had adequate and sometimes excess intake of macronutrients (kcal, fat and protein), intake of specific micronutrients, such as vitamin D, E, folate, calcium, and magnesium were lower than Dietary Reference Intakes for healthy individuals. However, DRIs were developed as guidelines for healthy individuals free of illness. Using the DRI as the recommendation for patients with chronic illness is not always appropriate given the potential for increased nutrient requirements for some patient populations. Therefore, simple nutritional comparisons to DRI levels, though informative, may be misleading.

More recently, Elalfy and colleagues (2020) conducted a 24-hour dietary recall in 200 young people with transfusion dependent β-thalassaemia (Average age 12 years) in comparison with 50 healthy young controls. They observed total caloric intake was on average 22% lower in patients with thalassaemia compared to healthy controls. Total protein, carbohydrates, calcium and phosphorus were also all reduced. Of note, the thalassaemia patients were also shorter and weighed significantly less than the controls, illustrating the point that patients in this sample were consuming a limited total number of calories that were not matched by increased nutrient density, yielding poor growth.

A few small studies sought to relate the commonly observed complications of stunting and wasting to inadequate intake. Soliman and colleagues (2004) placed 15 nutritionally deplete thalassaemia children (average age of 8 years) on a high calorie diet (130-150% of estimated requirement) for 8 weeks. Following 2 months of increased caloric intake they observed significantly higher body mass index (kg/m2), skinfold thicknesses, mid-arm muscle circumference and IGF-1. Fuchs et al (1996,1997) performed a similar study on toddlers with thalassaemia residing in Thailand a decade earlier. Linear growth did not improve after their short caloric intervention, though differences in weight gain, as well as plasma zinc, alpha tocopherol and IGF-1 were observed. Taken together, the improvements in IGF-1 in both of these studies suggest that some of the growth impairment in young thalassaemia patients may be partially corrected by addressing nutritional concerns.

There are a handful of studies which have investigated dietary intake in patients with thalassaemia in comparison with functional outcomes (ie. growth, weight gain) and/or biochemical markers of nutritional status. In 2018, Goldberg and colleagues assessed dietary intake in 41 patients with thalassaemia along with corresponding circulating levels of specific nutrients, to address the question, 'Is dietary intake sufficient to support adequate circulating nutrient levels?' Data from this study found that circulating levels of key nutrients were depleted, e.g. vitamin D and zinc, while dietary intake was at or above recommended levels. In Elalfy's et al (2020) observed reduced caloric and protein intake in children with TDT, associated with poor growth, altered lipid profiles, and depressed serum insulin concentration in comparison with healthy controls. They also noted that 25% of the young people were non-compliant with chelation, 20% had hepatitis C infections and pre-transfusion hemoglobin levels averaged 7.4 mg/dL - much lower than current standards of care. These combined data suggest that dietary intake is insufficient to support circulating levels of nutrients in both optimally and poorly transfused patients with thalassaemia.

Protein, fluids and fibre

Protein. Consistent intake of dietary protein is necessary for optimal function of every organ in the body. Dietary protein deficiency typically occurs along with energy imbalance and is referred to as protein calorie malnutrition (PCM). Though uncommon, signs of PCM include fatigue, growth disturbances, decreased muscle mass, immune dysfunction, thinning hair, brittle nails and dry skin. Protein is not typically a limiting macronutrient in most middle to high income countries. Individuals who consume a strictly vegan diet may have more difficulty meeting their protein requirements. The suggested intake of 'good quality' dietary protein per kg body weight is 0.8 g/kg/day for adults (National Academy Sciences, 2006), so for an individual who weighs 75 kg, the recommended protein intake would be 60 grams per day. Good quality proteins are those that have all nine essential amino acids, for example animal and soy protein. The protein recommendation is based on the amount of protein needed to achieve nitrogen balance in a healthy individual. Protein requirements are higher during periods of rapid growth (e.g. children 4-13 y: 0.95 g/kg/d), catch up growth and/or tissue healing. Protein recommendations are also given in terms of the percentage of calories as protein, or 10 to 30% of total kilocalories. The most recent National Health and Nutrition Examination Survey data (2011 to 2014) reports that the average dietary protein intake in US adults 19-50 years of age in the US is 89 g/day, of which 67% comes from animal sources (CDC, 2021).

Protein intake was assessed in the Thalassaemia Clinical Research Network study of 221 US patients with thalassaemia (Fung et al, 2012). Dietary protein averaged 75 grams per day in adults or 16.5% of total kcal intake – this compared to 89 g/day in other US adults. Young children consumed relatively less total protein (66 g/day), though as a percentage of kilocalories in the diet, protein intake was not different by age group. Animal protein was the largest contributor to total protein intake as dairy sources were quite low in this cohort, limited to 1 serving of dairy per day. Elalfy et al (2020) also reported a similar percentage of calories as protein in the diet (15%) of children with thalassaemia compared to healthy controls. In summary, from the few studies where protein intake was assessed, it does not appear to be limiting in the diet of patients with thalassaemia, at least for those residing in the US or Egypt.

Fluids. The importance of water in the body and sufficient hydration for optimal health cannot be overemphasised. Water is essential to all metabolic processes in the human body. Inadequate hydration can lead to fatigue, headache, dizziness, elevated heart rate, muscle cramps, constipation, and altered kidney and digestive function. Moreover, inadequate fluid and fibre intake combined with inactivity can lead to chronic constipation and abdominal discomfort, thereby lowering appetite and dietary intake.

When it comes to guidelines for fluid intake, there is no one consistent recommendation. Optimal hydration will vary depending on the age, gender, size of the individual, activity level and environmental conditions. One of the most common public health messages is to drink "8 glasses of water per day," but this is too simplistic to be useful for all individuals in all situations. Current guidelines for hydration status by the American Academy of Pediatrics (AAP) suggest children aged 4-8 years should consume 5 cups of fluid/day = just over 1 litre, including water and milk (Kleinman et al, 2020). The fluid guidelines scale with the age and gender of the child. It is also recommended that children should limit their intake of sugar-sweetened beverages, including 100% fruit juice (WHO, 2016). While water is the preferred beverage for children over 3 years of age, the goal for children 7-18 years of age is to limit juice intake to 8 oz (~230 mls] or less per day (AAP). These recommendations arise from concern about the steady rise in obesity rates in developed countries. Most juice-based beverages add significant calories to the diet, with minimal nutritional density. Moreover, most fruit juices eliminate the skin and pulp, thereby reducing the fibre content and satiating effect that comes with fruit consumption. While the risk of obesity may be less of a concern for patients with thalassaemia and PKD, the lack of nutritional density in juice compared with fruit should be considered. Of concern too is the public's inability to discern 100% juice products, which are considered a serving of fruit, from juice drinks or fruit drinks and beverages with minimal nutritional value.

For adults who are not exercising, the National Academy of Science recommends water intake to be 9 cups for women (72 oz = just over 2 litres) and 13 cups/day for men (104 oz = ~ 3 litres). Recommendations have also been provided on a body weight basis for paediatric patients (see Table 6.1). Athletes and individuals residing in warmer climates should increase their fluid intake. Patients with thalassaemia and PKD may face challenges in achieving adequate hydration. Treatment with the oral chelator deferasirox can cause gastrointestinal distress, nausea and diarrhoea. This may lead to dehydration in some patients. Moreover, chelation may be more toxic to the liver and kidney during periods of dehydration, thus stressing the importance of adequate fluid intake. Additionally, historically patients have been advised to consume tea to decrease the absorption of iron from meals. Both black and some green teas contain caffeine which act as a diuretic. It has been suggested that consumption of moderate quantities of caffeinated beverages may actually lead to dehydration, though this has not supported by research (Killer et al, 2014). Therefore, caffeinated beverages (coffee and tea) may be consumed as part of the total fluid intake for people with thalassaemia & PKD. Alcohol consumption, on the other hand, can suppress the body's ability to reabsorb water, thus leading to dehydration. Patients with thalassaemia and PKD should be extremely careful about their alcohol consumption, not only because it can lead to fluid imbalance, but also because it may be contraindicated for those with hepatic dysfunction.

Table 6.1: *Recommendations for Fluid Intake in Healthy Populations: Age and Weight Based*

	Daily Adequate Fluid Intake
Age (years)	***National Academy of Science Recommendation***
1-3 years	4 cups, 32 ounces, 950 mls
4-8 years	5 cups, 40 ounces, 1.1 litre
9-13 years	7-8 cups, 56-64 ounces, 1.7 - 1.9 litres
14-18 years	8-11 cups, 64-88 ounces, 1.9 – 2.6 litres
Men, 19 and older	13 cups, 104 ounces, 3 litres
Women, 19 and older	9 cups, 72 ounces, 2.1 litres
Weight (kg)	**Holliday-Segar Recommendation**
1 – 10 kg	100 mL / kg
10 – 20 kg	1000 mL + 50 mL/kg for each kg above 10 kg
> 20 kg	1500 mL + 20 mL/kg for each kg above 20 kg

Adapted from: National Academy of Science. Water. Dietary Reference Intakes, National Academies Press, 2006. Holliday MA, Segar WE. The maintenance need for water in parenteral fluid therapy. Pediatrics 1957;19:5:823-832. Lower amounts may be needed for individuals with smaller body surface area. These are considered guidelines and should not be considered a daily prescriptive amount. Exercise and hot climates will increase fluid requirements.

In healthy populations, urine coloration is typically used to discern if an individual is well hydrated. High volumes of clear, light yellow colored urine are a sign of adequate hydration, while small volumes of darkly colored urine are an indication of dehydration. However, in patients on chelator medications, darkly coloured urine is a sign of chelator efficacy: iron compounds cause the dark colouration. Urine may also change color following supplementation with water soluble B vitamins, certain foods (e.g. carrots, beets) and other medications e.g. antimalarial medications. People with haemolytic jaundice, as in PKD and NTDT, may also pass orange urine, which increases in intensity with dehydration. Therefore, total urine volume, or frequency of urination by a patient, may be a better indication of hydration status than the colour of urine produced.

Fibre intake in the diet has been associated with cardiovascular health, weight maintenance, cancer prevention, glucose maintenance and overall gastrointestinal health (Ionita-Mindrican et al, 2022). Inadequate consumption of fibre can lead to constipation, haemorrhoids, weight gain, high blood pressure and elevated cholesterol. Conversely, adequate consumption of fibre without adequate fluid intake can also lead to constipation. The 2015-2020 USDA Dietary Guidelines for Americans (https://www.dietaryguidelines.gov/sites/default/files/2020-

12/Dietary_Guidelines_for_Americans_2020-2025.pdf) recommends adults regularly consume at least 3 servings of whole grains per day or a total of 25 to 38 grams per day. To put this in perspective, one slice of 100% whole grain bread has 16 grams of whole grains for a 1 oz [28 g] serving.

A decade ago, dietary fibre intake was assessed by food frequency questionnaire in a large cohort of U.S. patients with thalassaemia (Fung et al, 2012). Fibre intake averaged 14 grams/day in adults, and was not different by age group. Total dietary fibre intake was similar to the US National Health and Nutrition Examination Survey 20-25 report of average fibre intake for healthy US adults, but was far lower than recommendations. Whole grain intake was also assessed. It was significantly lower in both adolescents and adults with thalassaemia (0.8 servings/day) compared to the US dietary guidelines recommendation (3 to 5 servings/day). Of interest, fruit and vegetable intake, and the fibre within these food groups was consistent with recommendations for age (see Table 6.2). Limiting whole grains from the diet may be a conscious choice for some patients due to iron fortification of whole grain products in the US [6 mg Fe/0.1 kg flour]. For transfusion-dependent patients, the amount of iron contained in fortified foods is minimal in comparison with the iron from one transfusion (roughly 200 mg Fe in 1 unit of packed red cells). Therefore, the advice to patients is now to focus on chelation and less on the iron content from food.

Table 6.2

	Older Children and Adolescents to 18.9 years 3.0		Adults ≥ 19 years	
	USDA Recommendation, Range	**Thalassemia n=83 Mean ± SD**	**USDA Recommendation, Range**	**Thalassemia n=108 Mean ± SD**
Whole grains (servings	3.0 – 3.5	0.9±1.1	3.0 – 4.0	0.8±0.7
Fruits (cups)	1.5 – 2.0	1.3±1.2	1.5 – 2.0	1.0±0.8
Vegetables (cups)	2.0 – 3.0	1.7±1.4	2.5 – 3.0	2.9±2.6
Dark Green Vegetables (cups)	0.29-0.43	0.3±0.3	0.43	0.4±0.5
Orange vegetables (cups)	0.21-0.29	0.1±0.1	0.29	0.1±0.2
Dairy (cups)	3.0	1.3±1.0	3.0	1.3±1.2
Meat (oz)	5.0-6.0	4.4±4.0	5.0-6.5	5.1±4.7

USDA: US Department of agriculture

SD: Standard deviation

Table reproduced from Fung EB et. al. J Acad Nutr Diet. 2012;112:980-990.

Whole grains servings: 1 serving = 16 grams

Fruit, Vegetable and Dairy: 1 cup ~ 240 mls

Meat: 1 oz ~ 28 grams

Dietary intake and energy expenditure in Pyruvate Kinase Deficiency

Growth has not been well characterised in people with PKD, but in haemolytic anaemias like thalassaemia and sickle cell disease, lower BMI and shortness in stature have been identified (Arab-Zozani et al; 2021, Rani et al, 2019; Bulgurcu et al, 2021; Da Silva de Jesus et al, 2018) suggesting the possibility in PKD. BMI data from the Peak Registry, a global longitudinal study of patients diagnosed with PKD with participants from around the world (Grace et al, 2023), was compared to WHO standards to determine levels of thinness to obesity (WHO 2014). There was a wide range of BMIs for individuals with PKD aged 20 years or older (n=61). For men aged 20-24 years, the mean BMI was 26.1 (overweight), for 25-59 years mean BMI 22.9 (healthy weight); and men 60 years plus the mean BMI was 28.4 (overweight). BMI for women aged 20-24 years was 22.5 (healthy), for women 25-59 years mean BMI was 26.4 (overweight), and for women 60 years plus mean BMI was 20.8 (healthy). To summarise, adults with PKD tend to have a healthy weight-for-height or are overweight. However, BMI does not account for delayed stature, and for this, it is important to look at children's data.

Growth data (BMI and height) of 57 school aged children and adolescents from the Peak Registry was compared to the World Health Organisation's child and adolescent growth standards (WHO 2007), which represents age and sex specific growth curves derived from measurements of subjects in extremely diverse populations thus making it internationally applicable (de Onis et al, 2007). Overall, the mean BMI-for-age of children and adolescents with PK deficiency fell within the 3rd to 97th percentile of the WHO standards with the exception of the 15-year-old girls' BMI, which was higher. The 3rd to 97th BMI represents the BMI spectrum from thinness to obesity.

Girls' height-for-age fell within the WHO standards, while boys' height-for-age was below the WHO's 3rd percentile for ages 5, 6, 7, 8, and 9 years indicating short stature. At age 11, boys' height-for-age fell within WHO standards until age 18. Height that is less than the 3rd percentile or greater than the 97th percentile is deemed short or tall stature, respectively (Nwosu and Lee, 2008). Based on the current data available, school aged boys with PK deficiency appear to be affected by short stature, although this may be a reflection of a small sample size.

In the absence of research on dietary intake and energy expenditure on people with PKD, practitioners will need to modify energy balance formulas with the goal of maintaining children on healthy growth trajectories, and adults with healthy BMIs. Each person should be considered on an individual basis.

Summary and recommendations

- Total kilocalorie intake in patients with thalassaemia is consistent with dietary recommendations based on height, weight and age.
- Nutrient density, or the concentration of essential nutrients per kilocalorie, is however limited. In particular, dietary intake of vitamin D, E, folate, calcium and magnesium are less than healthy individuals.
- Historically, high calorie, high protein diets have improved weight, body composition and nutritional biochemistries in young children with thalassaemia. It is unclear if a dietary supplement would be beneficial for all patients, or simply an increase in nutritional density.
- Calculation of energy expenditure, developed for healthy individuals, may not be appropriate for use in patients with thalassaemia and PKD due to the reliance on total body weight without consideration of body composition.
- Measured energy expenditure, in small cohorts of patients with TDT, is elevated and correlates with haemoglobin concentration, decreasing post transfusion. It is unclear how kilocalorie needs may vary within a transfusion cycle.
- Protein intake does not appear to be limited in patients with thalassaemia.

- Patients with thalassaemia may face challenges in achieving adequate hydration which could lead to fatigue, constipation, altered kidney and digestive function.
- Reports suggest fibre intake is less than recommendations which could lead to constipation, weight gain, high blood pressure and elevated cholesterol.
- A diet rich in fibre and unsweetened beverages should be encouraged.
- BMI data for adults and children with PKD suggest that people are receiving adequate calories, but nutrient adequacy is unknown because of a lack of dietary research in this group.
- In the absence of disease-specific guidelines, people with PK deficiency should follow established guidelines for calorie, protein, fluid, and fiber intake.

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Supplement Facts
Serving Size 2 Tablets
Servings Per Container 180
Amount Per Serving
% Daily Value
Supplement Facts
1 Capsule
Amount Per Serving
% Daily Value
5 mg
1,667%
Supplement Facts
Size 1 Softgel
% Daily Value
GESTED USE: As a dietary
lement, take one softgel one to
imes per day with water.
Do not take this product
gnant or nursing.
DO NOT USE IF SEAL IS BROKEN
KEEP OUT OF REACH OF CHILDREN
STORE IN A COOL, DRY PLACE
DO NOT REFRIGERATE

CHAPTER 7
Micronutrients

Part 1 – Vitamins

Published reports suggest that patients with thalassaemia often have deficiencies in vitamins C, D, E and folate. In this chapter we highlight the function of each of the vitamins which patients with thalassemia and PKD appear to be at greatest risk for deficiency, where they can be found in the diet, how they are assessed, and summarise what is known about their importance in patients with these conditions.

Vitamin C

Vitamin C is an essential nutrient for humans given their inability to synthesise ascorbate from its precursor, gulono-1,4-lactone. Interestingly, humans are one of the few vertebrates that lack a critical enzyme necessary to make vitamin C; domestic dogs and cats do not need vitamin C in their diet. Ascorbate is the biologically active form of vitamin C, which serves as the most abundant and important water-soluble antioxidant and a substrate for numerous enzymatic reactions.

Ascorbate is integrally involved in iron metabolism, and therefore is of critical importance to patients with enhanced iron absorption like thalassaemia and PKD. Dietary ascorbate enhances non-heme iron absorption in the gut. Ascorbate modulates iron metabolism by stimulating ferritin synthesis, inhibiting lysosomal ferritin degradation, and decreasing cellular iron efflux. Furthermore, ascorbate cycling across the plasma membrane is responsible for ascorbate-stimulated iron uptake, relevant to astrocyte brain iron metabolism and tissue iron loading in conditions such as these. Ascorbate is a novel modulator of the classical transferrin-iron uptake pathway, which provides almost all iron for cellular demands and erythropoiesis under physiological conditions (Lane and Richardson, 2014)

Numerous reports have documented the importance of vitamin C for optimal health in the general population. Vitamin C supplementation improved endothelial function; its effect is dependent on health status, with stronger effects in those with higher cardiovascular disease risk (Ashor et al, 2014). The role of vitamin C in epigenetic regulation has also been suggested, dependent on its bioavailability. When readily available, vitamin C can influence the demethylation of both DNA and histone, further leading to different phenotypic presentations (Taira et al, 2023). Ascorbate deficiency might play a role in various diseases such as neurodegeneration and cancer through epigenetic dysregulation (Camarena and Wang, 2016). The effects of vitamin C administration on cancer, cardiovascular diseases, and infections however remain uncertain, and high doses can cause overt side effects in some susceptible patients, such as oxalate renal stones (Doseděl et al, 2021). Moreover, re-bound scurvy type symptoms can occur with abrupt discontinuation of high dose supplementation.

Dietary sources and recommended intake

Excellent dietary sources of ascorbate include citrus fruits, such as oranges, grapefruits, lemons, and their juice. Guava, peppers, strawberries, blackcurrants, broccoli, brussels sprouts and potatoes are also good sources. There is significant variability in the interpretation of scientific data, and therefore the recommendations for vitamin C intake vary depending on country of origin: the UK recommends 40 mg/day, India 110 mg/day and the US Recommended Dietary Allowance for vitamin C in healthy adults is 90 mg/day for males and 75 mg/day for females (Carr and Lykkesfeldt, 2021).

Table 7.1: *Selected Foods Rich in Vitamin C*

Food Source, serving quantity	Vitamin C, mg
Sweet red bell pepper ½ cup Raw	95
Orange, 1 medium	70
Kiwifruit, 1 medium	64
Broccoli, ½ cup cooked	51
Strawberries, ½ cup raw	49

1 cup = ~ 240 mls.

Assessment

Vitamin C status is assessed by monitoring plasma or serum ascorbate levels. Care should be taken to protect the specimen from light and avoid haemolysis. Thawing and re-freezing as well as exposure to light will reduce the vitamin C concentration in the sample.

Vitamin C and thalassaemia

Several studies have highlighted the importance of vitamin C to reduce body iron burden and improve oxidative stress. Oxidative stress induced by iron overload is one of the major precipitating causes of vitamin C deficiency in TDT patients. It has been shown that patients with TDT have significantly lower levels of vitamin C compared to healthy individuals. Adequate levels can facilitate the accessibility of iron to chelators through increase of iron release from the reticuloendothelial system (Darvishi Khezri et al, 2016).

In another study, vitamin C levels from 100 paediatric TDT patients (64% Male, median age: 9 years, range 7 - 13 years) were compared with 30 healthy controls (Bhat et al, 2021). 85 % of the patients had low plasma vitamin C levels compared to none of the controls, and clinical signs and symptoms of scurvy (bone pain, bleeding gums) were observed in 7% of the patients. Dietary intake of vitamin C was similar between the patients and the control group. Vitamin C (200 mg/day for 15 days) was then provided to 36 of the patients with low plasma ascorbate. Increasing serum ferritin values was correlated with vitamin C deficiency, while the mean level of malondialdehyde, a measure of

oxidative stress, was significantly reduced with vitamin C supplementation in almost half. It was recommended that dietary counselling, along with supplementation with vitamin C in those with low levels, may ameliorate oxidative stress.

Deficiency of multiple essential nutrients is not uncommon in TDT, including those that function as antioxidants. To determine the possible benefit of supplementation with 2 of these, vitamin C and vitamin E, Dissayabutra and colleagues (2005) recruited 20 transfusion dependent children with thalassaemia. All of them had high oxidative stress and low vitamin C and vitamin E level at recruitment. Three months after vitamin C and vitamin E supplementation, plasma vitamin C, vitamin E and glutathione were significantly increased, while total bilirubin was slightly decreased. Other parameters including total antioxidant status, plasma and erythrocyte MDA, haemoglobin and plasma free haemoglobin, did not change during the study period. Given their synergistic relationship, supplementation with both vitamin C and E may have more benefit than vitamin E alone in promoting antioxidant status and enhancing liver function.

Vitamin C and PKD

Although less researched, at least one study has reported that ascorbate was decreased in all PKD patients compared to controls, an important indicator of iron overload syndrome (Roy et al, 2021).

Vitamin C: Summary and recommendations

- Reduced circulating ascorbate levels are frequently observed in patients with thalassaemia and in one study, patients with PKD.
- Patients with thalassemia and PKD should not avoid vitamin C-containing foods for fear of enhanced iron absorption; the benefits of natural sources of vitamin C outweigh any risks.
- Severe vitamin C deficiency (scurvy) is rare, though it has occasionally been reported (Maikap D 2022).
- Vitamin C may be useful for reducing oxidative stress and improving chelator efficacy.
- Monitoring of serum ascorbate for both paediatric and adult patients is recommended.
- Vitamin C should be provided to those diagnosed with deficiency, though care should be taken to ensure adequate chelation when supplementing with vitamin C, and it is usual to delay starting vitamin C supplementation until a month after starting chelation.
- The usual dose is 2 – 4 mg / kg taken orally. High dose supplementation should be avoided.

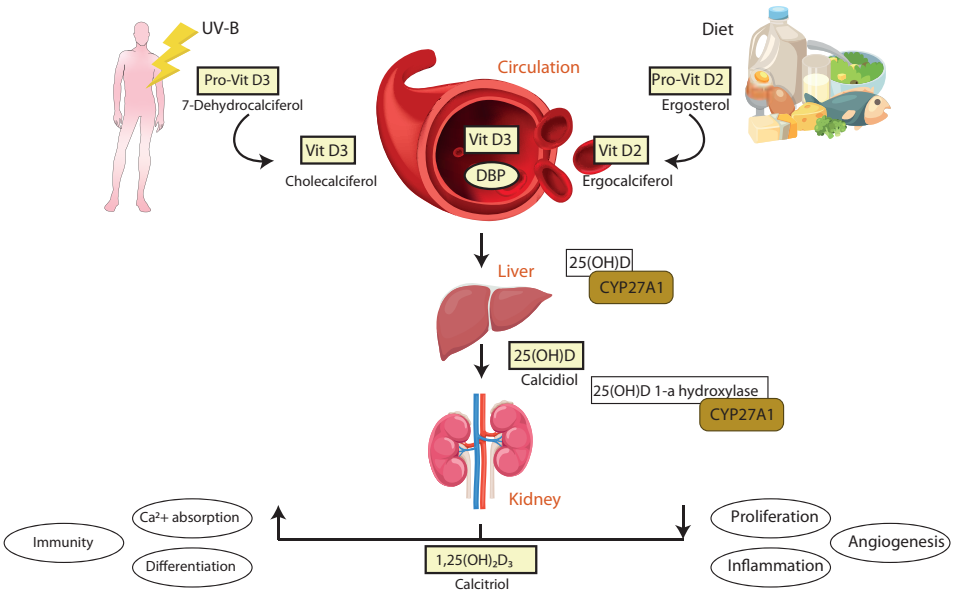
Vitamin D

Vitamin D is crucial for calcium homeostasis and for bone mineralisation, especially during periods of rapid growth, namely infancy and puberty. Vitamin D deficiency may lead to rickets - mineralisation defect at epiphyseal growth plates, and osteomalacia - mineralisation defect of bone tissue (Root and diamond, 2002)

Vitamin D is made through skin exposure to sunlight and thus is affected by sun exposure, season, skin colour, cloud cover and use of sunscreen. It is then transported to the liver and hydroxylated to the active form of 25-OHD. Parathyroid hormone regulates the additional hydroxylation to 1,25-dihydroxyvitamin D3 that takes place in the kidney. The major metabolite of vitamin D (serum 25-OHD) has a half-life of between 10 - 19 days (Condamine et al, 1994). This is the best indicator of vitamin D status and reflects levels from both dietary intake and skin synthesis. 1,25-dihydroxyvitamin D3 increases intra- and extracellular calcium concentrations by various mechanisms: it promotes intestinal calcium absorption, reduces calcium excretion through the kidneys and, in combination with parathyroid hormone, mobilises calcium from bone.

Fig 7.1: ***Diagram showing vitamin D synthesis pathway*** and signalling mechanisms relevant to PAD formation. The main forms of vitamin D in nature are vitamin D3 (cholecalciferol) that is synthesized in the skin of animals and humans in response to sunlight and obtained through diet. The vitamin D3 travels in the circulation bound to DBP. Vitamin D must undergo several hydroxylation steps to become an active metabolite. The synthetic pathway involves 25-and 1-alpha-hydroxylation of vitamin D3 and D2, in the liver and kidney, respectively. The first hydroxylation occurs in the liver resulting in the formation of 25(OH)D3 or calcidiol and the second hydroxylation occurs mainly within the kidneys and intestinal epithelial cells and immune cells and generates the most biologically active hormonal form of vitamin D: 1, 25(OH) 2 D, or calcitriol. The biologically active form of vitamin D3 is involved in the regulation of numerous cell cycle regulatory mechanisms protecting the vasculature from pathological conditions.

Abbreviations: Ca 2+ , calcium; DBP, vitamin D binding protein; PAD, peripheral arterial disease; Vit D3, vitamin D3; CYP27B1 is a gene that encodes the enzyme hydroxylase in the Kidney; CYO27A1 is a mitochondrial gene encoding for catabolic hydroxylase in the liver (also important for bile acid biosynthesis and regulation of cholesterol homeostasis)



Calcium, parathyroid and vitamin D homeostasis in children and adolescents

Adequate calcium intake and vitamin D during skeletal growth increases bone mass in adolescents and decreases bone loss in adults. In adolescents, there is an inverse relationship between serum 25-OHD levels and parathormone (PTH) levels (Johnston et al, 1992; Cheng 2005) and a positive association between serum 25-OHD levels and bone mineral density (BMD) (Johnston CC 1992; Cheng et al, 2005).

Serum levels of 25-OHD are directly related to bone mineral density with a maximum density achieved when the 25-OHD level reaches 40 ng/ml or more (Bischoff-Ferrari et al, 2006). Serum levels below 30 ng/ml are associated with a significant decrease in intestinal calcium absorption. In children, adolescents and adults this is associated with increased PTH and decreased insulin like growth factor-1, IGF-1 (Soliman et al, 2008a) and so low vitamin D levels inhibit linear growth as well as bone mineralisation. PTH enhances renal reabsorption of calcium, and stimulates the kidneys to produce 1,25-dihydroxyvitamin D while activating osteoblast cells within bone.

In young children with early-stage vitamin D deficiency, the reduced calcium absorption from the gut leads to an increase of PTH which stimulates the production of 1α(OH)2D3 therefore maintaining serum Ca within the normal range. With chronic or more severe vitamin D deficiency, PTH secretion becomes higher and continuous. This high PTH maintains normal serum Ca concentration at the expense of bones, with significant osteoclastic activity and a phosphaturic effect that leads to hypophosphatemia. Secretion of IGF-1 decreases further with slowing of bone growth (Soliman et al, 2008a; Bouillon, 2001; Soliman et al, 2010).

Clinical presentation of low vitamin D status

The clinical spectrum has a wide range of presentations of deficiency, starting from subclinical to frank vitamin D deficiency with levels less than 20 ng/dl. The presentation depends on the potent adaptation process that defends the body against hypocalcemia when vitamin D levels are low (Soliman et al, 2008a; 2010). It also depends on dietary calcium intake; when calcium intake is very low (<500 mg/day) with insufficient vitamin D (<20 ng/mL), full-blown deficiency occurs as the biological corrective mechanisms fail to compensate.

In children, vitamin D deficiency may present as delayed linear growth, teething and closure of the fontanel, broad wrist joints and 'bow legs'. In severe deficiency there may be hypocalcaemic tetany, and fractures. Radiological findings include absent or irregular line of ossification at metaphyseal fronts, excessive osteoid deposition (wide wrist space) with cupping, metaphysis decalcification, and the cortex of long bones with subperiosteal erosion of the shafts (Soliman et al 2008a; Bouillon et al, 2001; Soliman et al 2010; Vogiatzi et al, 2009).

In adolescents, presentation of severe and prolonged deficiency differs, and they may experience vague symptoms including pain in weight bearing joints, back, thighs and/or calves, difficulty in walking and/or climbing stairs and running, muscle cramps, facial twitches and carpo-pedal spasms. Improvement in back and joint pains and increased exercise tolerance has been reported in thalassemic adolescents with low 25-OHD levels after treatment with vitamin D (Soliman et al 2008a, 2008b). Radiological manifestations are less common and may present in the form of pseudo-fractures affecting the femoral neck or scapula, or generalised or metaphyseal osteoporosis (Vogiatzi 2009).

Dietary sources and recommendations

The richest sources of natural vitamin D in the diet are from fatty fish (e.g. sardines, salmon, swordfish), fish oils (e.g. cod liver oil), wild mushrooms and egg yolks (Table 7.2a). Given the widespread prevalence of vitamin D deficiency, some countries (Finland, Norway, Sweden, Canada and the US) have chosen to fortify foods such as cereals, milk, yoghurt and orange juice with vitamin D. In the US, fluid milk is typically fortified to 100 IU/cup. In the UK, margarine was previously fortified with vitamin D, but this practice ended in 2013.

Table 7.2a: *Selected foods rich in vitamin D*

Food Source, serving quantity	Vitamin D, mg	Vitamin D, IU
Cod Liver Oil, 1 Tbsp	34	1360
Salmon, cooked 3 oz	14.2	570
Mushrooms, white raw exposed to UV, ½ cup	9.2	366
Milk, fortified 1 cup	2.9	120
Egg, 1 large scrambled	1.1	44
Tuna fish, canned in water 3 oz	1.0	40

1 cup = ~ 240 mls.

Extracted from: Vitamin D Fact Sheet for Health Professionals: NIH Office of Dietary Supplements, 2022.

Assessment
The best indicator of vitamin D status is serum 25-OHD levels. Fasting is not necessary, though avoidance of haemolysis and lipaemic specimens is recommended. There has been much discussion regarding the threshold for vitamin D sufficiency, 25-OHD levels <25 nmol/L (10 ng/ml) are generally considered deficient; levels <80 nmol/L (32 ng/ml) are considered insufficient (Hock et al, 1988; Holick, 2009). Some clinical laboratories will assess 25-OHD2 and 25-OHD3 separately, the sum of D2 and D3 is preferred if available. Though 1,25-OHD is the metabolically active form of vitamin D, given its much shorter half-life (4-6 hours), it is rarely used (Holick, 2009).

Table 7.2b

New Zealand Lab Test Referance Range 2021

Referance range	Result
<25	Moderate to severe vitamin D defiency
25-50	Mild defiency
50-150	**Optimal target range for bone health**
>250	Vitamin D intoxication has been reported at this level, but is rare at levels <500nmol/L

Table 7.2c: *Serum 25-OHD Concentrations and Health*

nmol/L*	ng/mL*	Health status
<30 rickets	<12	Associated with vitamin D deficiency, which can lead to in infants and children and osteomalacia in adults
30 to <50 health	12 to <20	Generally considered inadequate for bone and overall in healthy individuals
≥50 health in	≥20	Generally considered adequate for bone and overall healthy individuals
>125	>50	Linked to potential adverse effects, particularly at >150 nmol/L (>60 ng/mL)

Reproduced from: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010

Vitamin D and thalassaemia

Vitamin D deficiency is common in people with thalassaemia in many countries despite availability of good sunshine exposure and routine prescription of daily vitamin D. There is impaired calcium homeostasis as a result of iron overload, and bone disease becomes an important cause of morbidity with pain, easy fracture, and sometimes vertebral collapse.

In children and adolescents with thalassaemia, many factors can compromise the adaptation process to Vitamin D deficiency including: IGF-1 deficiency, hypoparathyroidism, delayed puberty and hypogonadism, decreased bone mass and decreased synthesis of 25-OHD due to liver iron deposition (Soliman et al, 2008b; Vogiatzi et al, 2009).

Several studies have reported a higher risk of vitamin D deficiency due to genetic and ethnic factors, for example dark skin or concealing clothing may lead to limited sun exposure (Mahachoklertwattana et al, 2003). Decreased outdoor activities in thalassaemic patients can also compromise skin synthesis of vitamin D. Low Vitamin D, hypoparathyroidism and a blunting PTH response have been reported in many thalassaemic patients (Soliman et al, 2008a). In cases of iron overload and sub-optimal transfusion, osteopenia is caused by focal osteomalacia and reduced bone synthesis. In iron overloaded patients, bone density and histomorphometric studies indicated impaired trabecular and cortical bones.

Low vitamin D is associated with decreased cardiac function, muscle weakness, glucose insensitivity and refractory congestive heart failure. A proportional association between low vitamin D, high cardiac iron and increased ventricular dysfunction has been reported in a study of 24 thalassaemic patients (Lowry, 2008) although causation has not been proven. In addition, low vitamin D, in the absence of iron-induced hypoparathyroidism, results in secondary hyperparathyroidism, which exacerbates heart failure of any aetiology. In addition, elevated serum parathormone levels are associated with myocardial iron overload in patients with TDT (Dimitriadou et al, 2010).

Treatment of vitamin D deficiency

Adequate circulating levels of vitamin D are essential for optimal skeletal health in people with thalassaemia. There is increasing evidence that in general, in the absence of adequate sunlight exposure, at least 1000 IU dietary or supplemental vitamin D2 per day is required in adults to prevent deficiency.

Recent data has shown that inadequate vitamin D status in people with thalassaemia persisted despite daily supplementation of 400–1,000 IU vitamin D. Intermittent high-dose supplementation with oral 50,000 IU vitamin D2 every 3 weeks is an effective, safe and convenient way of increasing 25-OHD levels. Patients with inadequate vitamin D levels on screening <25nmol/L, are likely to need maintenance high-dose supplementation (Fung et al, 2011), and this may be achieved by 50,000 IU D2 or 2000IU of D3 weekly for 8 weeks (Soliman et al, 2013). Hypercalciuria is a concern at these high doses and monitoring is recommended (Auguste et al, 2019).

Vitamin D and Pyruvate Kinase Deficiency

There is no specific research investigating the relationship of vitamin D to morbidity in people with PKD. However, given that 24% of the general population in the United States, 37% in Canada and 40% in Europe are vitamin D deficient with levels of <20ng/ml (Amrein et al, 2020), it makes sense to check vitamin D status in people with PKD and treat if deficient.

Vitamin D: Summary and recommendations

- Bi-annual assessment of vitamin D status by measurement of 25-OHD in both children and adults with thalassaemia
- Levels of serum 25-OH vitamin D below 20 ng/ml (50 nmol/L) should be treated with vitamin D, 50 000 IU of vitamin D2 weekly for 8 weeks or 2000 IU of vitamin D3 daily for 8 weeks.
- Maintenance recommendations are oral daily vitamin D2 of 800–1000 units or 50,000 IU per month or a mega dose of vitamin D2 (10,000 U/kg, maximum 600,000 IU) every 6 months (either orally or intramuscularly) especially those who do not receive adequate sun exposure (Soliman et al, 2013).

Vitamin E

Many of the physiological functions of vitamin E, including its antioxidant effects, have been studied for over a century. Change in redox balance is involved in various diseases, and vitamin E is known to regulate this; however there is ongoing debate regarding the precise biological functions of vitamin E and its relationship with redox balance, as well as its non-antioxidant functions of (Miyazawa et al, 2019).

Only one of four tocopherols - RRR-α-tocopherol - satisfies the criteria of being a vitamin (Azzi, 2017). It has been shown to prevent the human deficiency disease 'ataxia with isolated vitamin E Deficiency', and is called vitamin E. It has documented immune boosting properties and activity against non-alcoholic fatty liver and low-grade inflammation. Epidemiological studies suggesting that vitamin E could prevent cardiovascular events, neurodegenerative disease, macular degeneration, and cancer did not, in general, carry through to clinical intervention studies (Khadangi & Azzi 2019; Brigelius-Flohé 2021). A major research focus on α tocopherol and its anticancer effects did not yield meaningful results (Alpha-tocopherol study group, 1994; Lippman et al, 2009) and there was a shift of focus to gamma-tocopherol, delta-tocopherol and tocotrienol. Preclinical research on non-alpha tocopherol isoforms of vitamin E have shown promising data on their anticancer effects (Abraham et al, 2019).

Dietary sources and recommended intake

Nuts and vegetable oils are rich in tocopherols, whereas barley, oat, palm oil, rice bran, rye, and wheat germ are rich in tocotrienol. In addition, natural sources of both vitamin E isomers are also found in other foods such as fruits, seafood, cheese, and eggs (Sookwong et al, 2010).

Recommendations for adequate intake in the general population is typically focused on α tocopherol (mg/day), with US RDA for both male and female adults of 15 mg (IOM, 2000). The WHO recommendations are slightly lower, 10 mg for males and 7.5 mg of α tocopherol for females (WHO, 2004)

Table 7.3: *Selected Foods Rich in Vitamin E*

Food Source, Serving Quantity	Vitamin E, mg
Wheatgerm oil, 1 Tbsp	20.3
Almonds, 1 oz	6.8
Sunflower Oil, 1 Tbsp	5.6
Hazelnuts, 1 oz	4.3
Peanut butter, 2 Tbsps	2.9

Extracted from: Vitamin E: Fact Sheet for Health Professionals: NIH Office of Dietary Supplements, 2022.

Assessment
Vitamin E status is monitored by serum α-tocopherol and gamma-tocopherol. Patients should abstain from alcohol for 24 hours prior to collection. There are differing normal ranges according to age.

Vitamin E and thalassaemia
Over the last two decades, more than 100 publications have focused on vitamin E status in thalassaemia. Many people with thalassaemia have lower levels of vitamin E. The excess iron in the body leads to higher risk of oxidative stress, a major mechanism contributing to complications. An increase in total oxidant capacity (TOC), superoxide dismutase, and a decrease in catalase activity, reflects the presence of a severe oxidative stress in transfusion dependent Eβ- and β-thalassaemia patients with changes in erythrocyte membrane proteins. All these changes were ameliorated to considerable extent when the patients were treated with vitamin E at a dose of 10 mg/kg/day for four weeks. (Das et al, 2004)

Vitamin E for 3 months did not reduce anaemia in nine NTDT patients, median age of 39 years, but it could be useful for reducing oxidative damage in other target organs (Pfeifer et al, 2008). In another study, 120 adult thalassaemia patients received either zinc (50mg/day) or vitamin E (400mg/day) supplements or both. Serum zinc levels and serum vitamin E levels increased significantly, mean glutathione peroxidase activity decreased significantly, but mean superoxide dismutase activity and total antioxidant capacity did not show significant change after supplementation (Rashidi et al, 2011).

The effects of vitamin E 10 u/kg/day orally versus N-acetyl cysteine 10 mg/kg/day orally on total oxidant status and total antioxidant capacity, were studied in 78 adult patients with TDT. At the end of the study, total oxidant status significantly decreased only in the vitamin E group, total antioxidant capacity significantly decreased in both supplemented groups while haemoglobin did not

significantly change at the end of the study (Haghpanah et al, 2021). Similar benefits of Vitamin E supplementation in promoting antioxidant status and also in improving liver function were found in an earlier small study (Hashemian et al, 2012).

Vitamin E and Iron Chelators

A randomised prospective trial with 180 Egyptian vitamin E deficient TDT patients aged 6-18 years, serum ferritin >1000-2500 µg/L, cardiac T2* > 10 ms and ejection fraction > 56 %, were divided equally into 3 groups, (n=60) to receive desferrioxamine (DFO), deferiprone (DFP) or deferasirox (DFX). They were further randomised to receive vitamin E supplementation (400 IU daily) or not. All patients were followed-up for one year. Baseline vitamin E level was negatively correlated to serum ferritin, liver iron content, and malondialdehyde (MDA), while it was positively correlated to glutathione, glutathione peroxidase, and catalase. After vitamin E therapy: transfusion index, serum ferritin, and liver iron concentration were significantly decreased, while haemoglobin and cardiac MRI T2* were elevated (improved) compared with baseline levels. MDA levels were decreased while all the studied antioxidants were increased post-vitamin E supplementation compared with baseline levels. DFX-treated patients had the highest haemoglobin and antioxidant levels with the lowest serum ferritin and liver iron concentration compared to DFO or DFP subgroups. Vitamin E as an adjuvant therapy possibly potentiates the efficacy of DFX more than DFO and DFP and it reduces oxidative stress in moderately iron overloaded vitamin E-deficient TDT patients (El Laboudy et al, unpublished data).

Vitamin E and PKD

In rat studies, pyruvate kinase levels increase in vitamin E-deficient diets; however, no similar studies have been conducted in patients with PKD and patients should be sure to include vitamin E-containing foods in their diet (Bessho et al, 1988; Chow, 1975).

Vitamin E: Summary and recommendations

- Reduced circulating α-tocopherol levels are frequently observed in patients with thalassaemia.
- Vitamin E may be useful in reducing oxidative stress, particularly for iron-loaded patients.
- Annual monitoring of serum α- tocopherol is a useful suggestion in paediatric and adult patients, but more evidence is needed to introduce this as standard practice.
- Vitamin E, as α-tocopherol, should be provided to those diagnosed with deficiency.

Vitamin K

The scientific interest in Vitamin K has grown exponentially in the past decade with nearly 8500 published articles focused on vitamin K related topics. Vitamin K is the general term used for a group of fat-soluble compounds with similar structures. Vitamin K exists naturally in two basic forms, vitamin K1 (phylloquinone) and vitamin K2 (menaquinone). Vitamin K1 is the primary form of the vitamin found in western diets, while K2 is produced by bacteria and has a longer half-life in the body.

Vitamin K is required for the carboxylation of glutamic acid within the coagulation cascade proteins. Calcium binds these proteins when they are carboxylated, which activates the clotting process. Vitamin K is also responsible for carboxylating glutamic acid residues in osteocalcin, a protein integral to bone formation. Matrix Gla-protein, also vitamin K dependent, has been shown to reduce vascular calcification (Hariri et al, 2021). Vitamin K2 is also now recognised as a 'calcium chaperone', enabling calcium to safely be deposited into bone while preventing its deposition into arteries (Karpinski et al, 2017). Interestingly, many calcium and vitamin D supplements also now contain vitamin K. The most common sign of severe vitamin K deficiency is bleeding, while vitamin K insufficiency is related to vascular calcification, poor cardiovascular outcomes and altered bone metabolism.

Dietary sources

The richest sources of vitamin K1 in the diet are from leafy vegetables, soybeans and soybean oils, nuts, seeds and a few fruits. Vitamin K2, the form synthesised by bacteria, is found both in the gut as well as from food processing - foods fermented by bacteria, pickled foods and some animal sources including eggs and cheese can be rich sources of vitamin K2. Given the unique properties of the 2 basic forms of vitamin K, some recommend separate dietary recommendations for K1 and K2 (Akbulut et al, 2020). However, it is difficult to obtain accurate food composition tables for K2 to inform decision making.

There are some important drug/nutrient interactions. Dietary intake of vitamin K can reduce the efficacy of vitamin K antagonist anticoagulant medications, in particular warfarin and acenocoumarol, so individuals taking warfarin should ensure a consistent intake of vitamin K. The best way to achieve this and reduce the day-to-day variability in vitamin K is through supplementation. Supplementation with 150 ug vitamin K1 has been shown to reduce the variability and improve anticoagulation control (Sconce et al, 2007). Newer non-vitamin K antagonist oral anticoagulant medications that directly inhibit thrombin or Factor Xa are not affected by vitamin K intake. Antibiotic medications also interact with vitamin K, as they can eliminate healthy vitamin K2-producing gut bacteria and decrease vitamin K absorption. For individuals prescribed antibiotics, especifically cephalosporins, for more than a few weeks and who have limited dietary intake of vitamin K, supplementation is recommended.

There is no RDA for vitamin K in the US, only recommendations for what is considered, 'Adequate Intake' or an AI. As for many essential vitamins, this varies by age - from 30 ug for toddlers to 90 ug for female adults and 120 ug for male adults (IOM, 2001). The average recorded dietary intake of vitamin K1 also varies significantly in US adults, ranging from 80 to 225 ug/day (NHANES 2011-2012) and reported intakes of vitamin K vary widely across the globe, largely dependent on vegetable and fermented food intake. Of interest, no upper limit of vitamin K has been determined (Institute of Medicine, 2001). No adverse effects of excessive consumption of vitamin K have been observed in humans.

Table 7.4: Selected Foods Rich in Vitamin K1 (phylloquinone)

(1 cup ~ 240 mls, 1 oz ~ 28 g)

Food Source, Serving Quantity	Vitamin K1, ug
Collard Greens, boiled ½ cup	530
Spinach, raw 1 cup	145
Broccoli, boiled, ½ cup	110
Soybean oil, 1 Tbsp	25
Pine nuts, 1 ounce	15
Cashews, 1 ounce	10

Source: Elder SJ 2006.

Food Source, Serving Quantity	Vitamin K2, ug
*Natto, 1 Tbsp (MK-7)	150
Chicken breast, 3 ounces (MK-4)	13
Cheese, cheddar, 50 grams (MK-4)	12
Egg, hard boiled, 1 (MK-4)	4

Source: USDA National Nutrient Database for Standard Reference Release, 2016.

*Natto, fermented soybeans, common in Japanese cuisine.

Assessment

Vitamin K1 can be assessed through either monitoring of prothrombin time (PT) or serum phylloquinone (K1) level. The measurement of vitamin K dependent proteins (e.g. carboxylation of osteocalcin) and the analysis of proteins-induced-in-vitamin K absence (PIVKA) have also been used. The gold standard assay with the most sensitivity to vitamin K status is suggested to be PIVKA (Card et al, 2019). Although there are limitations, namely that K2 function is ignored, in clinical situations the most commonly measured variable to assess status is serum vitamin K1.

Vitamin K in thalassaemia

Although vitamin K plays a critical role in bone health and cardiac function, little attention has been paid to its role in the health of patients with thalassaemia. In 2012, an observational study by Fung et al reported that roughly 40% of 221 patients with the condition consumed less than the recommended intake for age of vitamin K. At the time of that publication, vitamin K was rarely included in multivitamin supplements. In a more recent systematic review, vitamin K was not identified as a nutrient of concern for deficiency in patients with thalassaemia (Goldberg et al, 2022) although that review focused on published sources only. Given the challenges to assessment and the lack of focus on vitamin K in thalassaemia, the 'jury is out' on the true level of deficiency that may exist in this population and its possible importance.

An early study of 30 children with TDT, based on samples at least 20 days after the last transfusion, found that 30 to 57% of vitamin K dependent clotting factors (Factor IX and XI) were reduced in the cohort compared to a healthy control group TDT (Caocci et al, 1978). The findings were similar in a repeat study (Schettini et al, 1987), in which children with TDT, half of whom were splenectomised, were compared to an age and sex matched control group; Factors II, VII, IX and X were all reduced, particularly in the splenectomised patients.

The only interventional study focused on vitamin K in thalassaemia was a single arm trial of supplementation in 20 children (3-18 years) with TDT (Ozdemir et al, 2013). 20 children received 50 ug of vitamin K2 plus 200 IU of vitamin D for 12 months. There was no control group, but a significant increase in BMD Z-score in the lumbar spine by DXA was seen, from a mean of -1.2 at baseline to -0.8 at 12 months (p=0.003). More research in this area is needed to clarify the specific roles of K1 and K2.

Vitamin K in PKD

There is currently no research on the impact of vitamin K on the health of people with PKD.

Vitamin K: Summary and recommendations

- Despite the importance of vitamin K in bone health and vascular calcification, little attention has been paid to its role in the health of patients with thalassaemia.
- For patients prescribed vitamin K agonist anticoagulants, a stable intake of vitamin K is critical.
- For patients prescribed antibiotics for more than a few weeks, vitamin K supplementation should be considered.
- A few reported studies suggest possible vitamin K deficiency in children with TDT.
- Vitamin K supplemented with Vitamin D may be beneficial to bone health in TDT and PKD, though further research with larger patient numbers is needed to determine optimal doses and frequency of administration before any firm recommendation can be made.

Folate

Folate, an essential water-soluble vitamin, has a key role in cell division and effective erythropoiesis. Its primary function is as a cofactor in 'one-carbon transfers' for DNA, RNA and amino acid metabolism. The conversion of homocysteine to methionine as well as deoxyuridylate to thymidylate are both folate dependent. A block in the latter reaction is what ultimately leads to megaloblastic anaemia, one of the key signs of folate deficiency, as well as vitamin B12 deficiency.

In the general population, adequate folate has been shown to decrease the risk of some cancers (Vollset et al, 2013) stroke (Marti-Carvajal et al, 2017) and depression (Roberts et al, 2018). Most research, however, has focused on the relationship between folate deficiency and neural tube defects (Pitkin, 2007), for which the evidence was clear enough to justify folate food fortification two decades ago. Partly owing to the success of the programme, in the years following fortification programs in the US, neural tube defects have decreased by 28% (Williams et al, 2015).

Dietary sources

The general term 'folate' is used to refer to all folate compounds including naturally occurring food folate (tetrahydrofolate, THF), the folate found in fortified foods (folic acid) and supplements (5 Methylenetetrahydrofolate, 5-MTHF). Given folate can exist in a variety of forms, when food products list folate concentration, they provide dietary folate equivalents or DFE. DFE is determined by the form of folate, and its relative bioavailability. 1 µg DFE is equal to 1 µg of food folate, 0.6 µg of folic acid taken with food, or 0.5 µg of folic acid consumed on an empty stomach.

Folate is found in a variety of foods, the richest sources being dark green leafy vegetables, brussels sprouts, beans and peas. It can also be found in fortified breads and cereals. The US began fortifying foods with folate in 1998, with breads, cereals, flour, pasta and other grains enriched to 140 µg folic acid per 100 g of food product (USDA 1996). Other countries have followed although the practice is not universal.

Table 7.5: *Selected Foods Rich in Folate*

Food Source, Serving Quantity	DFE, µg
Spinach, boiled ½ cup (~ 120 mls)	131
*Breakfast cereals	100
Brussels sprouts, boiled ½ cup	78
Green peas, boiled ½ cup	50
Bread, white 1 slice	50
Kidney beans, ½ cup	46
Orange juice, ¾ cup	35
Banana, 1 medium	24

Source: USDA, Food Data Central, 2019
*These foods also relatively high in iron
DFE: dietary folate equivalents

The US recommended dietary allowance (RDA) for folate intake is 200 to 300 µg DFE for youths and adolescents, 400 µg for adults. For pregnant women, the recommendation is 600 µg DFE - the minimum amount found in prenatal supplements.

Drug nutrient interactions

Methotrexate, used in treatment for cancer, rheumatoid arthritis and other auto-immune disorders, is a folate antagonist. Supplementation, or high dose folate intake, can interfere with the efficacy of methotrexate and should be avoided if possible. Some antiepileptic drugs - valproate, phenytoin, carbamazepine - can decrease serum folate. Folate supplementation while taking these seizure medications may also decrease their efficacy. Additionally, sulfasalazine, a drug used frequently for patients with ulcerative colitis, can affect folate status.

Assessment

Serum folate is typically used to assess folate status. Levels below 3 ng/mL are reflective of deficiency, though always check with local lab for their normal range. Serum folate reflects recent dietary intake and may not be sensitive to long term folate status. Erythrocyte folate has been used as the gold standard to reflect long term folate status. Unfortunately, in patients who receive regular red blood cell transfusion therapy, such as those with thalassaemia and PKD, erythrocyte folate may be a poor indicator of status. An elevation in plasma homocysteine concentration has also been used as a non-specific functional indicator of folate status: plasma homocysteine increases when folate is deficient, as the conversion to methionine will be limited. However, other factors may affect plasma homocysteine levels including vitamin B6 or B12 deficiencies, low thyroid hormone, and concurrent kidney disease.

Folate and thalassaemia

Given the importance of folate for red cell metabolism, it has been presumed that patients with thalassaemia have an increased folate requirement, and a few historical studies documented impaired folate status (Castaldi et al, 1983; Saraya et al, 1984). Kumar et al (1985) reported that 40% of TDT adult patients had low serum folate. Tso reported in 1976 that ~ 30% of a non-transfusion dependent cohort of adult patients had reduced serum folate and red cell folate levels. In those who were deficient, folate supplementation improved red cell folate levels to within the normal range. Folate supplementation, usually 1 mg/day, has now become a common practice for patients with thalassaemia, although not universal especially in transfused patients. As a result, more recent studies have typically found serum folate to be similar to healthy controls (Sherief et al, 2014). Claster et al (2009) found serum folate to be negatively correlated with age in a group of heavily iron loaded transfusion dependent patients.

Inadequate dietary folate intake may contribute, in part, to poor folate status. Fung et al (2012) reported 50% of patients in a North American cohort of patients consumed less than the amount recommended for age. Though servings of vegetables and fruit were consistent with recommendations, intake of dairy and whole grains were limited, which may have contributed to the overall low folate intake.

There have been a few published studies of folate supplementation. In 2006, investigators provided 1 mg folate per day or placebo to 56 adolescent patients and followed them for 1 month. They observed a significant increase in serum folic acid, though no changes in erythrocyte parameters were observed (Mojtahedzadeh et al, 2006).

More recently Baghersalimi et al (2018) reported that in patients taking clinically prescribed folate supplements (1 mg/day) for a minimum of 6 months, cessation of supplementation led to significantly increased serum homocysteine, and decreased serum folate.

With folate fortification in place in many countries, investigators have attempted to determine what level of folate supplementation is necessary to maintain folate status (Paniz et al, 2020). Agarwal et al (2002) compared the effectiveness of 3 different doses of folate supplementation, 5 mg/day vs. 2.5 mg/day vs. 5 mg/week in 90 children (5-18 years) with TDT. After 9 months, median serum folate levels were comparable in all groups, suggesting that the lower dose of 5 mg of folate per week was sufficient to maintain stores.

Folate and PK Deficiency

Despite a lack of research of folate/folic acid in PK deficiency, folic acid supplementation is recommended as part of supportive treatment for people with PK deficiency, especially for those not receiving regular transfusion, because of expected higher requirements. Emphasis on choosing and eating foods high in folate is also recommended.

Folate: Summary and recommendations

- Patients with these conditions have an increased folate requirement.
- Supplementation with 1 mg/day or 5 mg/week of folic acid appears to be sufficient to maintain optimal folate status for most patients, along with increased consumption of foods containing folate.
- Annual assessment of serum folate is recommended.
- Folate supplementation should be avoided in patients prescribed methotrexate.

Part 2 - Minerals

Macro-minerals are necessary in larger amounts and include calcium, magnesium and phosphorus. Micro-minerals, also called trace minerals, are needed in much smaller amounts but are still necessary for good health: they include zinc, iron, manganese, copper, iodine, cobalt, fluoride, and selenium.

Of all the essential minerals, iron has of course received the most attention from clinicians and research scientists focused on the care of patients with thalassaemia and PKD. The focus of this chapter is on the other essential macro-minerals and trace elements important to the health of patients with these conditions.

By definition, an essential trace element cannot be synthesised, therefore must be obtained through the diet, typically in amounts between 1 – 100 mg/day (IOM, 2011). Patients with thalassaemia commonly have deficiencies in many of the essential trace minerals routinely assessed. The three trace minerals that have been studied most extensively in this population include copper, selenium and zinc. Calcium and magnesium are also important to the overall health of patients, but are considered macro-minerals, required regularly in the diet in quantities >100 mg/day. In this chapter we highlight the function of each of these minerals, where they can be found in the diet, how they are assessed, and summarise what is known about their importance in patients with thalassaemia and PKD.

Calcium

Calcium is the most abundant mineral in the human body and is crucial to the structure of bones and teeth where almost all the calcium in the body is stored. Skeletal calcium also acts as the reservoir to maintain calcium homeostasis and serum calcium in a tight range. As patients with thalassaemia are at risk for low bone mass, calcium is of particular importance. Outside of

its crucial function in skeletal health, the small pool of remaining calcium is important for blood vessel contraction, muscle function, blood clotting, nerve transmission and hormone secretion. Calcium may also be protective against hypertension (Cormick et al, 2021)

In recent years, there have been some reports of possible negative effects of calcium supplementation. Many older adults at risk for osteoporosis supplement with calcium, for its potential benefits to bone health. Bolland et al, however, reported in 2010 that calcium supplementation alone may not be benign. They reported a meta-analysis which summarised 11 students from adults > 40 years who supplemented with calcium, >500 mg/day, and had a greater incidence of myocardial infarction. It was hypothesised that calcium supplements may accelerate vascular calcification and increase mortality in certain patients; however this hypothesis has not been proven. Further, low vitamin D, which is frequently observed in tandem with low calcium intake, has also been related to increased cardiovascular risk. Subsequent studies were not able to reproduce the findings of Bolland and his colleagues (Lewis et al, 2011; Anderson et al, 2016) and all the studies that explored the relationship have used secondary analyses. Most recent reviews suggest that there may be a link, but limited to situations where calcium is supplemented alone, without vitamin D (Michos et al, 2021). Dietary calcium is not implicated in these associative studies with cardiovascular risk.

Dietary sources

Calcium is naturally found in abundance in dairy products, milk, yogurt, cheese (Table 7.6). It is also absorbed most readily from dairy products in the presence of the milk protein lactose (~ 30% absorption; IOM, 2011). Calcium is most bioavailable, and absorbed best, in an acidic environment. Therefore for older adults with low stomach acid, or those taking an antacid, calcium supplement is best consumed with a meal. Other sources of calcium include dark green leafy vegetables, tofu, beans and corn tortillas. Though spinach is a reasonable source of calcium, with 125 mg/1 cooked cup (~240 mls), due to the oxalate content only 5% of the calcium is absorbed (Weaver et al, 2014). Kale and broccoli are better alternatives with lower oxalate content.

As the need for calcium changes with skeletal growth, the dietary recommendations for calcium vary widely by age group; in the US it is recommended that adolescents consume 1300 mg, but adults only 1000 mg (IOM, 2011). Though the scientific information on which the recommendations are based is similar, they vary. In the UK, the recommendation for adults is lower, 700 mg/day, and the WHO suggests only 500 mg/day is sufficient. Average dietary intake also varies by country of origin, the mean calcium intake in Nepal is only 175 mg/day while those in Iceland consume on average over 1200 mg/day (Balk et al, 2017).

Table 7.6: Selected Foods Rich in Calcium

Food Source, Serving Quantity	Calcium, mg
Yogurt, plain 1 cup {~240 mls)	415
Cow's milk, whole fat, 1 cup	276
Tofu, ½ cup	253
Soybeans, cooked ½ cup	131
Kale, cooked 1 cup	94
Pinto beans, canned ½ cup	54

Source: US Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.

Assessment

There is no gold standard for the assessment of calcium status in an individual. Though calcium can be monitored by serum levels, they are typically maintained within a tight range (8.5 – 10.5 mg/dL; 2.1-2.6 mmol/L). Only with severe disturbance to calcium homeostasis will serum calcium change. Calcium is primarily bound to albumin in the circulation, therefore in protein deficient states when albumin drops, serum calcium may also be decreased. When necessary, serum calcium can be adjusted for albumin concentration (Corrected Ca= (4-albumin in g/dL x 0.8) + serum Ca in mg/dL). Ionised (free) calcium can also be assessed (normal range 4.6 – 5.3 mg/dL) and will not be affected by albumin concentration. Calcium balance can be assessed by bone mineral content using bone densitometry (DXA scan). This assessment is not specific to calcium however as bone mineral accrual can be affected by hormones, vitamin D homeostasis, physical activity and other nutrients.

Calcium and thalassaemia

The majority of patients with thalassaemia are at risk for low bone mass, regardless of phenotype or transfusion dependence (Vogiatzi et al, 2011), and the prevalence of low bone mass increases with age as patients have reduced bone formation and accrue bone mineral at a reduced rate compared to healthy individuals (Fung et al, 2022). The aetiology of low bone mass is multifactorial (Gaudio et al, 2019), as discussed in chapter 4. Part of the risk appears to be sub-optimal nutrition.

Over half of adolescent and adults with thalassaemia in North America have been shown to have inadequate dietary intake of calcium when compared to recommended levels (Fung et al, 2012, Goldberg et al, 2018) with calcium intake averaging less than 800 mg/day. Dairy intake is also limited, averaging 1.3 servings per day in both adolescents and adults.

Although hypocalcaemia occurs infrequently in the general population, it has frequently been observed in those with thalassaemia. Hypocalcaemia, corrected for albumin concentration, has

been observed in both adult and pediatric patients (Bulgurcu et al, 2021; Handattu et al, 2022) and related to low bone mass (Meena 2015). Hypercalciuria is also a common finding in thalassaemia and is also related to low bone mass (Lasco et al, 2001). These findings, together with the fact that most patients with also have insufficient 25-OH vitamin D levels, highlight the gravity of altered calcium homeostasis. Only one group has conducted an interventional study of calcium and vitamin D with bone as the primary outcome. Thigarajan et al (2019) gave 29 children (2-12 years) with thalassaemia 500 mg calcium plus 1000 IU vitamin D for a year and found an increase in bone mineral content; however there was no control group for comparison. More research is needed to confirm these findings. In the meantime, it is recommended that both serum calcium and urinary calcium be assessed on a regular basis in patients with thalassaemia.

Calcium and PKD

Mineral-specific research and its impact on bone health, chelator effects, hypogonadism, immunity, growth, and diabetes as it relates to PKD is lacking. People with PKD are at risk of early onset of osteopenia, osteoporosis and fractures; median age of 34 years (Al-Samkari et al, 2023) so efforts to prevent, delay and guide appropriate therapy are needed. Like other haemolytic anaemias, there are multiple reasons for the early onset of thinning bone including erythroid hyperplasia widening the marrow spaces, iron overload and iron chelation potentially resulting in dysregulated osteoclast and osteoblast function and increased bone resorption, endocrine disruptions, and genetic factors. To what extent dietary adjustments can impact bone health is unknown but for now, advice has to rely on practices used in thalassaemia, which is to optimise calcium from dietary sources, monitor calcium levels, and undertake bone density DXA scans.

While the preferred source for calcium is food, people with PKD who do not consume many foods containing calcium – for example vegans, and people with lactose intolerance; and those who have bowel diseases which interfere with calcium absorption, post-menopausal women, or those who consume large amounts of protein- and sodium-containing foods should consult with their physician or dietitian about supplemental calcium with vitamin D for increased absorption (NIHODS 2022a).

Copper

Copper, an essential trace element, is crucial for several enzyme functions including lysyl oxidase, elastase, cytochrome oxidase, tyrosinase and copper-zinc superoxide dismutase. Cytochrome oxidase is crucial for mitochondrial respiration, while lysyl oxidase and elastase are involved with collagen cross-linking, and tyrosinase is responsible for melanin production. Caeruloplasmin, a ferrioxidase and the primary transport protein of copper in circulation, is responsible for oxidising ferrous to ferric iron, allowing iron to bind with transferrin. This is of course relevant to patients at risk for iron overload. Copper is also needed for intestinal iron absorption and free radical scavenging. The transport protein responsible for exporting iron through the intestinal epithelium is copper dependent, so when copper status is low, less iron is absorbed. Conversely, copper and zinc compete for some intestinal transporters, therefore zinc supplementation may reduce copper absorption and vice versa (Nishito and Kambe, 2018). Copper also plays a role in free radical scavenging through the enzyme Cu-Zn superoxide dismutase. Signs of copper deficiency are related to these enzymatic functions and include anaemia, low bone mass, and decreased pigmentation

(Wazir and Ghobrial, 2017). Though rare, high levels of copper can also be detrimental, associated with liver disease and neurological disorders.

Dietary sources

Copper is found within a variety of foods in an omnivorous diet (Table 7.7). They include avocados, chickpeas, cashews, mushrooms, oysters, potatoes, whole grains, tofu, and chocolate. Copper is readily bioavailable, with an absorption ranging from 30-40% from a varied diet (Wapnir, 1998). Factors that enhance copper absorption include amino acids and some proteins, and factors that inhibit absorption include zinc, iron and fibre (Wapnir, 1998). Similar to other minerals, absorption varies inversely with the amount of copper in the diet; as dietary copper increases, % absorption decreases. The US Recommended Dietary Allowance for adults is 0.9 mg. Dietary copper intake is similar in thalassaemia when compared to healthy controls, and rarely deficient (Fung et al, 2012).

Table 7.7: *Selected Foods Rich in Copper*

Food Source, Serving Quantity	Copper, mg
*Oysters, 3.5 oz	4.85
Potatoes, 1 medium cooked	0.68
Shiitake Mushrooms, 0.5 cup	0.65
Cashews, 1 oz	0.63
Dark Chocolate, 1 oz	0.50
Chickpeas, ½ cup	0.29
Avocados, ½ cup	0.22
Sesame Seeds, ¼ cup	0.15

*Note, these foods are also relatively high in iron

Source: US Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.

Assessment

Copper status is assessed from serum copper which is a reliable marker of severe copper deficiency. Ideally, samples should be drawn in the morning as there is a diurnal variation with serum copper levels highest in first morning blood draws. Care must be taken to collect the sample in trace element free vacutainers. Serum copper can be artificially elevated by oestrogen replacement therapy (Fischer et al, 1990), smoking (Kocyigit et al, 2001), and inflammation (Brown et al, 1993). When caeruloplasmin, the primary transporter of copper in the circulation and an acute phase protein, is elevated, inflammation should be considered. Therefore, copper and ceruloplasmin assessed together may assist in the identification of true copper deficiency.

Copper and thalassaemia

Copper deficiency in the general population is rare, except in cases of acute zinc toxicity or rare genetic forms of copper imbalance, such as Menke's Syndrome. Studies which have evaluated copper status in patients with thalassaemia are varied and inconclusive. Roughly half of all reports have observed low serum copper levels in people with thalassaemia compared to healthy controls (Ferdaus et al, 2010; Fung et al, 2012; Sherief et al, 2014). From studies which report prevalence statistics, approximately one third of subjects measured were deficient (Goldberg et al, 2022). A couple of studies conducted in Iran and the US found no copper deficiency despite low zinc levels (Claster et al, 2009; Mahyar 2010). The remaining studies have reported elevated serum copper and/or elevated copper:zinc ratios in comparison to healthy controls (Bashir, 1995; Fuchs et al, 1996; Widad et al, 2003; Al-Samarrai et al, 2008; Mashhadi, 2013; Zekavat et al, 2018). Confounding these observations is the fact that few studies have controlled for the influence of inflammation, hormone replacement or ceruloplasmin level.

Shamshirsaz et al (2003) reported a high prevalence of low bone mass in 220 adolescents with TDT. Though altered bone metabolism has been observed in severe copper deficiency in other populations, no association between copper deficiency in thalassaemia and bone morbidity was found. A recent study explored the effect of curcumin (tumeric) supplementation (1000 mg) on copper and zinc levels in 15 adults with NTDT (Saeidnia et al, 2021). In addition to its proposed antioxidant and anti-inflammatory effects, curcumin reportedly has a high affinity to chelate both iron and copper (Zhang, 2016). Saeidnia and colleagues observed serum copper significantly decreased and zinc increased compared to placebo following 3 months on curcumin.

To date there have been no copper supplementation studies. It is recommended that serum copper be monitored on an annual basis in transfusion dependent patients with thalassaemia. If low, 2 mg/day is typically sufficient to replace low stores. If elevated, consideration of zinc status and alternative explanations for the increase should be considered, for example inflammatory processes, sex steroids.

Copper and PKD

There is no research or outstanding concerns related to copper deficiency in people with PKD except that copper deficiency may occur with zinc supplementation, which should be monitored.

Zinc

Zinc is an essential trace element, indispensable for the structural integrity, function and regulation of hundreds of enzymes and metalloproteins in the human body. It is critical for optimal immune function, growth and pubertal development, insulin metabolism, and bone mineral accrual. Zinc deficiency manifests in children as growth failure and increased susceptibility to infection. In adults, it is associated with hypogonadism, diabetes, and poor bone mineralisation, all of which are coincidentally common complications of thalassaemia.

Dietary sources

Meat, fish and seafood provide the richest sources of zinc. Oysters, especially those farmed from the Atlantic Ocean, are extremely high in zinc. Given that iron is also found in many of these foods, individuals who purposefully limit dietary iron may inadvertently also be limiting zinc intake. Other

good sources of dietary zinc include beans, eggs, legumes, dairy products and whole grains (Table 7.8). Zinc is readily bioavailable from a mixed omnivorous diet. It has been estimated that between 26 to 34% of zinc is absorbed, while absorption declines to between 18 – 26% when consumed within unrefined grain-based diets, probably due to phytate content. Phytate, a natural plant-based substance, is known to bind zinc and decrease intestinal absorption, dependent on the concentration of each in the meal. A phytate to zinc molar ratio above 5 will reduce zinc absorption. As an example, lentils have a phytate:zinc molar ratio of 28 which has a much greater effect on zinc absorption than, for example, refined wheat flour, with a phytate:zinc molar ratio of 6 (Lazarte et al, 2015; Ma G et al, 2005).

Breakfast cereals in the US are often fortified with zinc, and therefore may be a good source of zinc for young children. However, these same foods are typically fortified with iron. The US Recommended Dietary Allowance for zinc for healthy adults in the US is 11 mg for males and 8 mg for females.

Table 7.8: *Selected Foods Rich in Zinc*

Food Source, Serving Quantity	Zinc, mg
*Oysters, Atlantic Ocean, 3oz	32
*Beef, roasted 3 oz	3.8
*Turkey breast, 3 oz	1.5
*Lentils, cooked ½ cup	1.3
Cheese, Cheddar 1.5 oz	1.5
Yogurt, 1 cup	1.0
Whole wheat bread, 1 slice	0.6

*These foods are also relatively high in iron

3 oz = 85 g

Source: US Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.

Assessment

Zinc status can be assessed from plasma or serum zinc. Ideally, samples should be drawn fasting and in the morning as zinc is affected by food intake and diurnal variation: zinc levels are highest in the early morning. Care must be taken to collect the sample in trace element free vacutainers, avoiding haemolysis – noting that this may be difficult in people with red cell disorders. Serum zinc can be artificially increased by oestrogen, haemolysis and contamination during sampling. Zinc can be artificially depressed by infection and systemic inflammation. Calculations have been proposed to adjust plasma zinc values in pre-school children with co-morbid inflammation (McDonald et al, 2020). For fasting samples drawn in the morning, adequate serum zinc for adult females is > 70 ug/dL and for adult males > 74 ug/dL (http://www.izincg.org).

Zinc and thalassaemia

Deficient levels of plasma zinc have been documented in both TDT and NTDT patients (Arcasoy et al, 2001; Kajanchumpol et al, 1997; Kassab-Chekir et al, 2003; Shamshirsaz et al, 2003). However, plasma zinc may not be a sensitive marker of zinc status, particularly in patients with these conditions due to sample haemolysis (Fung et al, 2002). The best indicator of zinc deficiency in children is to assess linear growth response to zinc supplementation. Over 2 decades ago, a significant increase in height velocity was reported in 21 regularly transfused, non-chelated children with thalassaemia supplemented with 22-90 mg Zn/day, providing evidence of a functional zinc deficiency (Arcasoy et al, 1987). Similarly, in 1996, Fuchs et al found that young people with thalassaemia with wasting had significantly lower zinc than those without wasting, and others reported height for age and BMI positively correlated with serum zinc (Bekheirnia et al, 2004). More recently, it was observed that height, weight, mid upper arm circumference and waist circumference positively correlated with zinc status in 140 TDT youth (Mirhosseini et al, 2013). The association between zinc and growth is not universal, however, as three separate cross-sectional studies found no correlation between zinc and height in Iranian children with TDT (Eshghi et al, 2007; Mehdizadeh et al, 2008; Banihashem et al, 2013). However, a recent Cochrane review suggests zinc supplementation improves growth in children with thalassaemia (Swe et al, 2013).

The aetiology of zinc deficiency in thalassaemia is likely to be a combination of inadequate intake, elevated urinary zinc excretion, iron overload and reduced zinc binding capacity to serum carrier proteins in the face of increased requirements (Arcasoy et al, 2001; Goldberg et al, 2018; Mousa et al, 2021; Uysal et al, 1993,). It has been estimated that roughly 30% of US patients with thalassaemia have inadequate dietary zinc intake when compared to the Institute of Medicine's published recommendations for healthy individuals (Fung et al, 2012). If elevated requirements of zinc are considered, it is likely that prevalence of zinc deficiency is far greater.

Another mechanism for deficiency has also been suggested: sequestration of zinc in the liver secondary to upregulation of metallothionein from iron loading. Though relationships between iron loading and zinc deficiency have been observed (Goldberg et al, 2018), there has been limited objective data to support the sequestration hypothesis. In a 1990 study, autopsy samples from 12 Hb Eβ thalassaemia patients with unclear transfusion history were compared with 28 non-thalassaemic controls who suffered accidental deaths (Shuler et al 1990). Zinc levels were found to be elevated in brain and heart tissue, with trends towards elevations of zinc in the kidney, pancreas and liver of those with thalassaemia. Of interest, copper and selenium were also elevated in liver tissue.

Chelator use has been implicated in the aetiology of zinc deficiency in thalassaemia though conclusive clinical evidence to support this claim is limited (Maclean et al, 2001). Al-Refaie et al reported in 1994 that up to 14% prevalence of zinc deficiency in people treated with deferiprone (DFP), with 67% of those with low serum zinc developing symptoms of zinc deficiency (dry skin, itching, alopecia) which improved with supplementation. Erdogan et al (2013) found that individuals taking desferriosamine or DFP excreted more urinary zinc than controls or subjects taking deferasirox (DFX). Widad et al (2003) found serum zinc to be significantly lower in 52 Iraqi children with thalassaemia who were taking DFO, in comparison to 30 who were unchelated. By contrast, Bekheirnia et al (2004) observed that within a cohort of 131 Iranian patients with TDT, serum zinc was significantly lower in individuals who had started chelation later in life. Additionally, Uysal et al (1993) found no correlation between urinary zinc excretion and DFO dose. The relationship between

zinc status and chelation use is complex and depends not only on chelator type but also chelator dosing regimen, adherence and hepatic iron concentration. Regardless of these conflicting results, some centres provide 50 mg/d of zinc in supplemental form to patients prescribed DFP regardless of serum zinc level.

One of the most frequent complications of adults with thalassaemia is low bone mass. Zinc directly affects bone growth and formation through growth hormone and osteoblast proliferation (O'Connor et al, 2020). A positive association between zinc and bone health has been observed in patients with thalassaemia. With a cohort of 220 patients with TDT, serum zinc levels were found to significantly correlate with lumbar spine bone mineral density (BMD) (Shamshirsaz et al, 2003). Female patients with thalassaemia and low serum zinc had significantly lower lumbar spine BMD than those with normal zinc status (Bekheirnia et al, 2004). In 2013, Fung et al reported that 25 mg/day zinc taken for 18 months increased whole body BMD in adolescents and young adults with low bone mass after controlling for pubertal stage. Moreover, zinc stabilised spine and hip BMD Z-score.

Zinc is also proposed to be related to immune function. Low serum zinc levels in 18 transfusion dependent (TDT) subjects were found to correlate with low thymulin levels, and the addition of ZnCl2 to patient sera in vitro significantly increased active thymulin levels (Consolini et al, 2001). 10 of these 18 patients had significantly higher numbers of CD19+ cells, and lower numbers CD3+ cells in comparison to healthy controls, biomarkers of improved immune function. In contrast, two years earlier, in a cohort of slightly older Italian thalassaemia patients, no association between zinc deficiency and abnormal immune function was found (Malizia et al, 1998).

The literature is rich with illustrations of the direct role zinc has in the development of, and treatment for, diabetes in the general population (Jansen et al, 2009; Fukunaka and Fujitani, 2018; Ruz et al, 2019). Zinc appears to be involved in the reduction or delay in the onset of diabetes by decreasing TNFα and IL-1, cytokines known for their role in B-cell destruction. Additionally, zinc may be involved not only in the protection of islet cells, but it is also crucial to the many steps of glucose metabolism. This is of obvious interest for people with thalassaemia. Dehshal et al (2007) assessed serum zinc and oral glucose tolerance test (OGTT) in 70 individuals with a recent diagnosis of TDT. They found that fasting insulin was depressed in zinc deficient subjects, low zinc was common in those with abnormal oral glucose tolerance test, and there was a positive correlation between plasma zinc and insulin concentration. Fung et al (2015) mirrored many of these results, and further demonstrated that 2 hours post OGTT, subjects with low serum zinc had higher plasma glucose than subjects with normal circulating zinc. They also observed that subjects with zinc deficiency had a 30% lower insulinogenic response than those with adequate zinc. In a subsequent study, a positive association between β cell function and serum zinc was observed (Fung et al, 2020). Similarly, Mousa et al (2021) found that young people with TDT and zinc deficiency had a higher prevalence of both pancreatic exocrine and endocrine dysfunction. Most recently, both Fung et al and Matter et al found that zinc supplementation (25 – 40 mg/day for 3 months) improved markers of insulin sensitivity and glucose homeostasis (Matter et al, 2020; Fung et al, 2020).

Given the overall importance of zinc to growth, bone health and glucose homeostasis, and the reports of deficiency in many patients, it is recommended that zinc be monitored on at least an annual basis in all thalassaemia patients (see Table on monitoring, Appendix 12.2); for those on

iron chelation more frequent checking, 6 monthly, is often recommended. For patients found to be deficient, supplementation with zinc (25 mg/day) has led to significant increases in serum zinc. Higher doses of zinc should not be necessary to remedy deficiency. Short term regimens (3 to 6 months) are preferred with reassessment given the propensity of zinc to also interfere with copper absorption. Nausea has been reported in some who take zinc supplements, attributable to the sulphate salt in the usually prescribed zinc sulphate compound. Alternate forms of zinc which have not been associated with stomach upset include zinc picolinate, zinc citrate, zinc acetate, and zinc gluconate.

Zinc and PK deficiency

Zinc's contribution to cellular metabolism, immune function, protein and DNA synthesis, wound healing, cell signaling and division, and overall support of healthy growth and development makes it a key nutrient of need for people with PKD (NIHODS 2022, Zinc). A food eating pattern should be established where dietary iron restrictions do not restrict foods rich in zinc, and monitoring of zinc levels is recommended.

Magnesium

Magnesium serves as an important co-factor to enzymes involved in oxidative phosphorylation, neuron and myocyte function, blood glucose control, blood pressure regulation and, of particular importance to patients with thalassaemia, bone formation. In general, magnesium status is strongly associated with bone health and supplementation with magnesium has been shown to enhance bone density in those who are magnesium deficient (Rondanelli et al, 2021).

The primary site of magnesium homeostasis is the kidney. When dietary intake is limiting, status is maintained by decreasing urinary excretion. However, with chronically low dietary intake, alterations in absorption – for example coeliac or Crohn's disease, or excretion – as in diabetes, deficiency may result. Signs of magnesium deficiency include fatigue, weakness, muscle cramping and, in severe deficiency, seizures and abnormal cardiac rhythms.

Dietary sources

Magnesium is found in a wide variety of foods, but most national dietary surveys in the US suggest that healthy adults consume less than the amount recommended for age (Moshfegh et al, 2009). Rich sources of dietary magnesium include seeds, nuts, dark green leafy vegetables, legumes, dairy nuts, whole grains and even chocolate (Table 7.9). Magnesium is also found in some laxatives and over-the-counter heartburn medications. The Recommended Dietary Allowance for healthy adults in the US is between 400 and 420 mg for males and between 310 and 320 mg for females.

Table 7.9: Selected Foods Rich in Magnesium

Food Source, Serving Quantity	Magnesium, mg
Pumpkin seeds, 1 oz (~28 g)	156
Almonds, 1 oz	80
Spinach, ½ cup cooked (~120 mls)	78
Soymilk, 1 cup	61
*Black beans, ½ cup	60
Yogurt, 1 cup	42
Dark chocolate, 1 oz	41
Banana, 1 medium	32
Milk, whole fat 1 cup	25
Whole Wheat Bread, 1 slice	23

*Note, these foods are also relatively high in iron

Source: US Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.

Assessment

Accurate assessment of magnesium status is challenging as less than 1% of total body magnesium is in the circulation. The majority of magnesium is intracellular, or imbedded in bone. Despite the limitations, serum magnesium remains the main method for monitoring status. Low serum magnesium is typically defined as levels less than 0.75 mmol/L (1.8 mg/dL).

Magnesium and thalassaemia

Dietary magnesium has been reported to be inadequate in over half of patients tested (Fung et al, 2012), worsening with age. Poor intake may be in part explained by low dairy consumption as yogurt and milk are good sources of dietary magnesium.

A number of studies have reported magnesium status in patients with thalassaemia; most have observed serum or erythrocyte magnesium concentration to be lower in subjects with thalassaemia compared to healthy controls (Al-Sammarrai et al, 2008; Al-Hakeim, 2018; Aslan et al, 2012; De Franceschi et al, 1998; Naderi 2013) while two reported no difference compared to literature values (Ferdaus et al, 2010; Genc et al, 2016). Of interest, in 2020, Sahin et al reported that 41 Turkish patients with TDT had elevated serum magnesium in comparison to age and gender matched controls (Sahin 2020). Incidentally, iron, zinc, and manganese were also significantly elevated in this cohort.

To date there has been only one, now quite old, study in which magnesium was given to thalassaemia patients. Supplementation with magnesium (0.6 mEq/kg/day or 300 mg/day for a 50 kg subject) for one month improved erythrocyte descriptors and circulating magnesium, while reducing erythrocyte density (De Franceschi et al, 1998). Given the short study length, bone outcomes were not explored. During a second month of supplementation, at a higher

dose of 1.2 mEq/kg/day, a level roughly twice the current RDA for healthy adults, all subjects experienced mild, though tolerable, diarrhoea. Given the general importance of magnesium to bone health, and the reports of deficiency in some patients, it is recommended that magnesium be monitored on an annual basis along with the other essential minerals.

Magnesium and PKD

In a small study, the impact of iv infusion and oral administration of a magnesium-containing medication on erythrocyte pyruvate kinase activity was measured in people with PKD (Wzewska-Czyzewska et al, 1975). Even though magnesium ion concentration and enzyme activity increased, it was not sustainable in the long term. Magnesium infusion or oral administration is not part of the typical protocol for people with PKD though anecdotally some patients do report receiving orders for magnesium infusions from their haematologists.

Body aches and cramping (similar to nocturnal cramps) are frequently reported among people with PKD. In a Cochrane review of magnesium for skeletal muscle cramps it was found unlikely that magnesium supplementation provided clinically significant reduction in cramp frequency or intensity in adults > 60 years (Garrison et al, 2020). This does not preclude the use in people with PKD but offers caution. Diarrhoea was the most common side effect of regular magnesium supplementation.

Dietary intake of magnesium is lower than recommended both in the US and Europe largely due to the use of demineralised water, processed foods, and agricultural practices that deplete the soil of magnesium (Fiorentini et al, 2021). It is suggested that people with PKD living should be monitored for magnesium deficiency.

Selenium

Acting through various selenoproteins, selenium has a critical role in thyroid hormone metabolism, DNA synthesis, and protection against free-radical damage. Given the high levels of oxidant stress experienced by most patients with chronic anaemias, adequate selenium status is likely to be important.

Though selenium deficiency is uncommon, certain individuals are at risk including those living in geographic locations with poor selenium content in the soil, dialysis patients, and individuals with HIV. The most well-known form of selenium deficiency is Keshan disease, a cardiomyopathy particular to the Keshan region of North Eastern China where selenium concentration in the soil is extremely low. In the 1970s, China began fortifying salt with selenium to reduce selenium deficiency in the population (Cheng and Qian, 1990).

Dietary sources

In general, animal proteins provide the best sources of selenium (Table 7.10). Many of these foods are also high in iron, therefore, selenium may be limited in the diet of a patient who is trying to limit dietary iron. Good sources of selenium include red meat, seafood, eggs, organ meats, and Brazil nuts. Selenium concentration in the soil will alter the amount of selenium found in plant-based foods, so the amount found in vegetarian diets may vary widely based on geographic location. The Recommended Dietary Allowance for healthy adults in the US for Selenium is 55 µg for both males and females, The mean intake of selenium in adult Americans is over 100 µg/day (USDA 2009-10).

Table 7.10: Selected Foods Rich in Selenium

Food Source, Serving Quantity	Selenium, µg
Brazil nuts, 1 oz (~28 g)	544
Tuna, cooked, 3 oz	92
Shrimp, 3 oz	40
*Beef steak, 3 oz	33
Brown rice, cooked 1 cup (~240 mls)	19
Egg, 1 large	15

*These foods are also relatively high in iron

Source: US Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.

Assessment

The traditional static measures are plasma or serum selenium. Urinary selenium can also be measured as it reflects recent selenium intake. Glutathione peroxidase or Selenoprotein P are functional measures of selenium status. Adequate level of serum selenium is typically above 8 µg/dL.

Selenium and thalassaemia

Inadequate intake of selenium is rarely observed in US patients with thalassaemia (Fung et al, 2012). Serum selenium, as well as plasma activity of glutathione peroxidase, are commonly found to be lower in adult subjects with thalassaemia compared to healthy controls, regardless of what chelator type they are using (Bartfay and Bartfay 2001; Nasir et al, 2018, Sherief 2014). Nasr et al also reported a negative relationship between selenium and serum ferritin in transfusion dependent young Egyptian patients with thalassaemia (Nasr et al, 2002).

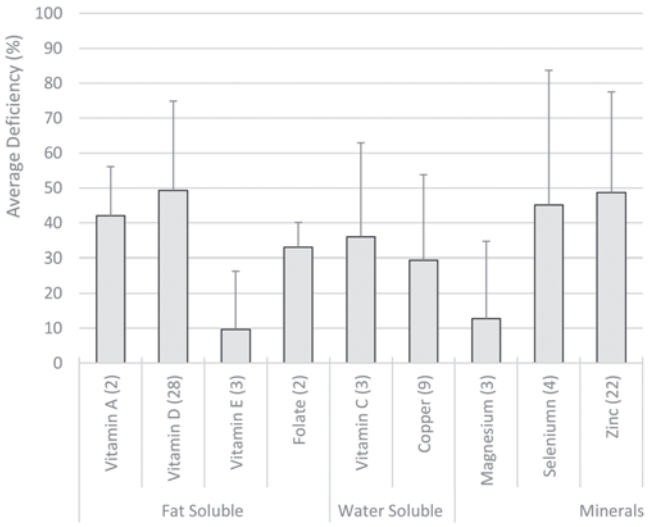
Only one supplementation study has been published in people with thalassaemia. In a single-arm study, 200 ug selenium derived from yeast was given to 34 adult Iranian patients for 1 month (Aboutalebi et al, 2020). Outcomes included increased serum selenium, reduced hair loss, lowered alanine transaminase (ALT), and reduced serum creatinine compared to baseline. A small percentage (6%) suffered gastrointestinal side effects from the ingestion of selenium supplements.

Despite the limited data available on this essential mineral, given its role in reducing oxidant stress, it is recommended that selenium status be monitored on an annual basis in patients with TDT.

Selenium and PK Deficiency

Research investigating the effects of vitamin E and selenium supplemented diets in rats found that diets lacking these nutrients were more susceptible to erythrocyte haemolysis (Chow, 1990). Research investigating the protective role of selenium in its contribution to glutathione and haemolysis in sickle cell disease is interesting (Delesderrier et al, 2019) but its broader applicability remains unclear.

Figure 7.2: *Summary findings: nutrient deficiencies in patients with thalassaemia*
(reproduced from Goldberg EK et al. J Pediatr Hematol Oncol. 2022;44:1-11)



Summary and recommendations:

- Zinc deficiency is frequently observed in patients with thalassaemia. Overt deficiencies of calcium, copper, magnesium and selenium are less frequently observed.
- Serum copper, magnesium, and selenium should be monitored on an annual basis in patients with TDT, NTDT and PKD and replaced if deficiency is observed.
- Given the importance of calcium to bone health, and the frequency of hypocalcemia and hypercalciuria observed, at least annual assessment of serum and urinary calcium are recommended.
- If increased calcium intake is needed, given the potential risk of cardiovascular disease and nephrolithiasis, dietary sources of calcium are preferred to supplemental calcium.
- For some individuals with PKD and low bone mass who do not consume dairy products, calcium supplementation (500 mg or less/day) combined with vitamin D may be appropriate.
- Zinc is beneficial to linear growth in children, bone health and reducing the risk of diabetes in some adult patients with thalassaemia.
- If serum zinc is low, 25 mg/day or 50 mg every other day, is typically sufficient to replace stores. Higher doses are usually not necessary. It is recommended to re-measure serum zinc after 3 to 6 months of supplementation.
- If zinc supplementation is prescribed, monitor serum copper and replace if deficient.

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CHAPTER 8
Special considerations

Tea and iron absorption

Phenolic compounds including polyphenols, (e.g., tannic acid and tannins) inhibit the absorption of non-heme iron. They are especially high in tea, coffee, cocoa, red wine, and some herb teas. In one study a cup of tea reduced iron absorption from a test meal by 64% and a cup of coffee by 39% (Morck et al, 1983). These findings may be relevant to patients with non-transfusion dependent thalassaemia and PKD, who may absorb a large percentage of dietary iron; inhibitors of iron absorption, such as tea, may be useful in their management.

The first study to assess the effect of tea on iron absorption in patients with thalassaemia was published over 40 years ago (de Alarcon et al, 1979). They found that when black tea was consumed with a meal in 6 patients (5 with TDT), iron absorption decreased between 41 to 95%. More recently, the consumption of green tea (three cups/day after meals daily for 12 months) in addition to chelation therapy in 29 adults with thalassemia intermedia resulted in a decrease in liver iron concentration compared to 28 adults prescribed chelation therapy alone (Al-Momen et al, 2020). Both groups of patients were heavily iron loaded at the beginning of the study (liver iron concentration by MRI 19 mg/g dry wt) despite consumption of low iron diets. They also observed slightly higher haemoglobin levels at the end of the study in those who consumed green tea, possibly related to the antioxidants within tea leading to reduced hemolysis.

Green tea extracts (GTEs) have gained popularity as an ingredient in dietary supplements, sometimes referred to as "nutraceuticals". They are rich in polyphenolic compounds belonging to the class of catechins, such as epigallocatechin-3-gallate (EGCG) which has been shown to have iron-chelating and pancreato-protective properties (Koonyosying et al, 2018). Green tea leaves contain higher amounts of monomeric catechins compared to black tea. Indeed, EGCG has beeen shown to be effective in improving ineffective erythropoiesis, iron dysregulation and oxidative stress in iron-overloaded β-thalassemic mice (Settakorn et al, 2022).

However, after an extensive review of recent published data by the US Pharmacopeia, risks of hepatotoxicity due to GTE intake were discovered, including serious liver injury, especially when ingested under fasting conditions or in cases where there is underlying liver vulnerability (Oketch-Rabah et al, 2020). These findings resulted in a requirement for powdered GTE products sold in the US to have the label: "Do not take on an empty stomach. Take with food. Do not use if you have a liver problem and discontinue use and consult a healthcare practitioner if you develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the skin or eyes)."

Turmeric

Curcumin is an ingredient in turmeric, which in many studies show health and medicinal benefits for humans (Kunnumakkara et al, 2023). Curcumin constitutes 2-5% of turmeric, and is claimed to possess several pharmacological properties including antioxidant, iron-chelating, and anti-inflammatory activities. However, turmeric may have effects which are not directly associated to curcumin including antimicrobial, insecticidal, and anticancer (Gupta et al, 2013).

In β thalassaemia patients there have been limited studies, which nevertheless claim benefit against oxidative stress. In one study 21 patients with HbE/ β thalassaemia were treated with 500 mg daily turmeric in capsular form for 12 months. An improvement in parameters of oxidative stress was noted (malonyldialdehyde, superoxide dismutase, glutathione peroxidase, reduced glutathione in red blood cells) as well as a reduction in non-transferrin bound iron (NTBI), however all values returned to baseline 3 months after stopping curcumin administration (Kalpravidh et al, 2010). Similar findings were confirmed in a recent study using doses of 500 and 1000 mg/day for 24 weeks in 29 adult patients with HbE/Bo thalassemia. Iron loading parameters were recorded in patients with baseline ferritin > 1000 ng/ml who received 1000 mg/day curcuminoids (Hatairaktham et al, 2021). They observed reduction in markers of oxidative stress and inflammation after 24 weeks in both groups. Since such results are reported in several studies it is suggested that curcumin could be effective in decreasing plasma NTBI and myocardial iron, alleviating lipid peroxidation and improving cardiac function. No adverse effects were reported. However, randomised clinical trials with large numbers of patients sufficient to support a firm recommendation, and confirm the most effective dose, are still lacking.

There is no research to date suggesting similar benefits in people with PKD.

Wheat grass

Wheat grass is prepared from the common wheat plant (Triticum aestivum) harvested from newly sprouted leaves at roughly 8 to 14 days. It is prepared and sold in a variety of forms including as a fresh or frozen juice or concentrate or as a powder or in tablets. It provides a heavy concentration of chlorophyll, amino acids, vitamins and minerals with relatively high amounts of vitamins E and K (Table 8.1).

A wide range of health benefits have been attributed to the use of wheat grass including its ability to lower cholesterol, prevent cancer, regulate blood sugar, aid in digestion and decrease inflammation (Banerjee et al, 2021). Researchers have explored the potential benefits of wheatgrass in patients who suffer from cancer, rheumatoid arthritis, ulcerative colitis, diabetes, obesity, and of relevance to this review, haematological diseases (Bar-Sela et al, 2015). Many of the studies performed in human subjects had small samples sizes, therefore broad claims regarding efficacy cannot be made. No adverse events of wheatgrass have been reported.

Table 8.1: *Nutritional Composition of Wheatgrass*

	Wheat Grass Powder 1 Tbsp (8 grams)
Calories	25 kcal
Protein	1 gram
Fiber	4 grams
Vitamin K	86 ug
Iron	1 mg
Calcium	24 mg

(Taken from USDA)

Note: Wheat grass is reportedly safe for patients with coeliac disease as it is a gluten free product. Only the kernel of the wheat grain, used in making wheat flour has gluten, wheat grass does not.

It is important to note, however, that wheatgrass is considered a food or nutraceutical, not a drug, and therefore is not regulated by the Food and Drug Administration (FDA) in the US or other European regulatory agencies. As such, there is no assurance that the amount of wheat grass reported on the label is consistent with composition of the product (tablet, powder, juice) or that the product claims on packaging are true.

There have been a handful of studies exploring the potential benefit of wheat grass for patients with thalassaemia. The primary outcome considered in most of the studies was the potential to decrease the transfusion requirement. The majority of the studies were conducted by investigators in India and performed in youth with TDT. The first study was a pilot, published by Marwaha et al in Indian Pediatrics (2004). Sixteen children completed the year-long study in which each child was asked to consume 100 mL of fresh wheat grass daily. The transfusion requirement was reduced by 25%, the definition of success, in half of the children. A similar study in 53 slightly older TDT patients (average age 16 years) did not observe a change in blood requirement after one year of wheat grass therapy (Choudhary et al, 2009). Singh et al (2010) gave wheat grass tablets in 40 TDT patients (2-16 years) for one year. They observed a decrease in the transfusion requirement in 60% of the patients. Most recently, Muhta and colleagues (2018) performed a study again using wheat grass tablets, 12 pills per day given over 18 months in 69 young children (4-6 years) with TDT. They could not demonstrate a reduction in transfusion requirement, but did observe an increase in HbF and stabilisation of serum ferritin in the wheat grass group. In all of these studies, subjects acted as their own controls, no randomised trials were conducted.

It has been proposed that because chlorophyll has a similar structure to haemoglobin, the ingestion of foods high in chlorophyll, such as wheat grass, may stimulate the synthesis of globin, and thereby haemoglobin molecules (Hammet et al, 1970; Pole, 2006). The extract may also have iron chelating effects, though these properties have only been explored in vitro (Das et al, 2016).

The limitations of these studies include limited sample size, lack of generalisability to older patients, inconsistent type of intervention (juice, pill, powder) and lack of a consistent definition of transfusion requirement reduction. In summary, it appears that wheat grass may have an effect of reducing transfusion requirement in a sub-set of young TDT patients, though the predicting variables of whom will respond are unclear. More studies would be necessary to elucidate a mechanism of action and determine efficacy in a larger cohort of adult patients with TDT.

Wheat grass therapy has primarily been researched in thalassaemic populations and not in people with PK deficiency. Its impact on transfusion frequency, HbF, serum ferritin, and quality of life in people with this condition is unknown.

L-carnitine

L-Carnitine plays an essential role in fatty acid beta-oxidation, a process especially important in the organs that preferentially use fatty acid as a source of energy such as the myocardium and the skeletal muscles. It also acts as an anti-oxidant. In thalassaemia there is down-regulation of carnitine biosynthesis, which in turn, results in reduced availability of carnitine in the blood and tissues; this might impair fatty acid oxidation and thus result in muscular weakness, and cardiac dysfunction in the patients with β thalassaemia (Kumar Sarker et al, 2018).

Over a decade ago, investigators from the New Cairo University Children Hospital enrolled 30 patients with β thalassaemia and followed clinical, laboratory, and cardiopulmonary exercise testing before and after 6 months of oral L-carnitine therapy (50 mg/kg/day). The oxygen consumption, cardiac output, and oxygen pulse at maximal exercise significantly increased after L-carnitine therapy ($p<0.001$, $p=0.002$ and $p<0.001$, respectively) (El-Beshlawy et al, 2007).

More recently, 60 β-thalassaemia patients (6 to 33 years) without advanced heart disease were given the same dose of L-carnitine (50 mg/kg/day) in a randomised controlled trial for 6 months. A significant decrease in left ventricular dilitation and left ventricular hypertrophy were observed (Shahidi et al, 2020).

Anti-oxidants

Oxidative stress is a primary phenomenon in thalassaemia and other haemolytic anaemias, including PKD and G6PD deficiency. In the pathogenesis of these conditions oxidative stress leads to early cell death (apoptosis), both in the red blood cells and in other vital tissues leading to irreversible organ damage. The oxidant stress in erythrocytes is countered by an internal defence system which includes enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and some other components such as vitamin E and ascorbate. These systems react with oxidising reactive species such as O2-, H2O2 and ONOO- . However, in congenital anaemias these protective mechanisms are overwhelmed, the cells are exposed to both endogenous and exogenous oxidants, and cell damage occurs. In addition, red cells stored for transfusion suffer from oxidative damage, therefore decreasing the length of time blood is stored between donor to recipient is important (Möller et al, 2022).

Iron accumulation, whether from increased absorption from food or from transfused red cells, promotes the formation of reactive oxygen species (ROS) that can damage DNA, proteins, and lipids within cells. This mechanism results in cell damage in various tissues causing cirrhosis, diabetes,

cardiomyopathy, and endocrine failure. This oxidative tissue damage is countered by chelating agents, which as well as removing excess iron also have anti-oxidant properties.

Oxidative stress is the imbalance between oxidants and anti-oxidants, which is an issue in the congenital anaemias especially if transfused (Atmakusuma et al, 2021 Bou-Fakhredin et al, 2022). The evaluation and maintenance of anti-oxidant defence can be useful in protecting β thalassaemia patients. The challenge is whether, beyond iron chelation, the provision of anti-oxidants in the diet or as supplements can counter this imbalance and have a beneficial effect. Various molecules have been tested as anti-oxidants to scavenge and inactivate ROS, leading to cellular protection against oxidation. Examples of nutrients and substances tested in thalassaemia include Vitamins C, E, A, and zinc, curcumin, silymarin, green tea, N-acetylcysteine, α-lipoic acid, L-carnitine and fermented papaya. In various trials the benefits of these substances on oxidative stress are reported. However, clinical trials have been generally on small samples, and the therapy provided for short periods 3-12 months (Table 8.2). For more information on how these nutrients function as antioxidants, refer to chapter 7, sections on vitamins C, E and zinc.

There is no clear recommendation on supplementation unless more extensive trials are conducted with possible long-term toxicity considered.

Table 8.2: *Summary of Anti-oxidant Trials in Patients with Thalassemia*

Anti-oxidant Studied	Subject Population, Study Length	Reference
Vit E	15 NTDT, 9 months	Tesoriere L, D'Arpa D, Butera D, et al. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Radic Res. 2001;34(5):529-40.
N-Acetylcysteine	75 children with TDT randomised to 3 group for 3 months	Ozdemir ZC, Koc A, Aycicek A, et al. N-Acetylcysteine supplementation reduces oxidative stress and DNA damage in children with β-thalassemia. Hemoglobin. 2014;38(5):359-64.
Vit C & E	20 children with TDT, 3 months on both vitamins	Dissayabutra T, Tosukhowong P, Seksan P. The benefits of vitamin C and vitamin E in children with beta-thalassemia with high oxidative stress. J Med Assoc Thai. 2005 Sep;88 Suppl 4:S317-21
Vit E	18 TDT, 4 weeks	Das N, Das Chowdhury T, Chattopadhyay A, et al. Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. Pol J Pharmacol. 2004;56(1):85-96.

Vit E	10 TDT, 4-8 weeks	Suthutvoravut U, Hathirat P, Sirichakwal P, et al. Vitamin E status, glutathione peroxidase activity and the effect of vitamin E supplementation in children with thalassemia. J Med Assoc Thai. 1993;76 Suppl 2:146-52.
Vit E	45 TDT, 3 months	Hashemian Z, Hashemi A, Fateminasab M. The Benefits of vitamin E on liver function and the hemopoietic saystem in thalassemia patients. Iran J Ped Hematol Oncol. 2012;2(4):153-8.
Vit E	25 TDT, 3 months	Unchern S, Laoharuangpanya N, Phumala N, et al. The effects of vitamin E on platelet activity in beta-thalassaemia patients. Br J Haematol. 2003;123(4):738-44.
Vits E, C and A combined	39 with TDT and low levels of Vit A, C and E, for 1 year	Elalfy MS, Adly AA, Attia AA, et al. Effect of antioxidant therapy on hepatic fibrosis and liver iron concentrations in β-thalassemia major patients. Hemoglobin. 2013;37(3):257-76.
N-acetylcysteine, deferiprone, and either curcuminoids or vitamin E	60 patients with β-thalassemia/HbE, NTDT, 12 months	Yanpanitch OU, Hatairaktham S, Charoensakdi R, et al. Treatment of β-Thalassemia/Hemoglobin E with Antioxidant Cocktails Results in Decreased Oxidative Stress, Increased Hemoglobin Concentration, and Improvement of the Hypercoagulable State. Oxid Med Cell Longev. 2015;2015:537954.
Silymarin	69 TDT, 12 weeks	Darvishi-Khezri H, Salehifar E, Kosaryan M, et al. The impact of silymarin on antioxidant and oxidative status in patients with β-thalassemia major: A crossover, randomized controlled trial. Complement Ther Med. 2017 Dec;35:25-32.

Silymarin

Silymarin is a phytochemical derived from the milk thistle, syn Silybum marianum, with polyphenols, flavonolignans, and flavonoids as its constituents. It inhibits ROS formation, functions as a scavenger of ROS once formed, increases the hepatic level of glutathione, decreases lipid peroxidation, stimulates the synthesis of proteins and phospholipids within the hepatocytes, and inhibits hepatic NF-κB activation (Aghemo et al, 2020). Silymarin acts as a free radical scavenger and modulates enzymes associated with the development of cellular damage, fibrosis and cirrhosis (Gillessen et al, 2020).

Despite optimistic studies performed in patients with drug induced liver injury and non-alcoholic liver disease, very few studies have evaluated the effects of silymarin on thalassaemia liver disease. There are reports that combined treatment with silymarin and conventional iron chelators is effective

in reducing iron overload in β thalassaemia major patients (Darvishi-Khezri et al, 2018; Hagag AA et al, 2015). More recently a study suggested that the use of the substance as monotherapy may also reduce iron load, even though only 6 patients were followed for 6 months (Reisi et al, 2022). A similar small study showed favourable iron chelation in hereditary haemochromatosis (Hutchinson et al, 2010). Apart from chelation, silymarin in thalassaemia is reputed to protect cells from ROS damage and so to be hepatoprotective, cardioprotective, osteoprotective and renal protective as well as anti-inflammatory – all these benefits are reviewed by Darvishi Khezri et al in their 2015 review. Such benefits may suggest supplementation to all patients with thalassaemia or with any iron loading anaemia; however more studies, especially over longer periods, would be required before such a recommendation can be made.

Summary and recommendations

- Tea consumption, either black or green, to inhibit iron absorption may be of benefit if taken with meals for patients with NTDT and other iron loading anaemias such as PKD.
- Green tea extracts have been shown to be of benefit in iron overload but should be used with caution and regular monitoring because of possible liver toxicity. Avoid their use if there is prior liver disease.
- Turmeric and its ingredients appear to have some benefits in the case of iron toxicity although the beneficial effect is uncertain in daily food consumption.
- Wheatgrass is not regulated, therefore, there is no assurance that the amount reported on the label is consistent with composition of the product (tablet, powder, juice) nor that the product claims are true.
- The in-vivo evidence on the efficacy of wheatgrass in contemporary patients with thalassaemia is equivocal. For patients who choose to consume wheatgrass, it should not take the place of fruit and vegetables in the diet. A daily variety of fruits and vegetables provide rich sources of fibre, vitamins, minerals and phytochemicals not found in wheatgrass alone.
- L-carnitine has been reported to benefit thalassaemia patients in small studies which as yet not provide adequate justification for carnitine supplementation or provide guidance as to which patients are likely to benefit.
- Based on the hepatoprotective effect of silymarin in other conditions, a case could be made to use it prophylactically in thalassaemia and PKD in which liver complications are a major concern, but at this stage further studies, especially long term, are required.

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CHAPTER 9

Practical approach to nutrition counselling

Nutritional assessment and counselling should ideally be conducted in the context of a comprehensive medical evaluation, by a nutrition professional in collaboration with the clinical team. The dietitian will need to know the patient's medical history, list of current medications and co-morbid conditions to best plan for the nutritional evaluation. It is good to start a nutrition counselling session by asking about the patient's questions or concerns about their diet and nutritional status, paying particular attention to any misinformation that may have been gathered from the internet or other media sources. When patients' nutrition concerns are addressed up front, they will be more inclined to participate in the counselling session.

As outlined in Chapter 5, the holistic nutrition assessment will rely heavily on the ABCDs: anthropometry, biochemical parameters, clinical signs and symptoms along with a focused dietary intake assessment. Care must be taken to consider current medications, for any possible drug-nutrient interactions, as well as the patient's dental care which may affect intake and food preferences. When all these data are gathered and reviewed, an individualised care plan can be developed that is respectful of the person's personal needs and preferences, sensitive to any cultural or socioeconomic barriers, and considerate of their nutritional knowledge and motivation, while recognising their clinical challenges. Involving the patient in the development of their care plan leads to the greatest long-term success.

When should supplementation be recommended?

Most research suggests that obtaining essential nutrients from foods first is optimal. Essential nutrients are often found within the foods we eat in a form intended for optimal absorption. As an example, calcium is best absorbed along with lactose, therefore dairy is an optimal source of dietary calcium (IOM, 2011). The concentration of essential nutrients in foods also allows for optimal absorption. Dietary sources of calcium rarely exceed 500 mg per serving, which appears to be the highest amount of calcium that can be absorbed from a single meal without down-regulating calcium transport proteins. This is in contrast with the high doses of supplemental calcium often prescribed which, if consumed in a single dose, leads to down regulation of absorption. Food also contains much needed fibre as well as other substances (phytochemicals, flavonoids) that are important for optimal health. No doubt there remain some substances in the diet that are important to human health, but have yet to be completely understood.

When healthy foods are replaced by foods with poor nutrient density ('empty calories') combined with high dose single nutrient supplements, patients miss out on all of the benefits of food intake, not to mention the simple joys of consuming delicious, nutritious foods. The challenge for patients with thalassaemia and PKD comes when nutritional requirements outweigh the amount that can feasibility be consumed from a healthy diet alone. Vitamin D

falls within this category. Natural sources of dietary vitamin D are limited to oils from fatty fish, some mushrooms and egg yolks, and it is therefore difficult to obtain sufficient intake through natural food sources alone. Vitamin D is fortified in the food supply, mostly in dairy products, in some countries. Yet even with fortification, most patients who live in northern latitudes, who wear clothing that covers much of their skin, and / or otherwise have limited sun exposure will require supplemental vitamin D.

There may be other nutrients for which supplementation will be beneficial for some patients - these are outlined in the relevant earlier chapters and in Appendix 12.6. Given increased nutritional requirements, potential lack of access to food variety, and challenges in maintaining a nutrient dense diet on a consistent basis, many comprehensive thalassaemia programmes recommend routine multivitamin supplementation without iron for all transfusion dependent patients (Standards of Care Guidelines, Oakland, 2012); this should extend to patients with PKD also. Supplementation of any kind can never replace a healthy diet, but should be used in addition to food intake for optimal nutritional status.

Focus on a varied, nutrient dense diet

For patients to consume a diet rich in vitamins and minerals, the counselling focus needs to be on dietary variability and nutrient density. Translating the science behind dietary variability to patients in a way they can understand, and make use of in practice, is valuable. The phrase "eating around the rainbow" can be used to explain to patients that a colourful diet is healthier than a monochromatic one. (Figure 9.1). Healthy foods, rich in vitamins and minerals, come in a variety of colours: kale (green), carrots (yellow or orange), beets (red), red cabbage (purple). Each food derives its colour from the rich concentration of antioxidants in its skin and flesh. The variety of colours come from the diverse range of anthocyanins and antioxidants, for example, carrots and sweet potatoes are orange because of the antioxidant, beta-carotene. By eating a variety of foods of different colours throughout the day (eating around the rainbow), patients will be consuming more antioxidants, substances which are important for reducing the damage that can be caused by the free iron in the body. It is, however, important to differentiate between naturally vibrant healthy food and processed foods with colour additives e.g. breakfast cereals such as 'Fruit Loops' – these processed foods should not be considered part of the healthy rainbow. That said, framing certain foods, or food groups, as 'good' or 'bad' can result in significantly limiting food choice and thereby dietary variety. If patients become extremely selective in food choice, for example by eliminating all foods rich in iron, they may also be limiting foods rich in other important minerals, such as zinc. By limiting the variety in the diet, the individual may also be unknowingly limiting nutrient density. Having exposure to broad food choice, within the individual's limits of food availability and access, while maintaining adherence to chelation therapy, will improve the likelihood of meeting nutrient requirements and maintaining body iron load.

Figure 9.1: *Eating around the rainbow*

Individualise the plan

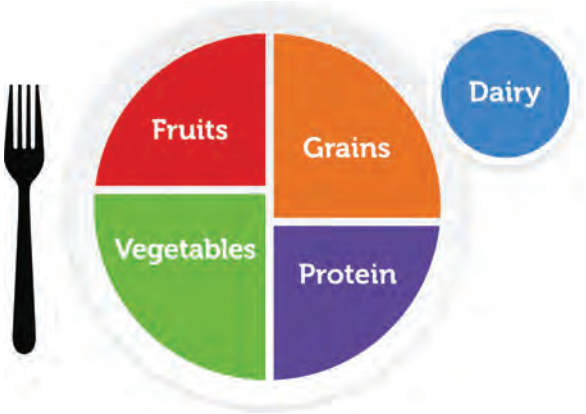
Individualised nutritional plans are particularly valuable. Not only will the dietitian incorporate the unique clinical situation of each patient, but also consider their schedule, home environment, food preferences and allergies, individual's socioeconomic status, food availability, personal, cultural and religious food practices. Dietitians may need to consider patients whose diets are vegetarian, vegan, gluten free, and/or follow kosher practices, or who do not eat pork products, and to be sensitive to the cultural and religious needs of the patient. These and other areas in which a nutrition professional can be especially helpful in developing the individualised plan are listed in Table 9.1.

Table 9.1: *Specific areas where a nutrition professional is helpful to patients with thalassaemia or PKD*

- Determining which nutrients may be lacking in the diet through a thorough nutritional assessment.
- Advising patients on practical strategies for meal planning and shopping.
- Strategising around meal planning specifically for transfusion or sick days.
- Managing dietary intake around feelings of fatigue.
- Suggesting tips for de-escalating meals.
- Ideas for how to encourage picky eaters to try new foods.
- Instruction on how to read nutrition labels.
- Identification of foods with high iron, or hidden iron content.
- Strategies for how to manage gastrointestinal stress from oral chelator medication.
- Identifying nutrient dense foods and those high in antioxidants.
- Recommendations for low glycemic index foods for patients at risk of diabetes
- Specific assistance with individuals who have specialised dietary needs (e.g. vegetarian, vegan, kosher, food allergies, low lactose, gluten free).
- Choosing an appropriate iron free nutritional supplement when needed.
- Assistance with specific dietary needs for diabetic patients.
- Awareness of current fad diets and potential misinformation geared towards patients.
- Assessment of disordered eating.

Simple nutritional guides may also be helpful to aid patients in increasing dietary variety. The 'My Plate' program is a visual guide developed by the USDA for healthy eating (www.choosemyplate.gov; Figure 9.2). It is a simple image of a small plate which is divided into portions, roughly ¼ for protein, ¼ for grain/carbohydrates and ½ of the plate set aside for fruits & vegetables. It comes with an online interactive website where individuals can learn more about what foods fall into each category, portion size, budget friendly food ideas, recipes, and suggestions for increasing nutrient density 'making every bite count'. Consuming meals with these proportions will increase vitamin, mineral and antioxidants intake. There are many other nutritional guidelines that have been developed internationally for healthy eating.

Figure 9.2: *'My Plate' Visual used in Nutrition Counselling*



The visual comes with an online interactive website where individuals can learn more about what foods fall into each category, portion size, budget friendly food ideas, recipes, and suggestions for increasing nutrient density 'making every bite count'.

Nutrition information and the media

Nutrition information is abundant in all forms of current media, and patients may be bombarded with advertisements about food products, nutritional supplements and weight loss programmes. Cooking shows on TV are prolific, and nutritional advice is freely shared by family, friends, neighbours and colleagues. Further, nutrition research often appears contradictory and difficult to interpret. This can all be quite overwhelming to digest, and to determine which information is accurate and relevant. This is where the dietitian is particularly helpful. They will be aware of the latest food trends and fad diets, able to answer patients' questions that arise from internet searches and well-meaning peer advice, as well as to interpret research findings. Directing patients and families to reputable sites for nutrition information is key, as is encouraging patients to maintain an open dialogue with the clinical care team before embarking on any major changes to their diet or supplementation. There are reputable published suggestions that may be useful for individuals when interpreting nutritional research findings, for example https://www.hsph.harvard.edu/nutritionsource/media/

Counselling strategies for paediatric populations

Promoting optimal nutritional status in children is integral to their health and immune function, and is critical to support growth. However, nutritional recommendations for the child or adolescent with these conditions must be considered in the context of the family. Nutritional issues commonly observed in this age group include food selectivity ('picky eaters'), food intolerances or allergies, food sensitivities (taste, texture, smell), swallowing dysfunction, dysfunctional relationships with food or food restriction e.g. anorexia nervosa, and altered mealtime dynamics; the dietitian with expertise in paediatrics is especially helpful here. They

can provide suggestions for how to encourage new food discovery for the picky child (Table 9.2). In general, other than in very low resource settings when sourcing sufficient food of any sort for the family may be a challenge, it is the parents' or carers' responsibility to provide food choice at each meal, and the child's responsibility to decide what to eat and how much to consume. If a child has extensive food allergies or sensitivities that limit overall intake, protein/calorie meal replacements can be discussed and the necessity for vitamin and mineral supplementation considered. There is extensive research on the relationships between food and family dynamics in some developed nations, for example dietician and family therapist Ellyn Satter has developed an institute dedicated to providing support to professionals and parents on childhood nutrition, feeding dynamics and eating competence (https://www.ellynsatterinstitute.org/) and some materials from this site may be useful for clinicians working with this patient group. As for adult patients, the focus is to encourage as varied and nutrient dense diet as possible.

Avoidant Restrictive Food Intake Disorder (ARFID) is a serious feeding and eating disorder formally recognised in the DSM-5 in 2013 and first included into the International Classification of Diseases in 2022. It affects children and is associated with individual, family, and social impairment along with potentially serious medical consequences. ARFID does not involve distress about body shape or size, but is more likely to be associated with the sensory characteristics of food and likely has a heritable component (Dinkler and Bryant-Waugh, 2021). Families affected by ARFID will need specialist professional support to manage this disorder.

Table 9.2: *Tips for 'picky eaters'*

Don't worry if the child is occasionally a picky eater. It is natural for children to refuse to eat some foods at some times, it is one of the ways they develop independence. It is an issue when they consistently avoid foods, food groups or adds stress to family dynamic and mealtimes.

- Where possible, offer many different foods at each meal, especially fruits and vegetables.
- When new foods are offered, be sure there are also familiar foods at the same meal.
- Don't give up. Children often need to experience a new food a number of times before acceptance.
- Provide regularly scheduled meals and snacks.
- Keep mealtimes constant using the same plates, utensils and location where the meal is provided.
- Avoid external distractions during the meal, remove phones, ipads, computers or TVs from the environment where the meal is served.
- Eat together as a family as often as possible.
- Model a healthy diet for the child.
- Avoid using food as a reward for behavior management.

Adapted from the Academy of Nutrition and Dietetics, Selective Eaters Nutrition Therapy.

Specific example of comprehensive nutrition management

Thalassaemia & Diabetes (see also chapter 4). Patients with thalassaemia and newly diagnosed diabetes will require a coordinated care plan between the haematologist, endocrinologist and dietitian to understand the clinical landscape and support optimal glucose control. First, there needs to be an assessment of beta cell reserve, often through the assessment of C-Peptide or HOMA-B (homeostasis model assessment of β-cell function). Despite the detrimental effects of iron on the beta cells of the pancreas, many patients will have some residual insulin secretion and this will have an effect on exogenous insulin or oral diabetes medication dosing and nutrition management (De Sanctis et al, 2016). The patient's ethnicity or country of origin must also be considered as there may be significant insulin resistance without elevated body mass index in some geographic regions, including China, Japan, and Thailand (Chan et al, 2009). Their total body fat to muscle mass ratio should also be considered as fat mass may be unusually high for body weight which may also affect insulin dosing (Patel and Abate, 2013). Finally, the macronutrient consumption of the diet (carbohydrate to protein) and frequent consumption of high glycemic index foods such as parboiled rice in some cultures should be considered. Patients with thalassaemia and diabetes will require focused nutritional education with coordinated meal and snack planning to support optimal glucose control. Unique to the care of these patients is also the consideration of reducing dietary iron while balancing chelation schedules with insulin dosing. Dietitians are attuned to consider patients' work or school schedules, household responsibilities, cooking abilities, economic stressors, cultural sensitivities, food preferences and beliefs, along with individual habits before making nutritional recommendations.

Eating Disorders (ED)

Disordered eating includes subtypes that all clinicians should be aware of: anorexia nervosa (AN), bulimia nervosa (BN), binge eating disorder (BED), avoidant restrictive food intake disorder (ARFID), rumination disorder (RD), pica, and other specified feeding or eating disorder (OSFED). Once thought of as occurring only in high income countries, EDs affect at least 9% of the population worldwide, appearing at any stage of life but concerningly, as early as pre-adolescence (https://anad.org/eating-disorders-statistics/) With globalisation, East Asia and South Asia have experienced the most significant increment in burden of disease from ED from 1990 to 2017. Its prevalence in people with thalassaemia and PKD is unknown. Those who develop eating disorders may also be at increased risk for co-occurring mental health concerns including anxiety, depression, substance use disorders, obsessive-compulsive disorder (OCD), and posttraumatic stress disorder (PTSD). Medical trauma has not been studied in relationship to eating disorders, though bullying and appearance-related teasing victimisation, which some patients with these blood conditions report, may be a risk factor.

BMI should not be viewed as the primary screening tool for ED as it fails to capture feeding and eating practices. Clinicians managing children and adolescents with signs of food restrictive behaviors, or females who develop secondary amenorrhea unrelated to iron toxicity, should be aware that referral to an eating disorder specialist is of the utmost importance.

Dietitians and the care team

Patients with thalassaemia or PKD have a complex set of medical issues that require a comprehensive team for optimal management. The haematologist with expertise in the condition often serves as the team lead, with input from endocrinologists, cardiologists, orthopaedics, hepatologists, dietitians, psychologists, social workers or case managers. A nutritionist or dietitian will have received extensive formal training in nutritional science and its application to clinical practice, and is best placed to provide evidence based medical nutrition therapy to patients and tailor counselling strategies to meet their needs. For more information on dietitians and their training please refer to: https://www.healthline.com/nutrition/dietitian-vs-nutritionist#dietitian <0r> https://www.unlockfood.ca/en/Articles/About-Dietitians/What-is-a-Dietitian

Summary and recommendations for nutrition counselling and monitoring

- Include a nutrition professional in comprehensive annual review of people with these conditions attending clinics, where available.
- When all the nutritional assessment data are gathered, nutritional support and counselling should come from a nutritional professional in collaboration with the clinical team.
- An individualised care plan should be developed that is respectful of the patient's personal needs and preferences, sensitive to any cultural or socioeconomic barriers, and considerate of their nutritional knowledge and motivation, while recognising their clinical challenges.
- 'Eat Food not Supplements' - it is recommended that essential nutrients be obtained from foods first, while acknowledging that supplementation may sometimes be necessary.
- 'Eating around the Rainbow' encourages variety in the diet, by eating a variety of colourful foods, patients will be consuming more antioxidants, substances which are important for reducing oxidative damage from iron overload.
- Framing foods as 'good' or 'bad' can result in significantly limiting food choice and thereby dietary variety. By limiting the variety in the diet, the individual may also be unknowingly limiting nutrient density.
- Direct patients to reputable nutrition information on the internet, and encourage open dialogue about nutrition information they are reading.
- Routine monitoring of growth is necessary, and nutritional factors such as caloric intake and micronutrient deficiencies should be considered in instances of poor growth.

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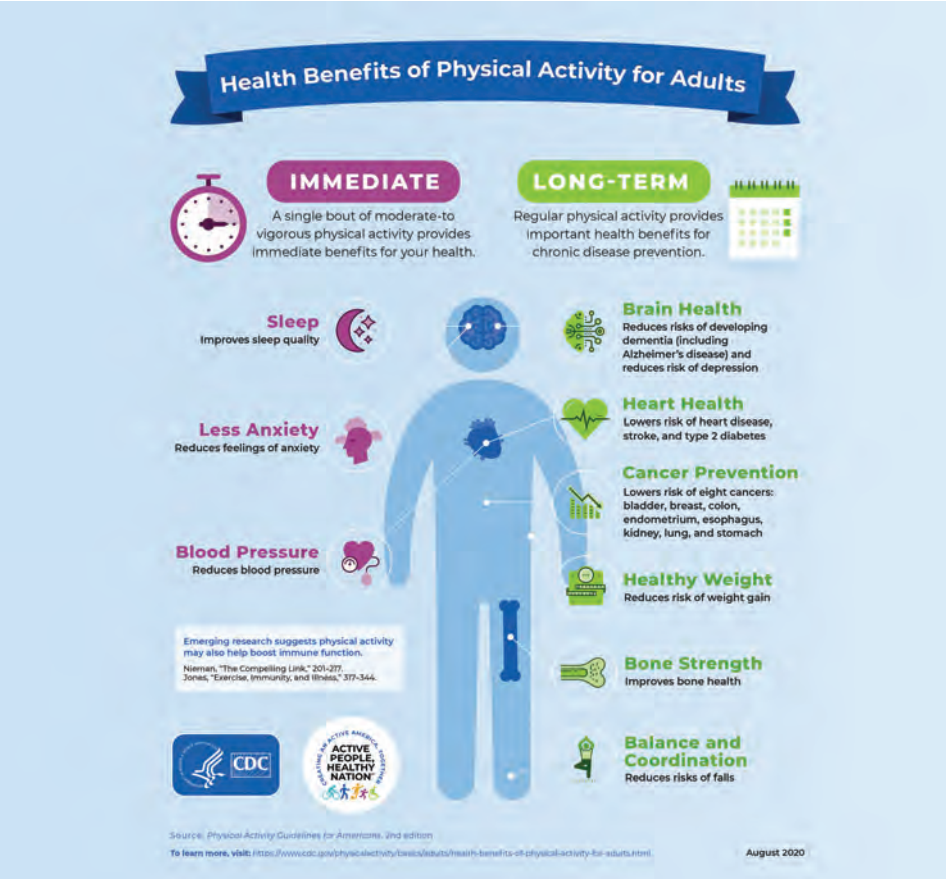
CHAPTER 10

Physical activity in thalassemia

Previous research in small cohorts has suggested that patients with thalassemia have reduced physical activity compared to controls (Nanas et al, 2009; Fung et al, 2015; Piatti et al, 2021). Anaemia, fatigue, and deconditioning, all of which are frequently observed in patients with thalassaemia, can hamper an individual from exercising. It is hypothesised that significant cardiac iron overload can further decrease exercise capacity by reducing stroke volume, cardiac contractility, and preventing exercise-induced increases in heart rate (Nanas et al, 2009; Sohn et al, 2013, Mavrogeni et al, 2009). Chronic pain, which is reported in over half of adult patients with (Haines et al, 2013), has also been shown to affect physical function and encourage sedentary behaviors, such as prolonged sitting and watching television (Fung et al, 2022a). These behaviors may contribute to osteoporosis, diabetes, depression, and a decreased quality of life.

Figure 10.1: *Health Benefits of Physical Activity for Adults*



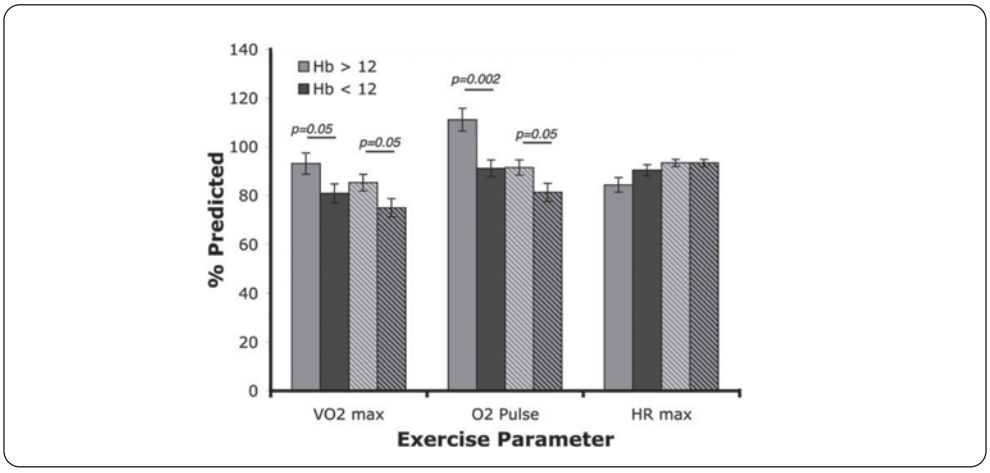
Reproduced from Center for Disease Control. Physical Activity Guidelines for Americans, 2nd Edition. August 2020.

The potential benefits of physical activity for individuals with congenital anaemias cannot be ignored. In people without thalassaemia, increased bone density and enhanced muscle mass (Gonzalez-Rocha et al, 2022), improved balance (Akbari and Naimi, 2022), improved glucose tolerance (Thomas et al, 2006; Quirk et al, 2014), reduced pain (Oesch et al, 2010), improved mood, decreased stress (Norris et al, 1992), and improved academic performance and long-term memory (Sibley et al, 2003; Pajonk et al, 2010) have been consistently reported in individuals who are physically active (and see Figure 10.1). In fact, some have purported that exercise is more effective and safer than prescription medications for a number of chronic diseases of adulthood (Pendersen and Saltin, 2015; Fiuza-Luces et al, 2013).

Reduced physical activity patterns in thalassaemia

People with thalassaemia are presumed to be inactive for many of the same reasons as healthy adults, though there are limited published data to support this claim and there are a number of other factors that may contribute to inactivity in this patient group. **Chronically low haemoglobin (Hb) levels, either in non-transfusion dependent patients or prior to-transfusion in those with TDT have been associated with reduced exercise performance (Sohn et al, 2013, Marinov et al, 2008) - see Figure 10.2.** Along with reduced haemoglobin, pain (Oliveros et al, 2013), depression (Yengil et al, 2014; Yahia et al, 2013) and for children, overprotective parenting, can result in reduced physical activity for many patients with thalassaemia. Additionally, those with TDT can have various complications which may inhibit or otherwise hamper participation in moderate to vigorous physical activity, including iron overload associated cardiomyopathy, hepatitis, diabetes, osteoporosis and hypothyroidism.

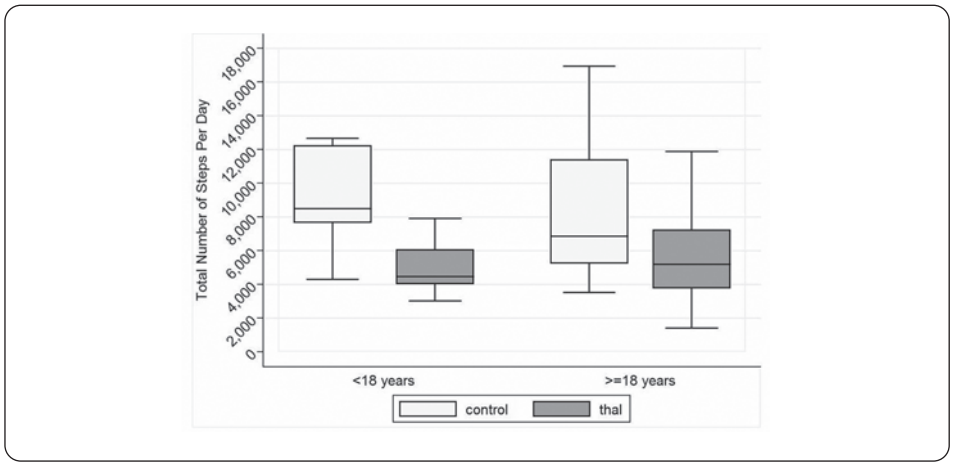
Figure 10.2: *Percent predicted VO2max, O2 pulse, and maximum HR (markers of exercise performance), reported by haemoglobin concentration in patients with thalassaemia. Females depicted by solid bars and males depicted by cross-hashed bars. P-values are reported for significant differences with respect to hemoglobin concentration. Better exercise performance in those with hemoglobin > 12 g/dL.*



Reproduced from Sohn EY AJH, 2013.

Physical activity patterns were explored in 37 subjects with thalassaemia (21 Female, 27.3±10.1 years) compared to healthy controls (17 Female, 28.0 ± 13.7 years) using data from accelerometers worn for one to two weeks (Fung et al, 2015). Accelerometers are small instruments worn on the hip used for measuring movement in 3 dimensions. They can not only measure the number of steps taken per day, but also the distance travelled and energy expended. In this study the thalassaemia subjects recorded a significantly fewer total number of steps per day compared to healthy controls with similar vocations (p=0.001, see Figure 10.3). None of the adult subjects met the recommended 10,000 steps/day and spent significantly less time in moderate (p=0.03) or vigorous (p=0.005) activities and more time in sedentary activity (p=0.006) compared to healthy controls.

Figure 10.3: *Total Number of Steps Per Day Assessed by an accelerometer (ActiGraph) in Patients with Thal (n=37) Compared to Healthy Controls (n=30) divided by Age Group.*
Step recommendations for adolescents is 12,000 steps/day and for adults is 10,000 steps/day (Tudor-Locke 2011).



Reproduced from Fung 2015.

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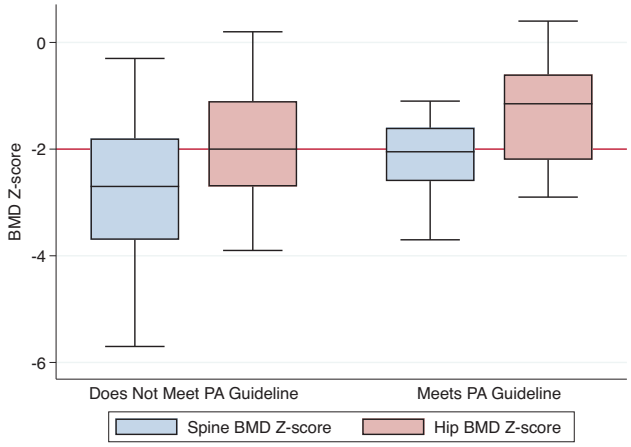
Figure 10.4: *Total Number of Steps Per Day Assessed by an accelerometer (ActiGraph) in Patients with Thal (n=37) Compared to Healthy Controls (n=30) divided by Age Group. Step recommendations for adolescents is 12,000 steps/day and for adults is 10,000 steps/day (Tudor-Locke 2011).*



Reproduced from Fung 2015.

Inadequate bone formation during adolescence

Bone accrual, or the amount of bone mineral gain during growth, has been characterised from DXA scans collected from 33 TDT youth (12.3+3.2 years, 5 to 10 years at initial exam) and compared to national reference data (Kelly et al, 2019). As has been shown before, bone mineral density (BMD) and bone mineral content (BMC) Z-scores declined with age. Specifically, whole body BMC Z-score decreased by 0.1 SD for every year increase in age ($p<0.001$). Lumbar spine BMD and whole-body BMC velocity Z-scores declined significantly with age in females ($p<0.02$ for both), but not males. Pubertal category, 25-OHD and dietary calcium intake were not related to bone velocity Z-score. 55% of patients had lower than predicted BMD Velocity Z-scores, even after adjustments for height, gender, baseline BMD, and pubertal status (Fung et al, 2022b). Children with TDT have inadequate bone gain during adolescence leading to low bone mass as adults and this may contribute to long term morbidity. Delayed puberty and low vitamin D stores do not explain bone accrual deficits in this population. Given that physical activity contributes heavily to bone formation during childhood, strategies to promote exercise in young people with thalassaemia are of value.

Special considerations and limitations

There are no published guidelines regarding how to exercise safely, nor are there recommendations regarding the types of exercises that would result in the greatest benefit to this population. For some patients, cardiac iron load and spleen size are significant risk factors which should be considered before initiating any new activity. For most patients, non-contact, weight bearing physical activity such as walking regimens can be encouraged, after consultation with the patient's haematologist and cardiologist. Physical therapists can also be helpful in translating a physician's recommendation for activity into safe daily patient exercises.

Given the significant bone deficits observed in patients with thalassaemia, and their relative inactivity, it appears that regular non-contact weight bearing physical activity has the potential to not only improve bone health but may also have positive effects on bone pain, glucose tolerance, iron load and overall quality of life.

Physical activity and PK Deficiency

Unlike thalassemia, there is no research investigating the impacts of exercise and physical activity in people with PKD. In a study measuring quality of life in adults and children with the condition, stronger fatigue scores were found in older adults, women, non-Amish people, those regularly transfused with iron overload and using chelation, people who also had pulmonary hypertension, children with non-missense/non-missense genotype, and children with a haemoglobin < 8 g/dL (Al-Samkari et al, 2022). Many children reported no problems at all with walking, but 63% had problems feeling dizzy, 60% short of breath, and 80% chest pain. Therefore, exercise and physical activity should be encouraged for bone health, at a rate that does not promote symptoms of fatigue. This will be different for each individual with PKD. Children and adults who receive blood transfusions may be better able to tolerate higher levels of physical activity and thus an improved quality of life as well as other possible benefits not yet researched for PKD.

Summary and recommendations

- There are many proven benefits of physical activity in non – thalassaemia populations including but not limited to weight maintenance, improved balance, improved bone and heart health, improved glucose tolerance, reduced anxiety and stress.
- Studies of patients with thalassaemia conducted in Greece, Italy and the US have reported they have reduced levels of physical activity compared to healthy controls.
- Factors that may contribute to reduced activity in thalassaemia and PKD include pain, anaemia, fatigue, deconditioning, and decreased exercise capacity.
- Physical activity in adults with thalassaemia has been associated with improved quality of life, less pain, reduced serum ferritin and higher bone density.
- Strategies to promote non-contact, weight bearing activity such as walking regimes in young people with thalassaemia and PKD should be encouraged.
- All patients with these conditions should consult their haematologist and cardiologist before initiating an exercise regime.
- Physical activity should not take the place of adequate chelation or optimal nutrition, but considered a synergistic activity for health promotion.
- More research is needed to determine the types of activities, including the frequency and magnitude of activity, which promote the greatest benefit while avoiding injury.

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CHAPTER 11
Summary and final recommendations

This is the first comprehensive guide focused on nutrition for patients with thalassaemia and pyruvate kinase deficiency (PKD). Though most would agree that optimal nutritional status is integral to high quality care for both of these diseases, little attention has been paid to nutritional status and its impact on clinical outcome and quality of life. Up until recently, clinical care has focused on the central pillars of care: blood transfusion, iron chelation, and managing related complications. Within the past decade however, there has been more focus on quality of life concerns for patients, including nutritional needs. The purpose of this book is to provide the scientific basis for optimising "nutritional health" in patients with these conditions.

Readers are referred to individual nutrient chapters for details, but for ease of use a summary of recommendations are highlighted below. The literature has been extensively reviewed and summarised for each essential nutrient where risk of deficiency has been observed. Recommendations are based on the literature reviewed, and the strengths and limitations of reviewed studies. Where there is a lack of information available specifically relating to thalassaemia or PKD, expert opinion is provided based on our knowledge of nutrition in the general population and other chronic diseases.

It is clear that nutritional deficiencies are rather common, though overt signs and symptoms may not be apparent. The aetiology of these nutritional deficiencies is multi-factorial. A careful, comprehensive nutritional assessment on an annual basis with follow-up by a nutrition professional is recommended. A varied, balanced, nutrient dense diet should be encouraged for all patients. The details of crafting meals and snacks that are healthy, economical, culturally appropriate with limited burden on the patient is the skill, creativity and responsibility and of the nutritional professional who works alongside the clinical team. Where feasible, consideration of a multivitamin mineral supplement without iron may be useful to reduce the risk of deficiency in the presence of increased requirements. This guideline also suggests supplementation with vitamin D, folate and an appropriate dose of zinc in almost all patients with thalassaemia. For patients with PKD, universal supplementation with vitamin D, folate and zinc is appropriate, with consideration of calcium supplementation and / or a daily multivitamin without iron if the patient has a suboptimal diet. For other single nutrients, there remains inadequate evidence to recommend routine supplementation for all patients in the absence of individual deficiency. Supplementation of any kind, however, should not take the place of a high quality, nutrient dense diet or adequate chelation. Therefore, it remains the job of the clinicians, and nutritional professionals working alongside them and the patient, to perform routine nutritional assessments and prescribe replacements to correct nutritional deficiencies when observed.

Limitations of this guideline

The recommendations provided within this guideline are based on, and therefore also limited by, published literature. Though clinical practice in some countries may consistently support the use of a particular nutritional supplement, experts who contributed to this guideline did their best to focus on published studies. Observational studies may be confounded by the lack of a control group, especially if conducted in environments where malnutrition and poor access to medical care are commonplace. As has been described before, the prevalence of deficiencies amongst thalassaemia populations was highly variable between studies, partly due to inconsistent reference values and nutrition assessment techniques. The error within these measurements likely contributes to an under-representation of the magnitude of the problem. These difficulties contribute to challenges in developing definitive conclusions regarding which patients are at greatest risk for nutrition deficiencies.

There are few well-designed, adequately powered, randomised controlled trials: the gold standard for scientific extrapolation. Clinicians should be aware of this, and be prepared to alter dosages in the event of any adverse reaction. While water soluble nutrients can be excreted from the body without harm, chronic high dose supplementation of fat-soluble vitamins and minerals, has been associated with organ damage in other population groups and should be used with caution, and adequately monitored.

Recommendations

- It is advised that a comprehensive nutritional assessment be incorporated into an individualised patient care plan for all patients with thalassaemia and PKD
- This assessment, composed of anthropometry, nutritional biochemistries, clinical and dietary assessment tools, should be conducted annually with the expertise of a nutrition professional where available.
- As energy expenditure may be increased in people with these conditions, it is recommended that the relationship between a patient's total caloric intake and energy expenditure be followed closely, particularly in children during periods of rapid growth.
- Protein intake is typically adequate in patients with thalassaemia and PKD, except in patients who are vegetarian, vegan or who avoid animal meat products due to high iron content. The suggested intake of 'good quality' dietary protein is 0.8 grams of protein per kg body weight per day for adults.
- Patients with thalassaemia may face challenges in achieving adequate hydration which could lead to additional fatigue, constipation, altered kidney or digestive function. Optimal hydration varies depending on age, gender, size of the individual, activity level and environmental conditions.
- A diet rich in fibre and non-sugar sweetened beverages should be encouraged. The US Dietary Guideline suggests an intake of 25 to 38 g of fibre per day for adults is adequate, and is a reasonable goal for adult patients with these conditions.

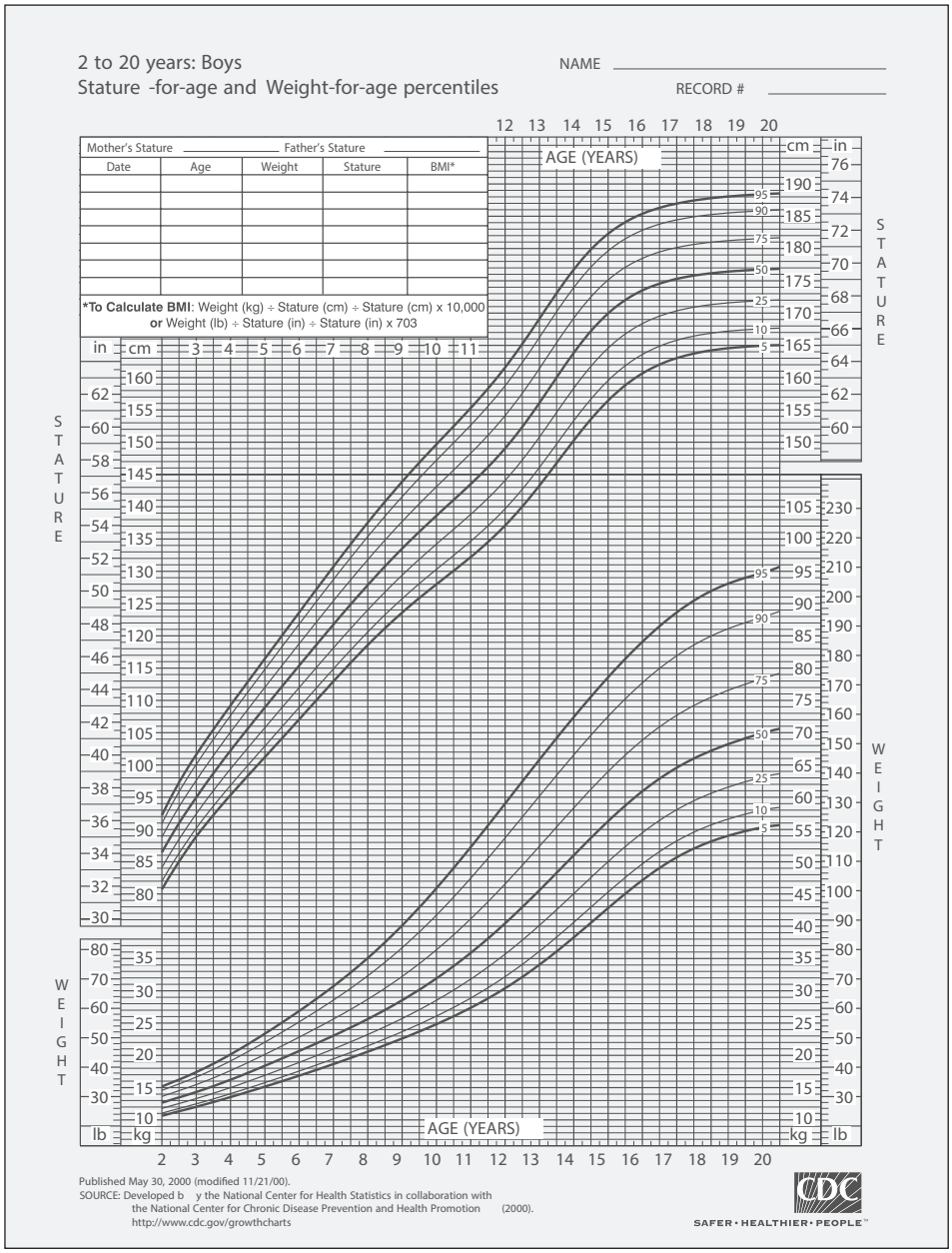
- Vitamin C may be useful for reducing oxidative stress and improving chelator efficacy. Supplementation is recommended in those with diagnosed deficiency, though care should be taken to ensure adequate chelation while supplementing with vitamin C.
- Folic acid supplements are recommended for all patients with thalassaemia and PKD given the nutrient's importance in cell division and effective erythropoiesis; the risk of toxicity is low. Annual assessment of serum folate is recommended. Supplementation with 1 mg/day or 5 mg/week appears to be sufficient to maintain optimal folate status for most patients with thalassaemia.
- Vitamin D supplements (oral formulations of vitamin D2, ergocalciferol) are recommended for all patients at a dose of 1,000 – 2,000 IU/day or 50,000 IU 3-4 weekly, along with measurements of vitamin D levels every 6 months to maintain a circulating 25-OH vitamin D level above 30 ng/mL (75 mmol/L)
- For patients with vitamin D deficiency (serum 25-OH vitamin D below 20 ng/ml or 50 nmol/L), high dose supplementation (50,000 IU/day) is recommended for 8 weeks followed by 2000 IU daily for a further 8 weeks.
- A diet rich in calcium (milk, yogurt, tofu, kale) is preferred over supplemental calcium to reduce the risk of nephrolithiasis. However, because of the high percentage of PKD patients affected by osteopenia and osteoporosis at young ages, supplemental vitamin D and calcium should be considered early on in this patient group.
- A diet rich in foods with high Vitamin E content, such as wheat germ, nuts and vegetable oils is recommended. Short term (3 month) supplementation of vitamin E (α-tocopherol) should be provided to those with identified deficiency; however, prolonged use of supplements requires further research.
- Despite the importance of vitamin K in bone health and vascular calcification, little attention has been paid to its role in the health of patients with thalassemia or PKD. There are not enough data to support routine supplementation unless deficiency is observed.
- For patients with thalassaemia or PKD prescribed vitamin K agonist anticoagulants, a stable intake of vitamin K is critical, and for those prescribed antibiotics for more than a few weeks, vitamin K supplementation should be considered.
- Serum copper, magnesium and selenium should be monitored annually in patients with thalassaemia and PKD, and replaced if deficiency is noted.
- Zinc supplements (25 mg/day or 50 mg every other day) may be given in cases of deficiency, poor growth, reduced bone mass or increased risk for diabetes. Zinc sulphate formulations may cause nausea in some and should be avoided. Alternative sources of supplemental zinc (e.g. zinc citrate, acetate, gluconate) are preferred.
- Zinc is commonly prescribed prophylactically to patients taking deferiprone (DFP); the present review finds zinc deficiency common, but no more frequent in patients given DFP than those prescribed other chelators.

- Dietary iron restriction may be considered in non-transfusion dependent patients but should not be a primary focus of nutritional counseling for transfusion dependent patients where the deposition of iron from transfused red cells far outweighs dietary iron absorption.
- Though routine supplementation without identification of single nutrient deficiency is not recommended (aside from folate, vitamin D and zinc), a daily multi-vitamin/mineral supplement without iron is suggested. Supplementation of any kind, however, should not take the place of a healthy, well-balanced, nutrient dense diet or adequate chelation.
- Though in-vitro evidence supports the scientific legitimacy of wheatgrass as a supplement, the in-vivo evidence on the efficacy of wheatgrass in contemporary patients with thalassemia is equivocal. For patients who choose to consume wheatgrass, a reputable source of wheatgrass (USP or other pharmaceutical grade) is recommended. Further research studies, performed in larger numbers of patients of differing ages and with consistent methodologies, would be necessary to determine efficacy and support any recommendation of broad scale use. There is currently no research to establish the efficacy or safety of wheatgrass in patients with PKD.
- There are many proven benefits of physical activity in the general population, although research to support benefits in people with thalassaemia have been limited to observational studies where activity was linked with improved quality of life, less pain, reduced serum ferritin and higher bone density.
- Factors that may contribute to reduced activity in thalassaemia and PKD include pain, anaemia, fatigue, deconditioning, and decreased exercise capacity.
- Given the potential for benefit, strategies to promote non-contact, weight bearing activity (e.g. walking regimes) should be encouraged in people with thalassaemia and PKD at all ages, after consultation with the patient's haematologist and/or cardiologist before initiating an exercise regime.
- Physical activity should not take the place of adequate chelation or optimal nutrition, but considered a synergistic activity for health promotion.
- More research is needed to determine the types of activities, including the frequency and magnitude of activity that promote the greatest benefit while avoiding injury.

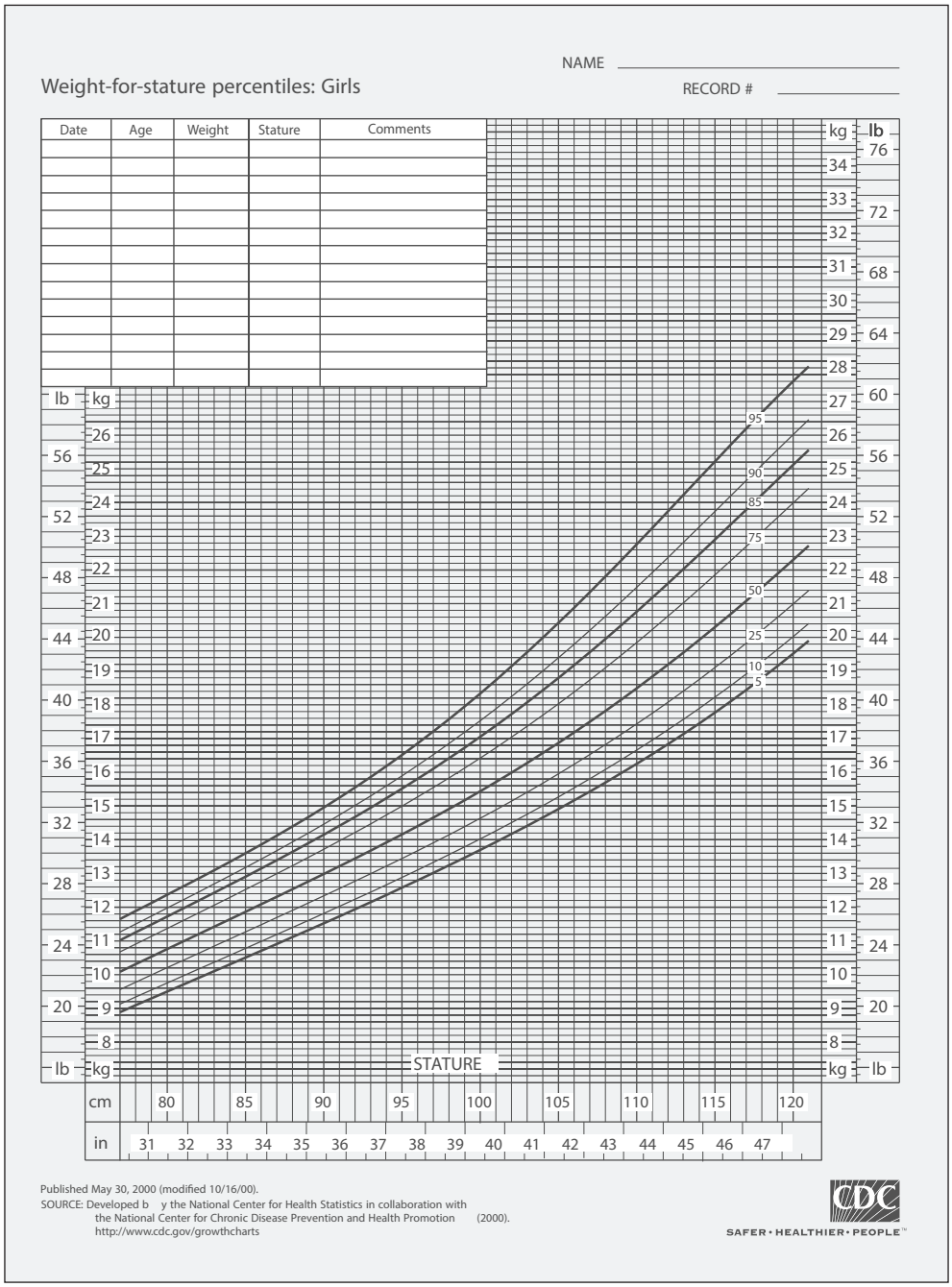
A detailed table of general and specific nutritional recommendations, including levels of evidence, can be found in Appendix 12.6.

CHAPTER 12
Appendices

12.1a: *Example of growth centile chart (boys)*



12.1b: *Example of growth centile chart (girls)*



12.2: *Table of suggested nutrition and clinical monitoring guidelines for patients with transfusion dependent thalassaemia*

All congenital anaemias require careful monitoring to detect the effects of anaemia, possible iron overload and the adverse events related to blood transfusion. The appearance of complications will vary according to the severity of the condition in each individual patient and the treatment that is necessary, including the frequency of transfusions and the need for iron chelation. Suggested schedules for haematological and microbiological parameters are outlined in the TIF guidelines https://thalassaemia.org.cy/publications/tif-publications/guidelines-for-the-management-of-transfusion-dependent-thalassaemia-4th-edition-2021-v2/

Growth/ Development	Test	Frequency	Adequacy
Weight, kg	Anthropometry	Monthly (Tx), Quarterly (Non-Tx) Quarterly (PKD)	>10th & < 90th centile
Height, cm	Anthropometry	Quarterly in youth till reach adult height then annually in adults	>10th & < 90th centile
Sitting height, cm	Anthropometry	Every 6 months till reach adult height	
Body mass index, kg/m^2	Calculation	Every 6 months in youth Annually in adults	>10th & < 90th centile
Head Circumference	Anthropometry	Every 6 months in youth	>10th & < 90th centile Monitor Change particularly < 2 yr
Growth velocity, cm/year	Calculation	Every 6 - 12 months till reach adult height	Infancy: 23-28 cm/yr Childhood: 5-6.5 cm/yr Puberty: 8.3 cm/yr (girls), 9.5 cm/yr (boys)
Puberty: tanner staging	Physical assessment	Every 6 - 12 months Starting at 8 till tanner 5	Tanner 2 by age 13 yrs in females Menstruation by age 16 yrs in females Tanner 2 by age 14 yrs in males
Bone Age	Hand wrist X-ray	Annually Starting at Tanner 2	Within 2 SD of chronological age
LH, FSH, Sex Hormones	Serum	Annually Starting at Tanner 2	Age and gender dependent
IGF-1, IGF-BP3	Serum	In patients with growth delay	Age and gender dependent

Nutritional Laboratories			
Albumin	Serum	Annually	3.5 – 5.0 g/dL
Calcium	Serum	Annually	8.5-10.5 g/dL, corrected for albumin when necessary
	Urine	Annually in adults, 24 hour sample calcium/creatinine (preferred)	24hr Ca/Cr ratio < 0.21 mg/mg or < 4 mg/kg/day
Folate	Serum or Red Blood Cell	Annually	>5.9 ng/mL >366 ng/mL
Vitamin C-Ascorbate	Plasma, Serum or RBC Ascorbate	Annually or more frequently if unsatisfactory response to chelation	Serum: > 0.4 mg/dL
Vitamin D, 25-OH	Serum	Every 6 months	30 - 50 ng/mL
Vitamin E- α tocopherol	Serum or Plasma (protect from light)	Annually	Age & gender dependent
Vitamin K	Serum or Plasma Phylloquinone (F)	Annually	0.13 – 1.19 ng/mL
Copper	Serum (TE, F)	Annually	> 70 ug/dL
Caeruloplasmin	Serum	Annually	Age & gender dependent
Magnesium	Serum (F)	Annually	Age & gender dependent
Selenium	Urine	Annually	10 – 35 ug/g creatinine
Zinc	Serum (TE,F)	Annually	>70 ug/dL
Diabetes, other endocrine and nephrolithiasis monitoring			
Glucose, fasting	Plasma	Annually	< 98 mg/dL
Insulin, fasting	Plasma	Annually	< 9 ug/mL
Oral glucose tolerance test (OGTT)		Initial assessment at 10 years, Annually if impaired glucose tolerance observed	0 min <100 mg/dL 60 min < 200 mg/dL 120 min <140 mg/dL
Fructosamine	Diabetic Patient Monitoring Only	Quarterly	< 400 mmol/L
Parathyroid Hormone (PTH), intact	Serum	Annually Starting at age 9 years	15 – 65 pg/mL

Thyroid Stimulating Hormone (TSH)	Plasma	Annually Starting at age 9 years	Adults 0.4 - 4.0 mU/L
Thyroxine Free (Free T4)	Serum or Plamsa	Annually Starting at age 9 years	Adults 0.9 - 1.7 ng/dL
Early morning cortisol	Serum	Annually starting at age 16 years	level < 3 ug/dl confirms adrenal insufficiency, if level 3 – 15 ug/dl proceed to corticotrophin stimulation test
Inorganic Phosphate	Serum or Plasma	Annually Starting at age 9 years	Adults 2.8 - 4.5 mg/dL
Alkaline phosphate	Serum	Annually Starting at age 9 years	Age and gender dependent
Uric acid	Serum or Plasma	Annually	Adults 3.5 - 7.2 mg/dL
Calcium, urate, citrate	Urine, 24 hour	Annually	24hr Ca/Cr ratio < 0.21 mg/mg or < 4 mg/kg/day
Other Assessments			
Bone Densitometry (DXA)		2-3 yearly from age 10	BMD Z-score > -2.0
Review of Dietary Intake		Annually	

Adapted from: Standards of Care Guidelines for Thalassemia, Children's Hospital & Research Center, Oakland, 2012,De Sanctis, 2013, Cappellini et al TIF guidelines 2021.

^adequacy of growth is dependent upon the child's genetic potential for growth determined by the mid-parental height index. Growth should be monitored regularly until the youth reaches adult height.

Height loss should be monitored in adult patients annually to assess spine health

Tx: Transfused patient; Non-Tx: non-transfused patient

TE: all trace elements need to be collected into trace element free vacutainers.

F: it is important that these are collected in the fasted state.

For transfused patients, best to draw laboratory values prior to transfusion, (e.g. morning of).

May be possible to use Red Blood Cell folate in the non-transfused patient as it is typically a better indicator of tissue stores than serum folate.

If age to begin assessment is not specified in table able, should be according to the discretion of the clinician

Slightly lower fasting glucose and insulin levels are suggested for thalassaemia patients given their high risk of diabetes (Noetzli et al 2012)

These are suggested normative ranges, though reference range may be slightly different at local clinical laboratories

12.3: Table of suggested monitoring for patients with PKD

	Chelation therapy	6+ transfusions within the last year and not on chelation therapy	5 or fewer transfusions within the last year and not on chelation therapy
Blood tests			
Iron levels (ferritin)	Every visit	At least every 3 months	Yearly
Bilirubin Complete blood count (CBC) Reticulocytes (new red blood cells)	Every visit	Every visit	Every visit
Vitamin D levels	Yearly	Yearly	Yearly
HIV Hepatitis B and C	Yearly for people who have had a transfusion	Yearly for people who have had a transfusion	Yearly for people who have had a transfusion

	6+ transfusions within the last year	5 or fewer transfusions within the last year
Heart, liver, and bone scans		
Heart and liver MRI	Yearly	These scans should be done yearly if your iron levels are more than 500 ng/mL You may need additional assessments depending on the initial results
	Children, especially young children, may not be able to stay still for an MRI without sedation. Talk to your doctor about the risks that sedation can pose versus the risk of not being able to conduct these tests.	
Bone density test (DXA scan)	Your doctor will schedule an initial scan at about the age of 18 You may need regular scans depending on what this first assessment reveals	

Lab tests: Normal ranges for adults*			
Ferritin	Females (ages 18-39): 10 to 120 ng/mL	Females (ages 40+): 12 to 263 ng/mL	Males (ages 18+): 20 to 250 ng/mL
Total bilirubin	Less than 1.2 mg/dL		
Reticulocytes	0.5 to 2.5%		
Vitamin D levels	Some experts recommend levels of 20 to 40 ng/mL, while others recommend 30 to 50 ng/mL		
DXA scan	T-score of -1 or higher		

*Different labs may have different definitions of "typical" or may use different measurements. Talk to your doctor about the meaning of your results.

Adapted from: Agios Pharmaceuticals. Monitoring your PK deficiency over time. Available at: https://www.knowpkdeficiency.com/pdf/hcp-refresh/PKD-Patient-Monitoring-Flashcard-PKD-US-0651.pdf.

12.4: Suggested monitoring of iron overload in patients with thalassaemia

	Test	Frequency	Adequacy
Serum ferritin	Blood test	1-3 months	< 1000 ug/l in TDT < 800 ug/l in NTDT
Liver iron concentration (LIC)	MRI using validated technique	stable level 3-7 mg iron/g dry weight : 1 – 2 yearly, > 7 mg/g : yearly, rapidly falling or < 3 mg/g : 6 to 12 monthly. Consider transient elastography as an indicator of fibrosis	1.8 mg/g dry weight (wt), up to 7 mg/g dry wt
Myocardial iron estimation	MRI T2*	From age 10. 2 yearly if result > 20 ms on last test, yearly if < 20 on last test, 6 monthly if < 10 ms on last test	>20ms

12.5: Notes on monitoring of patients with NTDT and PKD not on regular transfusion

The frequency of clinical and laboratory monitoring for these patients depends on the severity of the condition.

- In children close monitoring is essential until it is decided whether regular transfusions are going to be required. After that, those with a more severe phenotype and lower haemoglobin may need 3 monthly monitoring, whereas older children and adults with milder conditions and a well-maintained steady state haemoglobin may only require annual assessments. All should have the facility for immediate access for assessment if they become symptomatic between booked reviews.
- Clinical review of facial bone development, dental problems, and growth and liver and spleen size are especially important in the early years, as they may impact on decisions to transfuse.
- Clinicians also need to check for ankle ulceration, and be vigilant for any symptoms or signs which could indicate the presence of extra-medullary haematopoietic masses especially in the paravertebral areas causing neurological problems.
- All the same elements need to be monitored, as summarised above for those with TDT, noting that
  - ferritin levels may underestimate liver iron levels, especially in those with NTDT.
  - iron loading is milder and later in those who have had only occasional transfusion, or none, so cardiac iron assessments usually start later ~ age 15.
  - echocardiograms need to check especially for evidence of pulmonary hypertension [recording tricuspid regurgitant jet velocity].

- liver iron quantitation by MR usually starts from age 10, repeated 2 - 5 yearly or more frequently if abnormal.
- DEXA bone density scans typically start from age 15 and are run 5 yearly or more frequently if abnormal

12.6: *General and specific nutrient recommendations for patients with thalassaemia and PKD*

General Recommendations					
	RDA	**Recommen-dation**	**Level of Evidence[b]**	**Possible Adverse Effects**	**Comments**
Nutrition Assessment	n/a	Individualized		n/a	Annual comprehensive assessment including ABCDs.
Calories	Age, gender dependent	Individualized	Weak to Moderate	Excess caloric intake may lead to obesity. Limited caloric intake associated with poor growth.	Caloric needs may be increased, monitor closely especially during periods of rapid growth. Focus on foods with high nutritional value, nutrient dense foods.
Protein	0.8 g/kg (adults)	0.8 g/kg (adults)	Weak to Moderate	Excess protein associated with nephrolithiasis. Limited protein intake associated with poor growth.	Focus on high quality protein containing all essential amino acids.
Fiber	25 - 38 g/day (adults)	25 – 38 g/day (adults)	Weak	High fibre, low fluid intake may lead to constipation.	High fibre diets help with maintaining blood glucose. Ensure adequate hydration with high fibre diet. Increasing dietary fibre too quickly may result in intestinal gas and bloating.
Fluids	Depends on age, gender, size, activity level and environment.	72 oz (women) 104 oz (men)	Weak	Inadequate fluid intake associated with fatigue, constipation, altered kidney and digestive function.	If consuming anything other than water, non-sugar sweetened beverages should be encouraged.

Specific Nutrient Recommendations					
Nutrient	**RDA (UL)[a]**	**Recommendation**	**Level of Evidence[b]**	**Possible Adverse Effects**	**Recommended Assessment Strategy & Comments**
Folate	200-400 mg (400-1000 mg)	1000 mg daily	Weak	None Reported	Annually Serum Folate: > 3 ng/mL
Vitamin B-12	2.4 ug (no evidence)	Only if deficient	Weak	None Reported	Serum B-12, fasting >200 pg/mL
Vitamin C	25-90 mg (650 -2000 mg)	100 mg daily when deficient	Moderate	May mobilise iron if not taken in combination with chelator (Thal)	Annually, more frequent if unsatisfactory response to chelation For Tx dependent patients, ensure adequate chelation while supplementing with Vit C Plasma Ascorbate: > 0.4 mg/dL
Vitamin A	700-900 µg RAE 2,333-3,000 IU (3,000 µg RAE)	Only if deficient	Weak	None Reported	Plasma or serum retinol, fasting >30 ug/dL
Vitamin D	600 IU (3000-4000 IU)	50,000 IU weekly[c]	Strong/ Moderate	None Reported	Every 6 months Serum 25-OHD: 30 - 50 ng/mL
Vitamin E	7-15 mg (300 - 1000 mg)	200 mg daily if deficient	Moderate	None Reported	Annually Serum α & γ tocopherol: Reference values age and gender dependent
Vitamin K	90 – 120 µg (none available)	Only if deficient	Weak	None Reported	Special attention should be paid to vitamin K status for patients on anticoagulants and antibiotics
Copper	0.9 mg (10 mg)	Only if deficient	Weak	None Reported	Copper absorption may be reduced with high dose zinc supplementation Annually Serum Copper TE Free Fasting: >70 µg/dL
Magnesium	310 – 420 mg (350 mg-supplements only)	Only if deficient	Weak	None Reported	Annually Serum Magnesium: <1.8 mg/dL
Selenium	30-55 ug (150-400 ug)	100-200 ug daily if deficient	Weak	GI Reaction	Annually Serum Selenium: 70 – 150 ng/mL
Zinc	5-11 mg (12 - 40 mg)	20-25 mg daily	Strong/ Moderate	Nausea[d]	Annually or 6 monthly if on iron chelation. Serum Zinc, TE Free Fasting: >70 µg/dL

[a] RDA=Recommended Dietary Allowance. RDA is assigned by the Institute of Medicine (IOM) within the US and is the average daily level of intake sufficient to meet the nutrient requirements of nearly 98% of healthy individuals. RDA's listed here are based on recommendations for healthy females and males 19+ years.

UL: Upper Limit, the tolerable upper limit set by the IOM above which adverse effects have been observed in healthy populations, the range is provided for healthy females and males, 19+ years of age.

[b] Levels of Evidence generated from the 2016 publication by the ASPS available at: https://www.plasticsurgery.org/documents/medical-professionals/quality-resources/ASPS-Evidence%E2%80%90Based-Clinical-Practice-Guideline-Methodology.pdf. Strong: represents a recommendation based on two or more "high" quality studies with consistent findings, where benefit to the patient is evident. Care providers should implement these interventions if possible, vast majority of well informed patients would implement this patient-care strategy. Moderate: two "moderate" quality studies or one "high" quality study, with consistent findings. Care providers should consider these recommendations for a patient, but intervention is not paramount. Weak: evidence from one or more low quality studies, or a single moderate quality study. Benefit is unclear.

[c] Supplementation with 50,000 IU/week should continue until serum 25-OHD reaches 30 ng/mL. After reaching this threshold, a lower maintenance dose should be provided. 25-OHD should not be maintained above 50 ng/mL

[d] Nausea was frequently related to zinc provided as zinc sulfate. Alternate formulations may be advised to reduce potential nausea.

RAE: retinal activity equivalents

ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION (TIF)

Thalassaemia International Federation (TIF), a non-governmental, patient driven umbrella organisation, established in 1986, supports today, the rights of patients for access to quality health, social and other care through its work with over 200 national thalassaemia associations in 62 countries across the world. It was founded by a small group of doctors and patients/parents who represented National Patient Associations, mainly from Cyprus, Greece, Italy, UK and USA i.e. Countries where thalassaemia had been recognized early as a genetic, hereditary disorder with huge medical, public, health, social and economic repercussions if left unaddressed in terms of both effective prevention and management. Thus, these were the countries where strong research activity was initiated and the first control programmes were implemented in the early 1980s, with measurable success. The rationale of these founding members lay on the establishment of an international umbrella organization to build on the accumulated experience and the knowledge gained, aiming to support the efforts of the other countries since in the mid-1980s the worldwide prevalence of the disease had been well verified.

Our Mission: The prioritisation of thalassaemia on national health agendas and the development and implementation of effective disease-specific control (prevention and clinical management) programmes within national healthcare systems based on universal coverage

Our Vision: To support the provision of equal access of every patient with thalassaemia to high quality health, social and other care in a truly patient-centred healthcare setting

Our Values: Transparency, reliability, ethos, accountability, independence and patient-centredness

Our Work:
- Education • Advocacy • Collaborations/ Networking
- Research • Raising Awareness

Our Partners:
- World Health Organisation:
- United Nations: in special consultative status with the United Nations Economic and Social Council (ECOSOC) since 2017
- Council of Europe: participatory status in the Conference of International NGOs since 2019
- European Union:

Our Motto: Unity & Knowledge constitute our Strength!

THALIA

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