Expert Opinion

Growth hormone: does it have a therapeutic role in fracture healing?

Gui Tong Tran, Joseph Pagkalos, Evgenios Tsiridis, Amir Ali Narvani, Manolis Heliotis, Athanasios Mantalaris & Eleftherios Tsiridis†
†*Leeds General Infirmary, Leeds, UK*

Background: The role of growth hormone (GH) in augmenting fracture healing has been postulated for over half a century. GH has been shown to play a role in bone metabolism and this can be mediated directly or indirectly through IGF-I. *Objectives*: The use of GH was evaluated as a possible therapeutic agent in augmenting fracture healing. *Method*: A literature search was undertaken on GH and its effect on bone fracture healing primarily using MEDLINE/OVID (1950 to January 2009). Key words and phrases including 'growth hormone', 'insulin like growth factor', 'insulin like growth factor binding protein', 'insulin like growth factor receptor', 'fracture repair', 'bone healing', 'bone fracture', 'bone metabolism', 'osteoblast' and 'osteoclast' were used in different combinations. Manual searches of the bibliography of key papers were also undertaken. *Results*: Current evidence suggests a positive role of GH on fracture healing as demonstrated by *in vitro* studies on osteoblasts, osteoclasts and the crosstalk between the two. Animal studies have demonstrated a number of factors influencing the effect of GH *in vivo* such as dose, timing and method of administration. Application of this knowledge in humans is limited but clearly demonstrates a positive effect on fracture healing. Concern has been raised in the past regarding the safety profile of the pharmacological use of GH when used in critically ill patients. *Conclusion*: The optimal dose and method of administration is still to be determined, and the safety profile of this novel use of GH needs to be investigated prior to establishing its widespread use as a fracture-healing agent.

Keywords: bone metabolism, fracture healing, growth hormone, osteoblasts

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1. Introduction

Growth hormone (GH) is a single-chain polypeptide composed of 191 amino acids, intermittently released from somatotroph cells located in the anterior pituitary gland. Its regulation is controlled via a negative feedback system involving IGF-I, somatostatins and neurological signals [1]. Among its many essential physiological roles, it increases insulin-like growth factor I (IGF-I) secretion [2], which is primarily released in the liver [3] but also in local tissue sites such as adipocytes [4] and various skeletal sites [5,6].

GH and IGF have well-known effects on bone metabolism, although the mechanisms have yet to be fully elucidated. Many studies have shown that GH mediates its effect on bone via IGF-I, a theory termed the somatomedin hypothesis [7-9]. However, a subsequent study demonstrated the direct effect of GH on long bone proliferation following local injection into rat tibia [10], and this finding was further supported by other *in vivo* and *in vitro* studies [11-14]. These two contrasting findings

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were reconciled by the dual effector theory, put forward by Green and colleagues [15], which suggests that GH and IGF-I acted on different stages of the cell cycle. This theory remains controversial, as GH and IGF-I have both been shown to have synergistic effects on bone growth [16] and no additive effect [17]. Nevertheless, it has been demonstrated that GH can mediate effects on long bone via both IGF-I-dependent and -independent means [18].

GH has been shown to directly and indirectly affect bone metabolism. It stimulates osteoblast differentiation/proliferation [19,20] and positively influences osteoclast activity [21,22]. Furthermore, evidence on the pivotal role GH plays in bone metabolism can be observed in GH-deficient models. Hypophysectomised rats demonstrate an increase in bone formation markers upon administration of GH [21] and treatment of GH-deficient patients led to an increase in bone-remodelling activity combined with net gain in bone mineral density (BMD) [23]. Similarly, an increase in bone turnover was observed in elderly adults when administered with the GH secretagogue MK-677, which increases the release of GH [24].

IGF-I may act through endocrine and paracrine/autocrine routes. It increases osteoblast activation [25] as well as proliferation [26] and has been shown to inhibit collagenase activity when endogenously administered [27], increase mRNA osteoblast markers [26], and optimise the environment for bone mineralisation to occur [28]. IGF-I may also activate osteoclast activity [29]. Furthermore, IGF-I levels in humans were shown to be positively associated with BMD, following correction of confounding factors [30]. Such anabolic activity may be partly explained by IGF-I's ability to enhance ascorbic acid uptake in osteoblasts, an essential component for differentiation and collagen synthesis [31].

1.2 Physiology and biochemistry of the two mechanisms by which GH may affect bone metabolism

1.2.1 Mechanism 1: direct GH action

The physiological and biochemical effects of GH may be explained by the direct activation of various signalling pathways [32] upon binding to its membrane receptor GHR, which is expressed by chondrocytes and osteoblasts [33,34]. Such pathways are mediated by the phosphorylation of the enzyme Janus kinase 2 (JAK2), which can in turn lead to activation of two other enzymes, namely MAPK and phosphoinositide 3-kinases (PI3K), and their corresponding cascades (**Figure 1**). These pathways then go on to influence, among others, bone morphogenetic protein (BMP)-induced osteoblast differentiation [35,36], thyroid hormone-induced osteocalcin synthesis [37] and BMP-2 gene expression [38], which play a pivotal role in bone metabolism [39]. Additionally, GH-induced JAK2 may lead to tyrosyl phosphorylation, and therefore activate members of the signal transducer and activators of transcription (STAT) family 1, 3, 5a and 5b, which are involved in gene transcription. STAT 1 and 3 may then go on to mediate the metabolic effects of GH on bone by activating the proto-oncogene *c-fos*, which is associated with cell growth in response to GH [40,41]. Interestingly, GH-induced MAPK and PI3K have also been shown to influence *c-fos* expression by phosphorylating the transcription factor CCAAT/enhancer binding protein β (C/EBPβ), which is a vital component in the GH-*c-fos* pathway [41]. STAT 5 is thought to be involved in inducing the transcription of IGF-I. However, despite this knowledge, exactly how GH can affect bone metabolism remains unclear. Indeed, GH activation of the MAPK pathway appears to be cell-specific [42], and its specific mechanism on osteoblasts and osteoclasts has yet to be fully elucidated.

Among its many physiological functions, GH has been shown to induce growth hormone binding protein (GHBP), a molecule that binds to 40 – 50% of circulating GH under normal physiological conditions [43]. Upon binding of GH to GHR in humans, GHBP is produced as a result of proteolytic cleavage of the extracellular domain of the receptor by TNF-α converting enzyme (TACE) [44,45], a zinc-dependent metalloprotease. The function of GHBP remains largely unclear, but it is thought to enhance GH half-life and inhibit GH binding to GHR, cell proliferation and IGF-I production (**Figure 1**) [46].

1.2.2 Mechanism 2: indirect action mediated via IGF-I and IGFBPs

GH has been shown to enhance IGF-I gene transcription *in vivo* [47], but the manner in which this occurs has only recently been postulated [48]. GH may induce STAT5b homodimerisation via the JAK2 pathway, which then proceeds to translocate into the nucleus and in turn binds to a DNA segment, termed HS-7. HS-7 may then proceed to activate IGF-I promoters. This has been substantiated by further studies demonstrating an increase in IGF-I in response to GH and reduced serum IGF-I levels in mice carrying *gh* receptor mutations [2,40]. However, in a study involving STAT5 knockout mice, bone trabecular remodelling appeared to be normal, suggesting that its role in bone cells is not yet clearly understood [49].

GH has also been shown to indirectly affect the metabolism of bone by modulating the activity of 1α-hydroxylase and 24-hydroxylase, key enzymes in the production of 1,25-dihyroxyvitamin D_3, possibly via the action of IGF-I [50]. Interestingly, 1,25-dihyroxyvitamin D_3 has also been shown to increase the concentration of IGF-I receptor IGF-IR in osteoblasts [51].

1.2.2.1 IGF-I

Binding of IGF-I and IGF-II to IGF-IR is postulated to influence cell proliferation and differentiation through a complex series of intracellular pathways [52]. IGF-I binds to IGF-IR with greater affinity than IGF-II, although mutational models have not been able to completely elucidate the binding sites involved [53]. Nevertheless, it has been shown that each

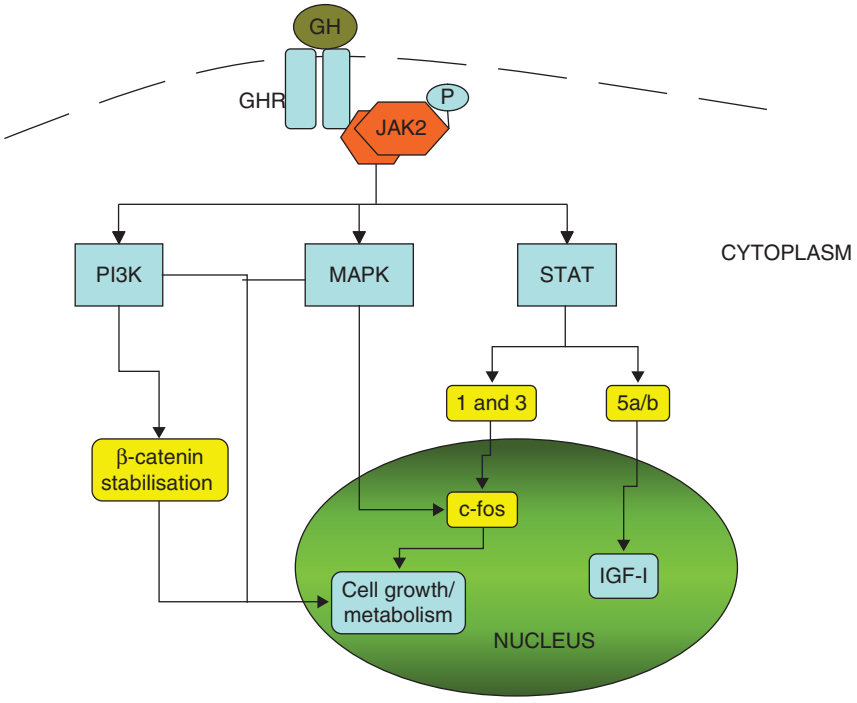

Figure 1. The direct mechanism by which GH induces cell metabolism and IGF-I production.

protein possesses individual unique activities and these cannot be compensated for by the other. Once bound to IGF-IR, autophosphorylation occurs at different tyrosine residues, which may proceed to activate the rat sarcoma (Ras)-MAPK signalling pathway. Also, upon autophosphorylation of IGF-IR, phosphorylation of other substrates occurs. This includes insulin receptor substrate-1 (IRS-1), a protein that may combine with other molecules containing SH2 domains, such as PI3K; GRB-2, which may go on to influence the Ras-MAPK pathway [54]; and Syp, which is involved in mitogenic signal transduction [55] and may act as an adaptor between growth factors and GRB-2 complexes [56]. In addition, IGF-I enhances calcium absorption [57] and has been shown to stabilise β-catenin [58], which is a component of the Wnt signalling pathway necessary for osteoblastogenesis [59]. Interestingly, IRS-1 is able to bind to β-catenin, leading to nuclear translocation and activation of genes that may induce mitogenesis and cell growth (**Figure 2**) [60].

1.2.2.2 IGFBPs: regulators of IGFs and direct effectors on bone metabolism

The physiological mechanism of IGF on bone metabolism has been further elucidated by the discovery of IGF interaction with IGF binding proteins (IGFBP), a family of six proteins that bind to 99% of circulating IGFs [53]. GH has been shown to influence levels of IGFBP, and these proteins in turn have been shown to both enhance and repress the activity of IGFs. GH may increase the levels of IGFBP-2 in chondrocytes [61], a protein that has been postulated to either inhibit [62] or enhance [63] IGF action, dependent on experimental conditions. GH administration has also been shown to increase levels of IGFBP-3 [64], a binding protein which, when combined with IGF, increases cortical bone formation in ovariectomised rats [65]. However, there has been *in vitro* evidence to suggest that IGFBP-3 may inhibit the IGF-I pathway in a dose-dependent manner, albeit independently of IGF-I [66,67]. In circulation, IGF-I forms a 150 kDa ternary complex with IGFBP3 or IGFBP-5 alongside an acid labile unit (ALS) [68] and, interestingly, GH has been shown to stimulate transcription of the ALS gene by the binding of STAT5a/b to the gene promoter site [69]. The function of ALS is not yet completely understood, but it is thought to be necessary for extending the half-life of IGFs and responsible for maintaining the high levels of serum IGF-IGFBP complexes [70].

Similarly, GH may decrease IGFBP-4, which may go on to inhibit IGF action by preventing its binding to IGF-receptor (IGFR) [71]. However, *in vivo* evidence suggests that, upon systemic IGFBP-4 administration, IGF-I levels are increased. This may be a result of IGFBP-4 proteolysis by the protease, pregnancy-associated plasma protein A (PAPP-A), found to be released by different cells including osteoblasts [72]. Proteolysis increases free IGF-I, leading to enhanced bone formation [73,74]. IGFBP-5 is the most abundant binding protein found in bone and the administration of GH has been observed to increase mRNA levels of IGFBP-3 and IGFBP-5 [75]. However, the role of IGFBP-5 in bone metabolism remains controversial. Studies have shown that it inhibits IGF function, possibly as

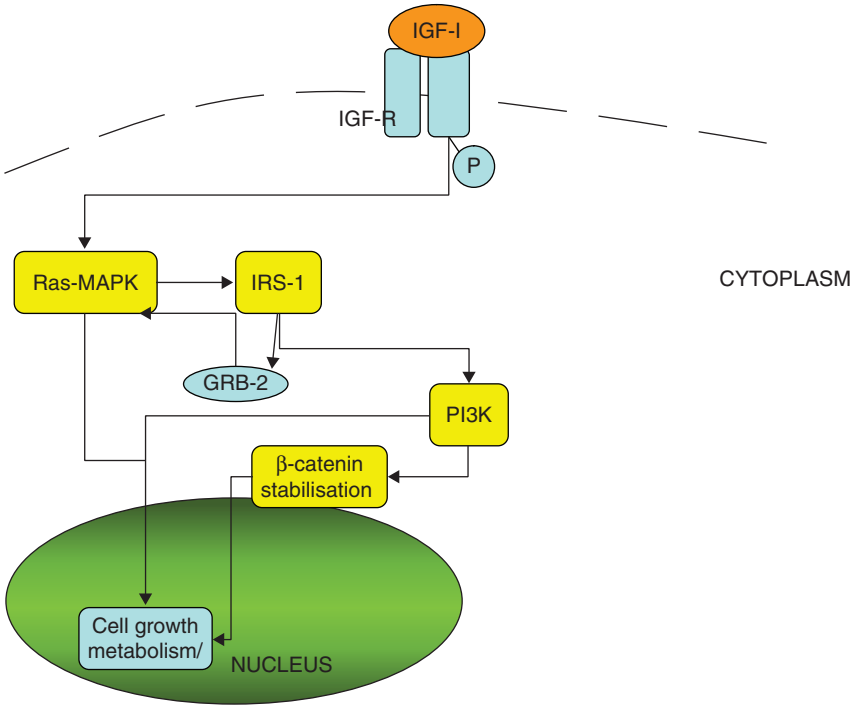

Figure 2. The mechanism by which IGF-I causes cell growth.

a result of sequestering IGF [76]. Interestingly, in the same study, IGF may impede IGFBP, resulting in prolongation of its bioeffectiveness. Other studies have shown the stimulatory effects of IGFBP-5 on bone cells [77,78], or even via an IGF-independent pathway [78]. Despite the contentious results regarding the role of IGFBP, there remains firm evidence that GH influences the expression of these proteins, and these then go on to play a pivotal role in the mechanism of IGF function and hence bone metabolism. IGFBP-6 is a specific binding protein for IGF-II, and so its role in relation to GH will not be considered here [79].

2. Results

Selected bibliographies regarding the evidence of GH on bone healing *in vitro* (**Table 1**) and *in vivo* (**Table 2**) animal studies have been presented. There have been few human studies undertaken to evaluate the direct effect of GH and fractures, with most used to determine its indirect effect, such as functional outcome (**Table 3**).

2.1 *In vitro* studies

2.1.1 GH on osteoblasts

GH's effect on osteogenesis *in vitro* was demonstrated in the late 1980s, with several studies showing increased chondrogenesis and osteogenesis in chondrocyte progenitor cells [12], as well as increased mitogenic effects and DNA synthesis on osteoblasts [80,81]. Since then, other studies have been published attempting to give an insight into the exact mechanism of this effect. Studies in cartilage progenitor cells have demonstrated the activation of JAK2 tyrosine kinase after binding of GH to the GH receptor, and the subsequent phosphorylation of GHR and JAK2 after its activation [82]. The phosphorylation and DNA binding of STAT5 after culturing rat osteoblast-like cells with r-hGH was demonstrated in 2000 by Gerland *et al.* [83]. GH also influence the extracellular signal-regulated kinase pathway (ERK1/2) and MAPK pathways that are essential for the function, growth and differentiation of osteoblasts [35,84-86]. Growth hormone has also been shown to induce the proliferation of human osteoblast-like cells, as well as increase the levels of alkaline phosphatase (ALP) and procollagen type 1 carboxy-terminal propeptide (PICP), both indicators of osteoblastic differentiation [11].

The effect of GH on *in vitro* osteogenesis and expression of mRNA in osteoblastic cells obtained from alveolar bone fragments from healthy women of three age groups (adolescents, young adults, and adults) was recently studied [87]. The authors used an osteogenic medium in which the cells were cultured, in contrast to previous studies [11,80] where non-osteogenic medium was used. Furthermore, the cells were cultured for a total of 21 days for alarazin red mineral quantification staining assays to be assessed, which was a significantly longer period compared with previous studies where culture periods were as short as 24 h [11]. The authors concluded that the GH effect on culture growth, ALP activity, collagen synthesis, mineral staining and mRNA expression of osteoblast markers was age-dependent. Cells from adolescents and young adults cultured with GH showed a significant

Table 1. *In vitro* studies.

Study	Cell model	Agent	Method	Measurements	Results	Conclusion
Kassem 1993 [11]	Human osteoblast-like cells	hGH	H-Thymidine incorporation assay: cells incubated for 24 h with 1% bovine serum albumin in the presence of hGH or vehicle. BrdUrd assay: 24 h after plating, the medium was replaced with serum-free medium containing hGH or vehicle, with 10 nM of BrdUrd. At 2, 8, 24 and 48 h the medium was drawn off and the cells fixed and stained for BrdUrd. Further assays for alkaline phosphatase activity, osteocalcin, collagen type I PICP	Cell proliferation Biochemical markers of differentiation: alkaline phosphatase, osteocalcin, type I collagen PICP. IGF-I, IGF-II, IGFBP-3	hGH stimulated hOB proliferation in a dose-dependent manner (min 0.1 ng, half-maximal 10 ng). Increased ALP activity, in a dose-specific way, increased PICP levels	hGH stimulated proliferation in subconfluent cultures and biochemical markers of differentiation. hGH did not cause significant IGF-I, IGF-II of IGFBP-3 level changes
Rubin 2002 [109]	ST2 murine stromal cells	IGF-I	ST2 cells were plated in a α-MEM media and penicillin/streptomycin and glutamine. One day later, 10 nM vitamin D was added if noted and 24 h later IGF-I was added for specified times. Northern probe for OPG and RANKL was used for PCR. For the half-life study of OPG, actinomycin was added to cells that had been treated with IGF-I for 24 h, and total RNA was collected at specified times. Real-time PCR for RANKL and 18S was performed and ELISA was used to evaluate OPG.	OPG and RANKL protein levels and mRNA expression, 18S mRNA levels	OPG protein and mRNA are decreased in the presence of IGF-I, with the effect being significant at 24 h and more profound at 48 h; the effect was transcriptional, as OPG mRNA half-life was unaffected. IGF-I has a stimulatory effect on RANKL expression that is significant at 100 and 200 µg/l	IGF-I appears to stimulate bone resorption by acting on the OPG/RANKL equilibrium through stromal cell expression of these two molecules
DiGirolamo 2007 [86]	Osteoblasts from calvaria of newborn $Igf1r^{flox/flox}$ or ROSA26 reporter mice	hGH	Cells infected with adenovirus-encoding AdCre in the absence of serum: IGF-IR deletion > 90% confirmed with real-time PCR and immunoblotting. Cells treated by GH and IGF-I. LacZ staining, cell lysis and immunoblotting analysis, quantitative real-time PCR. Osteoblast proliferation and apoptosis assays *In vivo* treatment of ΔIgf1r mice ($Igf1r^{flox/flox\ Cre+}$) and control littermates ($Igf1r^{flox/flox}$) with 3 µg/g body weight/day for 7 days	IGF-IR and GHR levels, p-STAT5, total STAT-5, p-Akt, total Akt, p-ERK1/2, total ERK, IGF-I and IGFBP-3 mRNA, osteoblast number, apoptosis (immunoblotting for Bax and TUNEL staining)	> 90% removal of IGF-IR and preservation of GHR. Upon removal of IGF-IR: GH induced ERK and Akt response was conserved while stimulation by IGF-I was abolished. GH stimulated IGF-I and IGFBP-3 mRNA production was maintained. GH inhibition of osteoblast apoptosis was maintained. GH induced osteoblast proliferation was abolished. *In vivo* GH failed to stimulate osteoblast proliferation	The *in vitro* antiapoptotic effect of GH is independent of IGF-I but cannot acutely increase osteoblast numbers *in vivo*

AdCre: Cre recombinase; ALP: Alkaline phosphatase; BrdUrd: Bromodeoxyuridine; GH: Growth hormone; hGH: Human growth hormone; hOB: Human osteoblast like cells; OP: Osteopontin; OPG: Osteoprotegrin; PCR: Polymerase chain reaction; PICP: Carboxyterminal propeptide; RANKL: Receptor activator of nuclear factor-κB ligand.

Table 1. *In vitro* studies (continued).

Study	Cell model	Agent	Method	Measurements	Results	Conclusion
Mrak 2007 [110]	Human osteoblast- like cells	GH	Primary cultures of hOB were seeded into six-well multiwall plates and allowed to grow. After 48-h serum-free medium incubation, the cells were treated for 24 h with increasing concentrations of GH (0.1 – 10 ng/ml; 4.5×10^{-12} - 4.5×10^{-10}) and/or in the presence of pegvisomant (5×10^{-8} M) or tyrphostin AG490 (10 µM). OPG was measured using a commercial kit and IGF-I using an ELISA kit. Real-time PCR was performed to measure OPG and IGF-I mRNA levels	GH receptor identified via RT-PCR. OPG levels and mRNA expression measured with different levels of GH. IGF-I mRNA levels	GH significantly induced OPG secretion after 24 h at 1, 5 and 10 ng/ml. Real-time PCR demonstrated significant increase in OPG secretion at 6 h of incubation with 5 ng/ml GH. Both antagonists (pegvisomant and tyrphostin) inhibited the effect of GH on OPG. IGF-I levels did not significantly change after 6 and 24 h of incubation with GH	GH directly affects OPG synthesis and expression on hOB. The effect is specific and does not require the involvement of IGF-I. The OPG/RANKL equilibrium achieved under GH stimulus may determine a positive bone mass outcome
Crippa 2008 [87]	Osteoblastic cells from human alveolar bone from adolescents, young adults and adults	hGH	Cells harvested from human alveolar bone explants. Osteoblastic cells were obtained using enzymatic digestion using type II collagenase. The cells were cultures primarily and the subconfluent cells were harvested and subcultured in 24 well-culture plates (density 2×10^4 cells/well) in culture medium with or without GH	Culture growth and availability, collagen content, ALP activity, bone-like formation (image analysis and colorimetric method), gene expression analysis (real-time PCR in nine donors: ALP, OC,COL, OP, Cbfa1 genes), RNA extraction and quantitative PCR	Number of cells, ALP levels, collagen content and bone-like formation in both adolescents and young adults, but not in the adult groups, increased at 7 days of GH treatment	The effect of GH on human osteoblast-like cells appears to be donor age-specific

AdCre: Cre recombinase; ALP: Alkaline phosphatase; BrdUrd: Bromodeoxyuridine; GH: Growth hormone; hGH: Human growth hormone; hOB: Human osteoblast like cells; OP: Osteopontin; OPG: Osteoprotegrin; PCR: Polymerase chain reaction; PICP: Carboxyterminal propeptide; RANKL: Receptor activator of nuclear factor-κB ligand.

Table 2. *In vivo* animal studies.

Study	Animal model	Agent	Method	Evaluation method	Results	Conclusion
Bak *et al.* 1991 [117]	3-month-old female rats (n = 105)	b-hGH	A unilateral, standardised, closed fracture was produced in the right tibia b-hGH was given twice daily s.c. 2.7 mg/kg to three groups of rat: for the entire period (n = 11); for the first 20 days (n = 11); for the last 20 days (n = 10) Saline was given to three groups of rat: for the entire period (n = 12); for the first 20 days (n = 13); for the last 20 days (n = 11) One group (n = 11) was not treated Animals were killed at 40 days	Destructive 3-point bending to detect: maximum load; maximum stiffness; deflection and energy absorption at maximum load; load, deflection and energy absorption at failure	Maximum load and stiffness of fractures significantly increased by 165% and 175% (respectively) in treated rats for entire period and 222% and 175% (respectively) for treated rats for first 20 days, compared with control; no significant difference observed between groups when treated for last 20 days Energy absorption was also significantly increased ($2p < 0.01$) in b-hGH treated rats for initial 20 days, compared with control	Fracture healing is stimulated when administration of GH is given in the initial period of healing or during the entire period
Carpenter *et al.* 1992 [127]	Mature, normal rabbit (n = 27)	Human GH	Standardised left tibial osteotomies created and externally fixed Study group (n = 14) received daily i.m. injection of 150 µg/kg hGH; control group (n = 13) received saline One-third of each group was killed at 4, 6, 8 weeks postoperatively	*In vivo* mechanical testing to assess bone compliance at 1, 1½, 2, 2½, 3, 4, 5, 6, 7 and 8 weeks post-op or until animal was killed Load versus displacement and load to failure using 4-point bending Weekly anteroposterior radiographs Weekly serum IGF-I	No rate of change of compliance of *in vivo* testing was observed between two groups No significant effect of strength could be observed between two groups during 4-point bending at 4, 6, 8 weeks No significant difference in healing between treatment groups observed radiographically No statistically significant IGF-I serum concentration between groups	No effect on the rate or strength of healing was observed between control and test groups No significant rise in IGF-I was demonstrated
Mosekilde and Bak 1993 [126]	3-month-old female rats (n = 64)	b-hGH	A unilateral, standardised tibial fracture performed in right tibia Study group received s.c. twice-daily 2.7 mg/kg b-hGH; control group received saline Administration was for initial 20 days post-op only 5/6 animals from each group	Histological	10 days: both groups showed extensive, similar-sized callus formation; massive stimulation of osteoblast/fibroblasts by way of extensive collagen fibres was observed in b-hGH group 20 days: b-hGH group had looser structure. No modelling	Short-term: b-hGH has initial stimulatory effect on callus formation Long-term: the callus formed in groups treated with b-hGH had delayed modelling and remodelling

bGH: Bovine growth hormone; b-hGH: Biosynthetic human growth hormone; GH: Growth hormone; hGH: Human growth hormone; r-pGH: Recombinant porcine growth hormone; r-rGH: Recombinant species-specific rat GH.

Table 2. *In vivo* animal studies (continued).

Study	Animal model	Agent	Method	Evaluation method	Results	Conclusion
			were killed on days 10, 20, 30, 40, 50 and 80		of periosteal surface of callus could be seen, in contrast to saline group. b-hGH group had bone marrow cell infiltration into external callus; in saline, this contained connective tissue with vascularisation 30 days: both groups demonstrated fracture healing; b-hGH group had large callus formation; control had small, dense formation. b-hGH group contained loose trabecular networks and exhibited signs of periosteal remodelling 40 days: trabecular bone in b-hGH group was significantly higher than control 50 days: small dense callus in saline group; large loosely structured callus in b-hGH group 80 days: callus visible in b-hGH group; healing complete in control	processes, delaying normalisation of fracture bone healing
Hedner *et al* 1996 [122]	Sprague-Dawley rats (n = 93)	hGH bGH	Transosseous defects were created in rat mandibles Four groups (n = 5) were administered constant systemic delivery of 200 µg/day of hGH, bGH, prolactin or saline via osmotic minipumps for 3 weeks to determine specificity Effect of hGH 200 µg/day over time was analysed at 2, 3 and 4 weeks Four groups (n = 5) of 0.2, 2, 20 or 200 µg/day systemic hGH administered via minipumps and evaluated after 3 weeks Local delivery via catheters to site of defect of 0.2, 2 or	Histological staining	Systemic hGH administration statistically increased bone union, maturity, marrow quality and peripheral bone (bone formed outside defect) compared with bGH, prolactin and saline. bGH-administered group had a statistically increased peripheral bone formation compared with saline and prolactin. All other parameters did not statistically differ between groups Systemic hGH administration over time showed stimulatory effect on bone healing at 3 and 4 weeks, but not 2 weeks 2 and 20 µg/day systemic	GH promotes bone healing GH delivered locally can enhance bone healing

bGH: Bovine growth hormone; b-hGH: Biosynthetic human growth hormone; GH: Growth hormone; hGH: Human growth hormone; r-pGH: Recombinant porcine growth hormone; r-rGH: Recombinant species-specific rat GH.

Table 2. *In vivo* animal studies (continued).

Study	Animal model	Agent	Method	Evaluation method	Results	Conclusion
			20 µg/day group (each group n = 6) for 4 weeks		hGH-treated rats showed stimulatory effects on bone union compared with other groups; 2, 20 and 200 µg/day hGH-treated group also showed increased bone maturity and peripheral bone Local 0.2 µg/day hGH increased peripheral bone and bone union ($p < 0.05$); 2 and 20 µg/day also showed increased bone union and peripheral bone as well as marrow quality and bone maturity score ($p < 0.01$)	
Schmidmaier *et al*. 2002 [17]	5-month-old female rats (n = 80)	Recombinant human IGF-I and TGF-β1 r-rGH	A standardised closed midshaft fracture of right tibia was produced Recombinant human IGF-I and TGF-β1 were given locally via implants Group 1 (n = 20) received uncoated implants with saline; group 2 (n = 20) received implants coated with IGF-I and TGF-β1; group 3 (n = 20) received s.c. injection of 2 mg/kg r-rGH alone; group 4 (n = 20) received implants coated with IGF-I and TGF-β1 and s.c. injection of 2 mg/kg r-rGH	Posteroanterior and lateral radiographs taken weekly for 4 weeks Mechanical testing to detect torsional force at 28 days from 10 animals in each group Histomorphometry from 10 animals in each group	Radiographs revealed local application of growth factors + systemic GH showed enhanced consolidation of fracture compared with control ($p < 0.05$) Biomechanical stability: group 3 showed statistical improvement compared with group 1 ($p < 0.05$); no difference seen between groups 2 and 4 Histomorphology: less cartilage was observed in groups 2 and 4 vs control; group 4 demonstrated more mineralised tissue in callus vs other groups; more mineralised tissue in cortices of GH treated groups vs control	Local administration of growth factor and systemic GH separately stimulated bone fracture healing Local growth factor healing had a greater effect than systemic GH No additive effects were seen when GH and growth factors were given in combination
Bail *et al*. 2002 [112]	Female micropigs (n = 20)	r-pGH	Distraction of tibia and fibula for 10 days Study group (n = 10) received daily s.c. 100 µg/kg injection of r-pGH for 25 days; control group (n = 10) received saline Killing occurred at day 25	Histomorphometry and *in vivo* polychrome labelling	Significantly larger callus and bone areas observed in GH group vs control ($p < 0.05$) No significant difference in cartilage tissue formation between groups Distraction gap in GH group showed significant ossification	GH increased bone formation in callus GH does not stimulate cartilage formation GH does not change structural parameters of new bone GH results in increased bone formation

bGH: Bovine growth hormone; b-hGH: Biosynthetic human growth hormone; GH: Growth hormone; hGH: Human growth hormone; r-pGH: Recombinant porcine growth hormone; r-rGH: Recombinant species-specific rat GH.

Table 2. *In vivo* animal studies (continued).

Study	Animal model	Agent	Method	Evaluation method	Results	Conclusion
Kolbeck *et al.* 2003 [113]	Female Yucatan micropigs (n = 24)	r-pGH	Osteomised left tibia and stabilised by plate fixation Study group (n = 12) received daily s.c. 100 µg/kg injection of r-pGH for 28 days; control group (n = 12) received saline Animals sacrificed after 4 weeks	Biomechanical testing assessing yield load and torsion stiffness Histomorphometry Serum IGF-I	Treatment group exhibited 91% higher mean torsional failure load and 61% higher mean torsional stiffness vs placebo ($p = 0.001$ and $p = 0.05$, respectively) Histomorphometry revealed 68% advance for total callus area and 60% increase in mineralised bone area in treatment group vs placebo ($p < 0.005$ and $p < 0.001$, respectively) IGF levels increased in GH-treated animals and decreased in control group daily up to day 12 and remained constant thereafter. The difference between the groups was significant at day 4 ($p < 0.001$)	Systemically applied GH stimulates callus formation and ossification in early fracture healing
Theyse *et al.* 2006 [115]	Mature Labrador retrievers (n = 4)	r-pGH	Critical sized bilateral ulna bone defect resulting in non-union was created, with periosteum preservation Continuous local s.c. drug delivery via osmotic pumps with 1.8 mg/day r-pGH was administered distal from the bone defect on the right side only for 28 days for one group (n = 4); controls (n = 4) received 0.03 mol/l sodium bicarbonate in 0.15 mol/l NaCl Animals killed at 6 weeks	Standardised mediolateral radiographs on day 0, 2-weekly post-op Segmental bone biopsy to measure expression levels of IGF-I, IGF-II, and GH receptor using PCR analysis Histomorphometry Radioimmunoassay to detect plasma GH, IGF-I, IGF-II, IGFBP-4 and IGFBP-6	Radiographically: both groups demonstrated bone formation after 2 weeks; bone formation progressed after 6 weeks in GH-treated group; very little progression was seen in control group after 6 weeks Histologically: bridging of defects with trabecular bones observed in GH group; in control, defects mainly filled with fibrous tissue IGF-I expression levels was lower in GH treated group vs control ($p = 0.03$); no differences were observed in IGF-II expression or GH receptor IGF-I and IGF-II plasma concentrations were greater in GH-treated group vs controls ($p = 0.02$ and $p = 0.008$) Plasma IGFBP-4 and IGFBP-6 were did not statistically differ between study groups	Continuous infusion with GH stimulates bone formation and healing Local delivery had no additional effect on bone healing IGF-I, IGF-II, and GH receptor expression in the bone regenerate did not increase at 6 weeks post-op GH induced increase in plasma IGF-I and IGF-II IGFBP-4 and IGFBP-6 did not play a role in enhanced bone regeneration

bGH: Bovine growth hormone; b-hGH: Biosynthetic human growth hormone; GH: Growth hormone; hGH: Human growth hormone; r-pGH: Recombinant porcine growth hormone; r-rGH: Recombinant species-specific rat GH.

Table 3. Human studies.

Study	Design	Population	Number	Agent	Dose	Methods	Measurements	Conclusion
Van der Lely *et al*. 2000 [138]	RCT	Hip fracture in patients aged > 60 years, operated (ORIF)	76	h-GH	0.01 mg/kg/day increased to 0.02 mg/kg/day within 6 days. Tapered off within 6 days	Placebo-controlled, double-blinded Daily s.c. injection for 6 weeks Follow-up 6 months	IGF-I, IGFBP-1, IGFBP-3, Modified Barthel Index, living situation	Significant IGF-I and IGFBP-3 response in treatment group. Increased percentage of return to prefracture state in > 75s
Boonen *et al*. 2002 [136]	RCT	Hip fracture in women aged > 65 years, first fracture	30	rh-IGF-I / IGFBP-3	Equivalent to IGF-I: 0.1 mg/kg/day and 0.2 mg/kg/day	Three treatment groups: Placebo-controlled Continuous s.c. 8-week infusion 24-week follow-up	IGF-I, IGFBP-3, U+Es, LFTs, glucose, procollagen peptide, OCA, deoxypyridinoline, type I collagen crosslinks of N-telopeptide, bone mineral density (DEXA), isometric grip strength, functional tests	Increased IGF-I levels for the duration of treatment (dose-dependent). Increased OCA and N-telopeptide. No difference in bone remodelling/BMD. Increased grip strength and function
Weissberger *et al*. 2003 [139]	RCT	Elective THR	33	rh-GH	0.01 U/kg/day increased to 0.04 U/kg/day in 6 weeks. Double dose post-op for 2 weeks	Two treatment groups: placebo-controlled, daily s.c. injection. For 18 weeks, follow-up 18 weeks	IGF-I, glucose, HbA1c, body composition (QuCT and total body K^+), muscle strength, walking ability	Increased IGF-I, increased lean body mass pre-op, increased mid-thigh muscle cross-sectional area
Yeo *et al*. 2003 [140]	RCT	Hip fracture in women aged > 75 years, operated within 48 h	31	rh-GH	0.05 or 0.025 mg/kg/day or placebo	Three treatment groups, placebo-controlled, double-blinded. Daily s.c. injection for 14 days; follow-up 18 days	Mid-arm muscle circumference, body weight, handgrip strength, body impedance, IGF-I, IGF-BP3, urea and electrolytes, LFTs, glucose, CRP, C-peptide, insulin, FBC, HBA1C, Prefracture Barthel index of daily activities	Brisker and greater rise in IGF-I and IGF-BP3 in treatment groups. Poor IGF-I response related to low weight, BMI, MAMC, handgrip strength and Barthel index. Thromboembolic adverse events

BMC: Bone mineral content; BMD: Bone mineral density; ESR: Erythrocyte sedimentation rate; FBC: Full blood count; FPM: Functional Performance Measures; hGH: Human growth hormone; LAQ: Living assistance questionnaire; LFT: Liver function test; MAMC: Mid-arm muscle circumference; OCA: Osteocalcin; RCT: Randomised control trial; SIP-NH: Sickness Impact Profile for Nursing Homes ; TFT: Thyroid function test; THR: Total hip replacement.

Table 3. Human studies (continued).

Study	Design	Population	Number	Agent	Dose	Methods	Measurements	Conclusion
Hedström *et al*. 2004 [137]	RCT	Hip fracture in patients aged > 65 years, operated on	20	rh-GH	0.1 IU/kg/day, reduced to 0.04 IU/kg/day	Placebo-controlled, double-blinded Daily s.c. injections for 21 – 28 days. Follow-up 3 months. Study interrupted and resumed at a lower dose of rh-GH	FBC, ESR, U+Es, LFTs, TFTs, IGF-I, IGFBP-I, BMD and BMC (both DEXA and quantitative CT, thigh muscle cross-section, subcutaneous fat, muscle power (quadriceps)	Significant IGF-I response in treatment group. Retention of lean body mass, loss of subcutaneous fat, retention of total body BMC
Bach *et al*. 2004 [143]	RCT	Hip fracture in patients aged > 65 years, medically stable	161	MK-0677	20 mg MK-0677	Two treatment groups Oral administration: once daily for 6 months Follow-up for 12 months	IGF-I, IGFBP-3, glucose, insulin, HbA1c, FPM, LAQ, SIP-NH.	Increased IGF-I response in treatment group vs placebo (84% vs 14% increase from baseline). No statistically significant improvement in FPMs, LAQ or SIP-NH
Raschke *et al*. 2007 [141]	RCT	Tibial fractures, intramedullary nail fixation (open and closed)	368	h-GH	0.015, 0.03 or 0.06 mg/kg/day or placebo, titrated to maximum dose within 3 weeks	Four treatment groups; double-blinded (GH vs placebo) Daily s.c. injection up to confirmation of healing or total of 16 weeks Follow-up 12 months	Radiologic confirmation of fracture healing (disappearance of fracture line and/or cortical bridging in 3 of 4 cortices seen on AP and lateral X-rays), clinical healing, secondary procedures, IGF-I, IGFBP-3, osteocalcin, serum crosslaps, FBC, glucose, HbA1c, blood pressure	Increased IGF-I levels (especially 0.06 mg/kg group), osteocalcin and serum CrossLaps increased significantly. Accelerated healing in the 0.06 mg/kg group for closed fractures: 26% decrease in healing time. No safety issues

BMC: Bone mineral content; BMD: Bone mineral density; ESR: Erythrocyte sedimentation rate; FBC: Full blood count; FPM: Functional Performance Measures; hGH: Human growth hormone; LAQ: Living assistance questionnaire; LFT: Liver function test; MAMC: Mid-arm muscle circumference; OCA: Osteocalcin; RCT: Randomised control trial; SIP-NH: Sickness Impact Profile for Nursing Homes ; TFT: Thyroid function test; THR: Total hip replacement.

rise in collagen content (day 7), ALP activity (day 7) and culture growth (4, 7 and 10 days), while cells from adult donors were not affected. Mineralisation at day 21 increased significantly only in cells from adolescents, with similar results regarding mRNA expression. mRNA expression of genes encoding ALP, osteocalcin, type I collagen, osteopontin and core binding factor alpha 1 was increased in cells from adolescent donors, while young adult cells only showed increased ALP mRNA expression. The transcription of these genes in cells from adults did not appear to be affected by GH. This study demonstrated an age-dependent response to GH. The exact mechanism of this is not understood; whether this effect is due to alterations in IGF-I levels is not known, but may be of significance when attempting to stimulate osteoblasts in adults to enhance healing.

2.1.2 GH-induced IGF-I and osteoblasts

The question of whether or not the effect of GH on osteoblasts is mediated via IGF-I has been addressed several times in the last decade. Utilising IGF-I knockout mice and GH mice, researchers have shown markedly impaired BMD in the deficient mice, with the effect being more severe in IGF-I-deficient mice [88]. A recent study utilised calvarial osteoblasts from genetically manipulated mice, in which the IGF-IR was selectively knocked out. The study demonstrated that IGF-IR-deficient cells retained the GH-induced phosphorylation of ERK and Akt, which are components of the GH signalling pathway, as well as IGF-I and IGFBP-3 production. The GH-induced osteoblast proliferation was abolished in IGF-IR-deficient cells, but GH-mediated osteoblast apoptosis inhibition was retained. It was therefore concluded that the anti-apoptotic effect of GH appears to be IGF-I-independent, but the main anabolic effect of GH appears to be mediated via local production of IGF-I [86].

The effect of IGF-I via the Ras/Raf-1/MAPK and PI3K pathways on osteoblast action have been demonstrated utilising IGF-IR deplete (Cre/loxP) mature osteoblasts that had severely impaired mineral apposition [89]. Furthermore, IGF-I has been shown to increase type 1 collagen synthesis, ALP activity and osteocalcin production in osteoblasts [90,91], while decreasing the levels of collagenase 3 [92,93]. IGF-I exposure decreased apoptosis of osteoblasts in a time- and dose-dependent manner [94], with normal human bone cells demonstrating decreased apoptosis after 6 h of exposure to IGF-I. Similarly, IGF-I doses of 0.1 – 20 ng/ml had a statistically significant effect, with a maximum effect observed at 2 ng/ml. IGF-I has also been shown to enhance the function of differentiated osteoblasts and adipocytes, but does not directly modulate the genes committing the multipotent precursor cells to a specific cell type [95]. In a study involving human marrow stromal cells (hMS(3 – 4)) cultured with 10 nM of IGF-I for 6 days, the expression of neither Cbfa1 (a transcription factor translating osteoblastic differentiation) nor PPARγ_2 (the transcription factor involved in adipocyte lineage) was affected.

Several recent studies have been able to demonstrate the pathway of differentiation of cells of the osteoblast lineage, allowing better understanding of the role of several key molecules involved in the process. The osteoblast differentiation pathway involves the gradual change in phenotype of the mesenchymal progenitor cell to the pre-osteoblast and finally to the mature osteoblast. Pre-osteoblasts express low levels of type 1 collagen (COL1A1) and ALP while the functional osteoblasts express high levels of COL1A1, bone sialoprotein and osteocalcin [96]. The regulation of this process is dependent on two transcriptional factors, runt-related transcription factor 2 (Runx2) and Osterix (Osx) [97,98]. IGF-I appears to be involved in the differentiation of the cells of the osteoblastic lineage via both of those transcription factors.

IGF-I can activate Akt, a serine-threonine kinase, via the PI3K/phosphoinositide-dependent kinase 1 (PDK-1)/Akt pathway [99]. This pathway is then deeply involved in Runx2-dependent osteoblast and chondrocyte differentiation and migration [100]. This activation enhances DNA binding and Runx2-dependent transcription of several genes including the PI3K and Akt genes, the expression of which is upregulated by Runx2, thus creating a positive feedback loop that further enhances differentiation and migration of cells. Furthermore, Celil and colleagues have demonstrated the involvement of IGF-I and MAPK signalling in mediating Osx, with the inhibition of MAPK component ERK1/2 not affecting Runx2 but inhibiting Osx expression, thus impairing differentiation [101]. GH also has a part in this differentiation pathway, by promoting the DNA binding of Runx2 but restraining its transcription potential [102].

2.1.3 GH on osteoclasts

GH has been shown to have direct effects on osteoclastic resorption, mainly via IGF-I. IGF-I increased bone resorption from pre-existing osteoclasts derived from rats as well as inducing multinucleated tartrate-resistant acid phosphatase (TRAP)-positive cells from pre-existing osteoclast-free cultures [29]. Another study demonstrated the inhibition of the stimulatory effect of GH and IGF-I on osteoclastic bone resorption by the addition of antiserum against h-IGF-I addition to the culture.[103] The IGF-I receptor has been identified on human preosteclastic cells [104] and IGF-I has been shown to promote osteoclast formation and culture at the dose range of 10^{-9} to 5×10^{-8} M [105] but did not stimulate bone resorption in cultured mouse calvarial bones at a dose of 100 nM. It was concluded that the effect of IGF-I on osteoclasts may not be direct and is possibly mediated by osteoblast-derived substances, on which IGF-I may have an inhibitory effect [106].

2.1.4 Osteoblast–osteoclast crosstalk

The process of fracture healing involves the regulated resorption of bone matrix by multinucleated osteoclasts and thereafter the formation of collagen and mineralisation of new bone by osteoblasts. The process appears to be regulated by a

cytokine system composed of osteoprotegrin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) and its receptor activator of nuclear factor κB (RANK). OPG is a receptor produced by cells of the osteoblast lineage and can bind to RANKL and inactivate it [107,108]. RANKL binds to its receptor RANK and acts on differentiation of osteoclast precursors, osteoclast apoptosis and activation [107,108].

GH appears to play a part in the regulation of this pathway, both directly and indirectly via IGF-I.

A recent study investigating the effect of IGF-I on mouse ST-2 stromal cells via the OPG/RANKL pathway demonstrated that IGF-I reduced OPG levels in a time- and dose-dependent manner [109]. It was observed that the estimated half-maximal inhibitory dose of IGF-I was 35 – 50 μg/l (4.5 – 6.5 nM), with little further effect after 100 μg/l (13 nM). Furthermore, a statistically significant inhibitory effect was seen between control and intervention groups, as well as between the two intervention groups, when cells were exposed to 100 μg/l at 24 and 48 h. The stability of OPG was not affected as measured by half-life studies, indicating that the effect on OPG was at the transcriptional level. Furthermore, the RANKL levels were increased, suggesting that IGF-I influences bone resorption by affecting the OPG/RANKL ratio.

The role of GH in the osteoblast/osteoclast crosstalk was demonstrated when human osteoblast-like cells were treated with GH and a dose- and time-dependent increase in OPG levels and on OPG mRNA production was observed [110]. GH induced OPG significantly at the doses of 1, 5 and 10 ng/ml, while larger or smaller doses did not demonstrate this. The effect of 5 ng/ml of GH on OPG mRNA expression was statistically significant at 6 h and was minimised at 24 h. Subsequently, the same procedure was repeated with cells treated with a GH antagonist (pegvisomant) or an antagonist of the JAK2 pathway, tyrphostin AG490. Both these substances inhibited the effect of GH on OPG levels and OPG m-RNA expression, indicating that the effect was mediated directly via GH. The discrepancy between the effects of GH and IGF-I on OPG, taking into consideration the positive effect of GH on serum IGF-I levels, is not well understood.

2.2 *In vivo* studies

2.2.1 *GH on fracture healing*

The positive effects of GH on animal models were initially demonstrated by Koskinen [111], who histologically showed an increase in fracture healing in 15 female rats when administering GH intramuscularly. Many studies have lent further support to the role of GH in fracture healing in animal models [112-122]. The stimulation of secondary fracture healing was demonstrated in a recent study involving micropigs, where 100 μg/kg weight recombinant porcine (r-p) GH was systemically administered for 4 weeks leading to both increased biomechanical strength and greater callus formation compared with untreated controls [113]. This finding was further supported by another recent study where, under the same experimental dose and delivery method, torsional failure and stiffness was 70 and 83% higher in the treatment group after 6 weeks [114].

r-pGH has also been shown to accelerate bone formation in a distraction osteogenesis model in micropigs [112]. Increased bone formation and resorption were observed after daily 100 μg/kg injection of r-pGH for 25 days. Interestingly, the structural parameters of the callus were histologically shown to be unaffected, suggesting that although bone fracture healing was accelerated, this was not achieved by altering the structural parameters of the bone.

2.2.2 *IGF-I on fracture healing*

Despite no studies directly implicating IGF-I mediating the role of GH in bone fracture healing itself, several experiments have observed a concomitant increase in IGF-I following GH administration [113-115]. There is evidence to suggest that the promotion of bone growth by GH is mediated by IGF-I [123], where administration of 0.25, 1 and 4 μg rGH into rat epiphyseal cartilage plate mediated growth, but this growth effect was nullified by the coadministration of antiserum to IGF-I. Furthermore, such an effect was demonstrated to be dose-dependent, with the highest dose providing greatest increase in mean epiphyseal plate width.

The positive effects of IGF-I on bone fracture healing were postulated in rats who were treated with either placebo, local administration of growth factors (recombinant human IGF-I and TGFβ-1) using poly(D,L-lactide)-coated Kirschner wires and 2 mg/kg weight of subcutaneous GH, or local growth factor and systemic GH in combination [17]. Bone healing was shown to be enhanced biomechanically, radiographically and histologically when GH alone or IGF-I/TGF-β1 were applied individually, but interestingly no additive effect was seen when they were given in combination. However, since TGF-β1 is itself a growth factor, it would be difficult to evaluate which of the two (IGF-I or TGF-β1) would be responsible for increased fracture healing. IGF-I has further been shown to positively affect bone healing, as infusion of 25 and 50 ng/day of IGF-I directly into rat bone marrow demonstrated an increase in the osteoblastic markers procollagen, osteopontin and ALP mRNA for the 25 and 50 ng groups [26].

Increased IGF-I mRNA expression has also been observed after 7 days of healing from a rat mandibular osteotomy, suggesting its importance in fracture healing repair [124]. In the study, four rat mandibles were assessed postoperatively on each of the days 0, 3, 5, 7, 9, 23 and 37 and mRNA expression was assessed using PCR and Southern hybridisation. It was noted that IGF expression peaked at day 7, with subsequent decreases on the following days. This finding supports a previous study where IGF-I mRNA expression levels in rat tibial fractures were analysed at days 4, 6, 8, 14, 18 and 24. Levels were found to be elevated and subsequently peaked at day 8 [125].

2.2.3 Timing of administration

The timing of administration of GH appears to be important, as the length of administration and period of fracture healing in which GH is delivered have been observed to play a vital role. In a 3-month-old rat model, maximum stiffness was observed to increase by 31% when 2.7 mg/kg GH was administered for 3 weeks, compared with 1 week [116]. In a separate study, administration of GH during the first 3 weeks of healing or throughout the entire 6-week period enhanced bone healing, but not when GH was administered in the last 3 weeks [117]. The authors demonstrated that maximum load was increased by 222% at 40 days in rats administered with b-hGH for the initial 20 days and 175% for rats treated for the entire period, compared with control. In contrast, there was no significant difference in fracture healing in rats treated with b-hGH in the last 20 days compared with control.

This positive effect of fracture healing was further validated by a later study [118], where the recombinant GH-treated group demonstrated increased ultimate load and stiffness by 100 and 200%, respectively, compared with vehicle at the end of the 3-week administration period. Interestingly, ultimate load was increased by 67%, 98 days post-fracture and 77 days after withdrawal of GH. Similar results have been observed demonstrating enhanced bone fracture healing post GH withdrawal, suggesting a potentially latent effect of GH on bone [119].

2.2.4 Dose-related effect

The effect of fracture healing may also be dependent on the dose administered [120,121]. Systemic biosynthetic human (b-h) GH s.c. injections twice daily at doses of 0.08, 0.4, 2.0 and 10 mg/kg into 3-month-old female rats showed a significant increase in strength of healing fractures in animals administered with the higher doses of 2.0 and 10 mg/kg only [120]. Using the lower doses a moderate, but not significant, increase in strength of healing fractures was observed. Similarly, hGH delivered locally via macroporous biphasic calcium phosphate (MBCP) implants at doses of 0.1, 1.0 and 10 µg in a rabbit femoral defect model for 3 weeks determined that MBCP ceramic resorption into the bone was significantly enhanced by 140% ($p < 0.001$) in hGH 1 µg only, with the 0.1 and 10 µg groups exhibiting a nonsignificant increase of approximately 60% increased resorption. In addition, bone formation was significantly raised in all three test groups compared with control, but interestingly the 1-µg hGH group demonstrated significantly increased bone ingrowth compared with the 0.1 and 10 µg groups ($p < 0.05$). This suggests that despite an increase in bone formation upon administration of a wide range of doses, there may exist an optimum dose in which hGH functions [121]. In both studies, the highest doses of either 10 mg/kg twice-daily s.c. injections into a rat model, or 10 µg locally released via MBCP implants into rabbit femurs, showed significantly increased bone growth without notable side effects.

2.2.5 Mode of delivery

The osteogenerative effect of GH through both local and systemic administration of human and bovine GH on rat mandibular defects has also demonstrated the mode of delivery as an important factor [122]. Histological analysis revealed that systemic administration of 200 µg/day hGH significantly increased bone fracture healing. Local delivery of 0.2, 2.0 and 20 µg/day of hGH for 4 weeks also showed highly significant enhancement in all healing parameters in the 2.0 and 20 µg/day groups, suggesting firstly a locally mediated IGF-I-produced effect on bone tissue formation, and secondly that such effects appeared to be dose-related. Furthermore, support for the importance of the timing of GH administration was shown when hGH administration on fracture healing over time demonstrated that the stimulatory effect of h-GH was discernible at 3 and 4 weeks, but not at 2 weeks.

The effects of GH on bone fracture healing have been further demonstrated on large animals [115]. Bilateral ulna bone defects were produced in eight mature Labrador dogs who then received continuous local s.c. 1.8 mg/day r-pGH infusion for 4 weeks in the right-sided defect only. Histological evidence showed that an increase in bone healing and formation in the critical-sized bone defects occurred, although the additional infusion of GH locally did not demonstrate any significant difference between contralateral fracture sites. In addition, a corresponding increase in plasma IGF-I and IGF-II was observed with GH administration, suggesting its role in bone healing.

2.2.6 Contradicting studies

However, not all studies have corroborated the findings of the positive effect of GH on bone fracture healing [126-131]. One study, through the histological profiling of healing rat tibial fractures, concluded that administration of 2.7 mg/kg b-hGH led to a delayed remodelling and normalisation of the fracture site, despite an initial stimulatory effect of callus formation [126]. This is in contrast to Bail and co-workers [112], who observed no effect on callus parameters in their study. Similarly, Northmore-Ball [128] was unable to demonstrate a reduced fracture healing time after administration of 5 mg bGH intramuscularly and testing for mechanical strength at 2, 3, 4, 5 and 7 weeks. The mechanical strength of healing fractured femurs compared with that of controls in mature rats did not statistically differ, although a statistically significant increase ($p = 0.011$), in torque indices, in the GH group was observed at 2 weeks only. This may suggest an early, albeit small, beneficial effect of GH and fracture healing.

No effect of GH on fracture healing has been observed in studies involving its systemic administration in rabbits [127,129-131]. In one study, no effect on accelerating healing time or tensile strength of the healing fractures could be demonstrated using 150 µg/kg r-hGH delivered intramuscularly [127]. In addition, no effect on IGF-I serum levels were observed upon GH administration. Such a finding contrasts with those of Raschke and colleagues, where GH-induced IGF-I

was observed [114], suggesting that the GH-IGF-I axis plays an important role and that its activation is a necessary part of the healing process. A further study has substantiated the reduced strength of healing fractures, where 0.46 IU/kg, approximately equivalent to 0.15 mg/kg, was administered via the i.m. route into rabbit tibial fractures. Testing every 10 days for 50 days did not result in increased tensile strength compared with control [131]. Additionally, no effect on healing time was noted when 4 mg bovine growth hormone (b-GH) was administered twice weekly i.m. in a rabbit model with bilateral resection of the radii [130]. Furthermore, no effect was seen on the rate of recovery of mechanical strength in previously atrophied long bone when h-GH was delivered 0.03 IE/kg i.m. [129].

2.3 Human studies

Attempts to elucidate the effect of GH on fracture healing in humans have been present in the literature since the early 1970s, when the serum GH levels of a patient with delayed fracture healing were compared with those of normal human volunteers [132]. The authors injected the patient with insulin, arginine and glucose, measuring GH levels at several intervals post each injection, and compared these with the results of normal volunteers. The study showed reduced GH levels of the patient compared with normal human volunteers. Further insight into the response of the GH axis in fracture healing was recently given by the prospective study involving measurements of IGFBP and ALS, which form a complex with IGF in circulation [133]. The study involved measurements of each of these components in the serum of patients with delayed fracture healing, compared with normal healing controls, over a period of 6 months and reported statistically significant low levels of IGFBP-3 (week 8) and ALS (weeks 1, 6 and 8) in patients with atrophic type of nonunion compared with controls with normal fracture healing.

The first interventional studies aimed at evaluating fracture healing with the use of GH were reported in 1977 with a case control study on the effect of GH on fresh fractures [134] and a case series on nonhealing fractures [135]. Human GH was administered at a dose of 16 IU, approximately 5 mg, on alternate days to 20 patients admitted with long bone nonunion or delayed union after a fracture. The study reported 100% healing rate, with 85% of the fractures considered stable within 12 weeks [135]. GH was also administered at the same dose to 12 patients with lower leg fractures for a period of 5 weeks, but the healing response did not appear to differ from that of the eight patients in the control group [134]. Recent human studies on the effects of GH on patients sustaining fractures place emphasis on the functional recovery of patients; therefore, most of the published data include indirect evidence of fracture healing. The intended benefit of GH administration in human fracture patients in most of the available studies has been to determine the systemic effect against the catabolic state that these patients enter after their injury/operation [133,136-140].

2.3.1 *GH effect on fracture healing*

The only human study evaluating GH on fracture healing is a recent randomised controlled trial (RCT) looking into the effect of r-hGH on 368 patients with tibial fractures treated with intramedullary nailing [141]. The main end point of the study was the radiological healing of the fracture, defined as the disappearance of the fracture line and/or cortical bridging in three out of four cortices seen in two perpendicular X-ray views. This double-blinded trial lasted for 16 weeks or until the healing of the fracture if earlier, with a follow-up of 12 months. Serum levels of IGF-I, IGFBP-3, osteocalcin and serum CrossLaps (CTX) were also measured during the 24-week period of the study. Clinical examination and safety measurements – including blood pressure, serum glucose and HBA1c – were also performed. The study involved four treatment groups consisting of three different GH dose groups (0.01, 0.03 or 0.06 mg/kg/day) and a placebo group. A reduced healing time (26%) for the patients in the higher dose group (0.06 mg/kg), along with significantly higher IGF-I, osteocalcin and serum CrossLaps levels, was observed. However, the beneficial effect was evident only in patients with closed fractures – perhaps, according to the authors, reflecting the longer healing period of the open fractures. There were no safety issues with the study, although an increased number of adverse events were reported in the treatment groups, especially in the higher-dose group. These events, possibly related to the intervention, were extraskeletal ossification (0.015 mg/kg/day), allergic dermatitis (0.03 mg/kg/day), increased plasma glucose, acute cholecystitis, pyrexia and hyperglycaemic hyperosmolar nonketotic coma (0.06 mg/kg/day).

2.3.2 *GH administration to hip fracture patients*

In 2000, a RCT was undertaken to determine the effect of 0.02 mg/kg/day of r-hGH on 76 hip fracture patients [138]. The intervention included daily s.c. injections of GH and lasted for 6 weeks, with follow-up for 6 months. The study reported a significant increase in the levels of IGF-I and IGFBP-3 in the treatment group (with maximum effect at 4 weeks), as well as an increased percentage of return to prefracture state, in patients aged > 75 (93.8 vs 75%, $p = 0.034$).

Similarly, an increase in IGF-I was demonstrated in another study undertaken to evaluate the use of GH on patients undergoing elective total hip replacement (THR) [139]. This study focused on administering r-hGH for 14 weeks preoperatively at a dose of 0.04 IU/kg/day with once-daily s.c. injection and doubling the dose for the first 2 weeks postoperatively. The authors concluded that this strategy increased the lean muscle mass preoperatively and increased function postoperatively, as measured by the 4-min walking distance. Again, dose-related adverse events of fluid retention and joint pains were noted in the treatment groups.

In 2003, a placebo-controlled double-blinded trial was undertaken to evaluate the effects of r-hGH on 31 women

sustaining hip fractures, where 0.05 and 0.025 mg/kg/day r-hGH was administered s.c. once daily for 14 days [140]. The results of this study, in accordance with the previous ones, demonstrated a rise in IGF-I and IGFBP-3 between the placebo and r-hGH groups but not between the two r-hGH groups. Furthermore, the pretreatment indicators of frailty were inversely related to the IGF-I and IGFBP-3 response to r-hGH. This study also demonstrated an increased rate of adverse events in the treatment group, including acute renal failure, pulmonary emboli (two nonfatal, one fatal), and a myocardial infarction, while no such events were noted in the placebo group. The small numbers of participants and events did not allow the authors to draw firm conclusions on safety, while previous trials on r-hGH have shown no increase in thromboembolic events.

Hedstrom and colleagues in 2004 [137] published their results on a RCT trial of r-hGH on 20 hip fracture patients. GH was administered at a dose of 0.1 IU/kg/day (equivalent to 0.03 mg/kg/day) via a daily s.c. injection, but the study was interrupted following safety issues that emerged from a recent trial [142] on GH for critically ill patients. Parallel RCTs on GH for critically ill patients requiring ICU admission demonstrated increased mortality in the GH treatment groups and caused concern regarding GH administration among researchers studying the effects of GH at the time. The study continued after 1 year with the dose reduced to 0.04 IU/kg/day (0.013 mg/kg/day). The parameters measured included bone mineral density and composition with the use of DEXA and quantitative CT. The authors report significant IGF-I response in the treatment group as well as retention of total body mineral composition, lean body mass and loss of subcutaneous fat in the treatment group. The only side effects reported in this study were two cases of soft-tissue oedema in the treatment group (improved after 50% reduction of dose) and a case of hypertension in the placebo group.

2.3.3 *Route of administration*

In an attempt to retain the beneficial effects of GH on hip fracture patients but avoiding the parenteral administration route, an oral GH secretagogue was studied in a multicentre RCT of hip fracture patients. A once-daily dose of 25 mg of MK-0677 was administered postoperatively for 6 months to 84 patients who were medically stable. The follow-up lasted for 12 months and the measurements included IGF-I levels as well as functional performance measures. The study showed significant IGF-I response, with the treatment group demonstrating an 84% increase compared with 14% in the placebo group at week 26, but no difference was observed in functional performance measures. The adverse events reported in this study include four thromboembolic events (although these were reported as not drug-related) in the treatment group compared with none in the control group. More reports of fluid overload and oedema were also reported in the treatment group along with significantly higher glucose, insulin and HbA1c levels, while the placebo group had more musculoskeletal adverse experiences [143].

2.3.4 *IGF-I*

Boonen and co-workers in 2002 published a RCT on hr-IGF-I/IGFBP-3 complex on patients aged > 65 years sustaining a proximal femoral fracture [136]. The complex used was reported to have a better safety and efficacy profile compared with that of IGF-I alone. The route of administration differed in this trial, as the patients had a continuous s.c. infusion for a period of 8 weeks during which the infusion site was changed every 48 h or less. The dose used was equivalent to 0.1 and 0.2 mg/kg/day of IGF-I in the two treatment groups. The study reported increased dose-dependent serum IGF-I levels as well as increased osteocalcin and N-telopeptide in the treatment group, while the BMD and bone-remodelling markers did not show any difference between the groups. The end points of the study also included functional tests such as grip strength and functional ability (standing from seated position), which were improved in the treatment group.

3. Discussion

There is robust evidence to suggest that GH has a multimodal effect on bone metabolism and fracture healing. *In vitro* studies have observed the role of GH in osteoblast differentiation, proliferation and maturation [11,86], as well as osteoclast activity [103] and the interaction between the two [109,110]. Given that the process of bone metabolism and fracture healing share common pathways, *in vivo* studies have been undertaken to investigate the positive effect of GH on fracture healing in both animal and human models.

Our understanding of bone cell biology has increased significantly in the last decade. Several pathways have been demonstrated to take part in bone cell metabolism and their interactions appear to be important when it comes to the coupled process of bone remodelling and fracture healing. Genetically mutated mice have been utilised to demonstrate the mechanism of GH on osteoblasts, indicating the crucial role of IGF-I in this process [86]. However, the exact mechanism of GH action on bone remodelling is not completely understood despite the better understanding of the crosstalk between osteoblasts and osteoclasts. Several intracellular systems appear to be a part of this, with the osteoprotegrin/RANK-L ratio playing a key role. The process of fracture healing depends on effective interaction between bone resorption and new bone formation, in both of which GH and IGF-I appear to have a role. GH and IGF-I appear to take part in the regulation of the process, but initial data present a discrepancy in their function, with GH increasing OPG levels and therefore reducing RANKL-related osteoclast activation, while IGF-I has the opposite effect [109,110]. Despite the difficulties in extrapolating the effects of GH and IGF-I on culture models to living organisms due to the

complex interactions of different hormones, there remains evidence to suggest a role of GH in bone healing.

The therapeutic dose of GH has produced conflicting results in animal studies [112-122,126-131,144] and such contrasting data may, in part, be due to the heterogeneity of methods employed in the different animal models. Indeed, two studies were able to show the importance of dosing in terms of strength of healing, with one demonstrating that the highest systemically administered doses (2 and 10 mg/kg) exerted the most beneficial effect in rats [120], and the other observing that local delivery of 1 µg GH exhibited significantly better healing than the 0.1 and 10 µg doses in rabbits [121]. This difference in the effective GH dose may be attributable to the animal model used. The majority of animal studies involving rabbits have observed no effect of GH on bone fracture healing, suggesting that animal species is an important variable when comparing results [145]. This can be explained when considering the differences in amino acid sequence homologies of both GH and GHR between different species [146,147]. It has been shown that key residues on the GHR and GH are vital in determining species-specific sensitivity of GH binding [147,148], and such differences may influence the affinity and potency of GH-GHR binding and activation. Furthermore, the production of GHBP, a protein that binds to GH and is thought to be important in influencing GH activity, has been shown to possess species-specific generation [46]. Therefore exogenously administered GH may not necessarily reflect true physiological function. Also, the production of antibodies to non-species-specific GH has been detected in animals [149], explaining its limited action [150].

The effect on the human studies also appears to be dose-dependent [136,140,141]. Dose-dependent IGF-I response to GH, as well as a dose-dependent effect on fracture healing and improved functional outcome, has been demonstrated [136,141]. Raschke and colleagues [141] showed accelerated fracture healing in closed tibial fractures treated with 0.06 mg/kg/day of GH via daily s.c. injections, while the administration of lower doses did not produce a statistically significant effect. Similarly, Boonen and co-workers [136] demonstrated a dose-dependent increase in IGF-I levels after treatment with 0.1 mg/kg/day of an IGF-I/IGFBP3 complex in elderly patients sustaining hip fractures. The effects on the human studies are in accordance with the *in vivo* animal data indicating the presence of an optimal GH dose, below which its effect cannot be obtained.

The direct delivery of GH to the fracture site offers the potential to expose local areas to relatively low dosing levels, thus reducing any potential side effects that may result from the increased doses required for systemic administration. It has been shown in animals that continuous locally delivered doses of 2 µg/day hGH by infusion pumps in rat mandibular fractures were able to promote fracture healing [122], and locally delivered 0.1 µg hGH via implants in rabbits has demonstrated significantly increased bone formation [121]. A further study showed an increased rate of bone healing in rat tibial fractures when 20 µg rGH was injected over the fracture site [118]. Studies evaluating the systemic doses in bone fracture healing have used in excess of these levels, ranging from 100 µg/kg [113,114] to 2.7 mg/kg [116], in order to demonstrate a positive effect. Furthermore, a recent study evaluating the effects of 0.08, 0.4, 2.0 or 10 mg/kg of b-hGH systemically administered via the s.c. route has shown that only the higher doses of 2.0 or 10 mg/kg were effective.

In the human studies, the effect of local administration has not yet been evaluated, as GH has only been administered systemically via either daily s.c. injections [137-141] or continuous infusion pumps [136]. In addition, the sole study where the agent was administered via a s.c. infusion pump utilised a different agent, namely the IGF-I/IGFBP-3 complex, and therefore cannot be compared with the studies in which GH was delivered via a daily s.c. injection. The systematic administration of GH in humans had positive effect on fracture healing [141], although dose-dependent, and caused increased serum IFG-1 levels [137-141]. Some of the human studies on fracture patients were designed for, and demonstrated, systemic beneficial effects such as retained lean body mass [137], increased return to prefracture state [138] and increased grip strength [136].

The length of GH administration is yet another contributing factor in influencing fracture healing, as it has been shown in rat models that a sustained administration of GH of at least 3 weeks at the initial stage of fracture healing is important in determining healing rate. When GH was either delivered systemically by s.c. injections at doses of 2.7 mg/kg or delivered locally over the fracture site at doses of 20 µg [116-119], an increased rate of bone formation was observed. Fracture healing is a complex and well-orchestrated process consisting of temporal, as well as spatial, sequences. Healing involves haematoma formation, angiogenesis, cartilage formation and calcification followed by bone formation and remodelling. Such healing properties are mediated by the sequential release of key molecules such as BMP and growth factors. The expression of such signalling molecules has been shown to occur over the first few weeks of fracture healing, and it may be that it is this stage at which GH may be most influential in augmenting its effect on healing via activation of these molecules [151,152]. Indeed, BMP-2 and BMP-4 mRNA expression has been shown to be induced by both GH and IGF-I [153]. However, the physiological differences between humans and animal models means that the optimum length of administration of GH in animal models cannot be applied to humans, and further studies need to be undertaken in order for such an optimum period to be elucidated.

It is interesting to note that the contrasting studies [127,128,131] used once-daily intramuscularly administered GH at 150 µg/kg [127,131] or 5 mg b-GH [128], whereas Bak and colleagues [117,144] were able to accelerate fracture healing by twice-daily s.c. administration of GH at doses of 2.0 kg or 2.7 mg/kg. This may be a result of the greater

doses administered by the latter two studies, but it is conceivable that the frequency of GH administration may have an important role in determining its effect on bone healing. Indeed, longitudinal bone growth has been shown to increase when GH is administered 2 – 8 times daily in hypophysectomised rats, possibly as a result of mimicking the pulsatile nature of GH release [154]. Furthermore, it has been shown that the pulsatile administration of hGh or bGH resulted in an increased bone growth compared with sustained intravenous GH infusion of the equivalent dose [155]. It can therefore be postulated that the application of GH to fracture healing in humans may benefit from frequent administrations.

Taken together, GH has been shown to have a positive role on bone healing, but this is dependent on the animal model and the dose and route of administration, as well as on the GH employed. Higher doses delivered systemically can enhance bone healing, as can lower doses delivered locally. The literature on human studies revealed only one study focusing on the direct effect of GH on fracture healing [141], with the remaining trials regarding GH administration on fracture patients being aimed at identifying the indirect effect of improved functional outcome, rather than the effect of GH on fracture healing itself. The sole RCT on fracture healing indicated a dose-dependent effect on fracture healing by demonstrating a promising 26% reduction in the healing time of closed fractures. A single daily dose of 0.06 mg/kg was administered for a period of 16 weeks or until the healing of the fracture. While the effect of lower doses was investigated, the frequency and duration of the intervention was fixed among the study participants [141]. This suggests a potential role in GH in accelerating bone healing in the clinical setting.

4. Expert opinion

The clinical question of whether GH is suitable for use as an agent to enhance fracture healing is not decisively answered by the available evidence. We have demonstrated the *in vitro* evidence of GH action on osteoblasts and osteoclasts and looked into the different pathways [11,29,86,109,110]. Furthermore, animal studies have demonstrated that factors such as the delivery method [115,122], the duration [116], the timing [117] and the dose [120,121] are also related to the beneficial effect on fracture healing. Finally, the effect on fracture healing after systematic administration of GH to humans was also demonstrated to be significant and dose-dependent [141].

The safety profile of r-hGH has received significant attention concerning its application in the clinical setting. Pharmacological administration to critically ill patients has demonstrated increased mortality in two parallel RCTs [142]. The physiological effect of GH on insulin metabolism – along with other adverse events such as fluid retention, increased lipoproteins, cancer risk and tumour recurrence – have also been noted when GH has been administered for replacement purposes [156]. The benefits of pharmacological administration of GH will have to clearly outweigh the potential risks; the evidence is that it does not in the case of critical care patients [142].

Human studies on fracture healing have demonstrated increased adverse events, such as fluid retention, joint pains, hyperglycaemia, and thromboembolic events, in the treatment groups [137-141], but firm conclusions cannot be made as these studies were designed to demonstrate morbidity and mortality effects and indeed had insufficient numbers to do so. Furthermore, these studies excluded patients with diabetes, renal failure, malignancy, diabetes mellitus [137-141] and congestive cardiac failure [138]. This has to be taken into consideration when trying to apply the outcomes to a population that would benefit from fracture healing-enhancing agents and could carry significant comorbidities.

Pharmacological therapy with GH, where the hormone is not intended as a replacement, has not yet been fully established: both the therapeutic window and the maximum safe dose of GH remain to be determined. Adverse events have been observed at various doses of GH administration, and these effects appear to be dependent on the patient groups. In critically ill patients requiring ICU admission, the possible harmful effects of GH were observed in two parallel trials. The GH dose used varied from 0.07 to 0.13 mg/kg/day, and significantly adverse effects were demonstrated on the treatment group with regard to morbidity and mortality [142]. Other studies on pharmacological administration of GH for several diseases such as AIDS wasting, postoperative patients, sepsis, abdominal aortic aneurysm repair, and cardiomyopathy utilised doses as high as 0.19 mg/kg/day, with the majority of the studies using a dose of 0.1 mg/kg/day. These studies had much smaller numbers compared with the study on critically ill patients, but did not show a problematic safety profile for GH [157]. Furthermore, when GH is administered as replacement in GH-deficient patients, the initial dose in adults is 0.15 – 0.30 mg/day, gradually increased, with the maintenance dose seldom exceeding 1 mg/day (0.014 mg/kg/day) [158]. In children, the dosage is more aggressive: 0.025 – 0.05 mg/kg/day, reaching a maximum of 0.1 mg/kg/day in adolescents [159].

The dose required to provide patients with the beneficial effect on fracture healing has not been demonstrated clearly. In tibial fracture patients, the effective dose of GH that enhanced fracture healing was 0.06 mg/kg/day, while patients who received a lower dose of 0.03 mg/kg/day demonstrated no significant effect compared with placebo. Other studies on human fracture patients administered with GH doses varied between 0.01 and 0.06 mg/kg/day [137,138,140,141] These doses in humans demonstrated a safe profile for the cautiously selected group of patients, although the treatment groups had more adverse events [139-141] such as fluid retention, as well as more severe thromboembolic events in two of the human studies [140,143]; however, these were not reported as intervention-related or significant.

The problem of therapeutic dose exceeding that of the recommended safe levels may be bypassed by the local

administration of GH directly into the fracture site, which would reduce the necessary levels of GH administration while maintaining high levels of GH exposure. Indeed, a recent animal study using MBCP implants to deliver GH was able to demonstrate increased bone ingrowth in rats at concentrations as low as 0.1 μg, with an optimum dose of 1 μg. Therefore, the local delivery of GH offers a realistic prospect for maintaining adequate concentration within the constraints of safety levels. However, human studies must be undertaken in order to resolve issues such as the effect local delivery would have on fracture healing; the optimum dose required; whether it is feasible or not; and how it can be implemented.

The consensus on adult GH treatment states that research for pharmacological intervention should be encouraged despite the adverse effects on critically ill patients, since these results cannot be extrapolated to other patient groups [156]. Clearly there is a long way to go before determining the optimal GH dose required to enhance fracture healing in humans and to determine the safety profile in this group of patients. The potential for local administration has not yet been explored in humans and could possibly eliminate some of the side effects of systematic administration. GH offers an exciting therapeutic option in enhancing bone fracture healing, but more data regarding its effect in humans are required before it can be fully utilised in the clinical setting.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Affiliation

Gui Tong Tran[1], Joseph Pagkalos[1], Evgenios Tsiridis[2], Amir Ali Narvani[1], Manolis Heliotis[3], Athanasios Mantalaris[4] & Eleftherios Tsiridis[†5]
[†]Author for correspondence
[1]University of Leeds School of Medicine, Academic Department of Trauma and Orthopaedics, Leeds General Infirmary, Great George Street, Leeds, UK
[2]Blue Cross Hospital, Cardio-Thoracic Anaesthetic Unit, Thessaloniki, Greece
[3]Northwick Patrick Hospital, Maxillofacial Unit, Wattford Road,Harrow, London, UK
[4]Imperial College London, Department of Chemical Engineering, South Kensington Campus,London, UK
[5]Leeds General Infirmary, Department of Orthopaedics, Great George Street, Leeds LS1 3EX, UK
Tel: +44 01133922621;
Fax: +44 01133922621;
E-mail: etsiridis@doctors.org.uk