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Skeletal Effects of Growth Hormone and Insulin-like Growth Factor-I Therapy

Richard C. Lindsey^{a,b} and Subburaman Mohan^{a,b}

^aMusculoskeletal Disease Center, Loma Linda VA Healthcare System, Loma Linda, CA 92357

^bDepartments of Medicine and Biochemistry, Loma Linda University, Loma Linda, CA 92354

Abstract

The growth hormone/insulin-like growth factor (GH/IGF) axis is critically important for the regulation of bone formation, and deficiencies in this system have been shown to contribute to the development of osteoporosis and other diseases of low bone mass. The GH/IGF axis is regulated by a complex set of hormonal and local factors which can act to regulate this system at the level of the ligands, receptors, IGF binding proteins (IGFBPs), or IGFBP proteases. A combination of *in vitro* studies, transgenic animal models, and clinical human investigations has provided ample evidence of the importance of the endocrine and local actions of both GH and IGF-I, the two major components of the GH/IGF axis, in skeletal growth and maintenance. GH- and IGF-based therapies provide a useful avenue of approach for the prevention and treatment of diseases such as osteoporosis.

Keywords

Osteoporosis; Skeletal development; Growth hormone (GH); Insulin-like growth factors (IGFs); IGF binding proteins (IGFBPs)

1. Introduction

The dynamic process of bone remodeling involves the complex and coordinated interaction of osteoblast lineage cells, which form bone, and osteoclast lineage cells, which resorb bone. This process occurs at all stages of growth and development throughout the lifespan, and myriad systemic and local factors participate in the regulation of osteoblast and osteoclast function. Of particular note, the growth hormone/insulin-like growth factor (GH/IGF) axis plays an important role in the regulation of bone remodeling.

The first major component of the GH/IGF axis is GH, a single-chain polypeptide hormone which is secreted in a pulsatile manner by the anterior pituitary gland in response to

Corresponding Author: Subburaman Mohan, Ph.D., Director, Musculoskeletal Disease Center, Loma Linda VA Healthcare System, 11201 Benton Street, Loma Linda, CA 92357, USA, Subburaman.Mohan@va.gov.

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hypothalamic stimulation by growth hormone releasing hormone (GHRH) and inhibition by somatostatin (SS) (Veldhuis and Bowers, 2003). Although GH can directly affect cells of various tissues, including osteoblasts and epiphyseal growth-plate chondrocytes (Wang et al., 2004), GH stimulates longitudinal bone growth primarily by activation of hepatic IGF-I production.

IGF-I and -II are small peptide hormones with significant structural similarity to insulin. While IGF-II is important for intrauterine growth, IGF-I regulates skeletal growth and maintenance during postnatal life. IGFs have endocrine actions, exerted systemically through the circulation, and local actions, exerted in an autocrine/paracrine manner (Mohan and Kesavan, 2012). GH-induced hepatic IGF-I secretion is responsible for systemic, circulating IGF-I while IGF-I production in peripheral tissues is controlled by a multitude of interacting endocrine and local signals, some of which are GH-independent (Mohan et al., 2003).

The GH/IGF axis significantly regulates both longitudinal bone growth, which is important during childhood, and appositional bone growth, which is important for bone maintenance in adulthood. This chapter will examine the role of the GH/IGF axis in the regulation of bone remodeling and the potential of GH/IGF-based therapies to treat and prevent osteoporosis and other diseases of low bone mass.

2. The GH/IGF axis

2.1 GH secretion and signaling

Many factors work together to produce distinct profiles of pulsatile GH secretion which vary depending on age, sex, and species (Clark et al., 1987; Edén, 1979; Saunders et al., 1976). Hypothalamic GHRH and SS respectively stimulate and inhibit pituitary somatotroph secretion of GH (Brazeau et al., 1973; Ling et al., 1984; Spiess et al., 1983). Additionally, several factors interact with the hypothalamus and pituitary to stimulate GH release in response to an organism's metabolic state, consistent with GH's dual role in regulating both metabolism and growth. Along these lines, free fatty acids act at the level of the pituitary to inhibit GH secretion, providing negative feedback to counteract GH's stimulation of lipid release (Imaki et al., 1985; Muggeo et al., 1975). Leptin, a so-called energy-sensing hormone released by adipocytes, modulates GHRH and SS action to trigger hypothalamic GH release (Carro et al., 1997; Tannenbaum et al., 1998; Vuagnat et al., 1998). Furthermore, a class of several peptide and non-peptide compounds which stimulate GH release have been identified and synthesized. These compounds have been termed GH-releasing peptides (GHRPs) or GH secretagogues (GHSs) (Smith et al., 1997). Compounds of note in this class include the hexapeptides GHRP-1, GHRP-2, GHRP-6, and hexarelin (Deghenghi et al., 1994) as well as the non-peptide GHRP mimetic MK-0677 (Patchett et al., 1995), some of which exhibit considerable oral bioavailability and activity (Ghigo et al., 1994). These compounds stimulate GH release via the GHS receptor (GHS-R) (Howard et al., 1996; Smith et al., 1996), a G protein-coupled receptor whose endogenous ligand, ghrelin, was later discovered and identified as a 28 amino acid peptide produced mainly in the stomach (Kojima et al., 1999). GH production in response to ghrelin has been demonstrated in both rats and humans (Takaya et al., 2000; Wren et al., 2000). In addition, sex steroids have been

shown to regulate the secretion and action of GH (Ho et al., 1996). A complex interaction of many factors regulates the secretion of GH, which exerts its effects on tissues throughout the body.

The binding of GH to the GH-receptor (GHR) initiates a signal transduction cascade responsible for the actions of GH on peripheral tissues. The GHR, a transmembrane protein which is almost ubiquitously expressed on cells of all tissues, undergoes several posttranscriptional and posttranslational modifications, the most notable of which is the formation of soluble GH binding protein (GHBP) from the extracellular ligand-binding domain of the GHR (Baumann et al., 1986; Leung et al., 1987). Activation of the GHR induces the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling pathway. GH binding to the GHR causes the GHR to associate with and activate JAK2, a receptor-associated tyrosine kinase. Activated JAK2 phosphorylates tyrosine residues on STAT-1, -3, -5a, and -5b (Brooks et al., 2014; Carter-Su and Smit, 1998), which, in turn, undergo nuclear translocation and act as transcription factors, binding directly to specific DNA sequences and modulating gene expression (Leonard and O'Shea, 1998; Takeda and Akira, 2000), leading especially to the upregulation of IGF-I expression. GHR is also known to interact with IGF and insulin receptor signaling pathways via phosphorylation of insulin receptor substrates IRS-1, -2, and -3, leading to activation of phosphatidylinositol (PI) 3'-kinase (Argetsinger et al., 1996; Herrington and Carter-Su, 2001; Souza et al., 1994). In addition, GHR can interact directly with IGF-IR, even in the absence of IGF binding, to modulate GH signaling (Gan et al., 2014a, 2014b). GH signaling may also occur through influx of extracellular calcium ions through voltage-dependent L-type calcium channels (Gaur et al., 1996) and increased intracellular diacylglycerol (DAG), leading to protein kinase C (PKC)-mediated activation of MAP kinases, p90rsk, and c-fos induction (Anderson, 1992; Campbell et al., 1992; Smal and De Meyts, 1987). Suppressor of cytokine signaling (SOCS) proteins accomplish further regulation of GH signaling through inhibition of the JAK-STAT pathway, leading to either suppression or augmentation of GH signaling (Corva et al., 2004; Greenhalgh et al., 2002; Krebs and Hilton, 2001; Ram and Waxman, 1999). GH signaling occurs through the interaction of several signaling pathways triggered by GHR activation.

2.2 GH effects: with and without IGF

Many of the actions of GH are mediated through production of IGF-I, widely accepted as a key regulator of skeletal growth, development, and metabolism (Locatelli and Bianchi, 2014; Mohan and Kesavan, 2012; Yakar et al., 2010). The preponderance of circulating IGF-I is produced by the liver in response to GH stimulation; thus, the original somatomedin hypothesis proposed that GH induces skeletal growth only indirectly via stimulation of hepatic IGF-I production (Salmon and Daughaday, 1957) (Fig. 1). However, additional studies suggest that GH can have direct effects on chondrocytes (Isaksson et al., 1982; Schlechter et al., 1986); the more recently proposed dual effector theory of GH action accounts for the inability of IGF-I to reproduce all of GH's effects (Green et al., 1985). Besides the effects of increased IGF-I expression, GH can directly stimulate the differentiation of prechondrocytes (Lindahl et al., 1987, 1986; Ohlsson et al., 1992), leading to longitudinal growth at the epiphyseal growth plate, data which have been recently

confirmed by growth plate-specific postnatal knockout of the IGF-IR in mice (Wu et al., 2015). Additionally, targeted disruption of the GHR in osteoblasts suggests that, besides its stimulation of local IGF-I action, GH may play a role in directly regulating the development of skeletal sexual dimorphism in mice (Singhal et al., 2013). The intracellular molecular mechanisms that contribute to IGF-independent, growth-promoting effects of GH are yet to be identified.

2.3 IGF expression and signaling

Systemic regulation of IGF-I expression occurs via the effects of several hormones in addition to GH. Notably, parathyroid hormone (PTH), 17β -estradiol, and thyroid hormone (TH) are known to upregulate IGF-I expression in osteoblasts (Bikle and Wang, 2012; Ernst and Rodan, 1991; Gray et al., 1989; McCarthy et al., 1997; Xing et al., 2012) while glucocorticoids and 1,25-dihydroxyvitamin D3 [$1,25-(OH)_2D_3$] can downregulate IGF-I expression (Canalis, 2005; Chen et al., 1991; Scharla et al., 1991). The ability of PTH to stimulate osteoblast proliferation, differentiation, and survival as well as collagen synthesis, alkaline phosphatase (ALP) activity, and osteocalcin expression appears to be dependent upon local IGF-I production (Linkhart and Mohan, 1989; McCarthy et al., 1989), data which have been confirmed *in vivo* by genetic disruption of IGF-I (Bikle et al., 2002; Miyakoshi et al., 2001a) or IRS-1 (Yamaguchi et al., 2005) in mice. Sex steroids stimulate a rise in IGF-I expression during pubertal growth (Christoforidis et al., 2005; Styne, 2003; Veldhuis et al., 1997); the effect of androgens is dependent on aromatization (Weissberger and Ho, 1993), and the effect of estrogens is dependent on dose with high doses decreasing circulating free IGF-I (Veldhuis et al., 2005; Weissberger et al., 1991). In prepubertal mice, TH has been shown to have a greater effect on skeletal growth than GH, and this effect is mediated by an increase of IGF-I expression in both the liver and bone (Xing et al., 2012). Similarly, clinical studies in humans have shown that correction of serum TH levels with methimazole treatment in hyperthyroid patients is sufficient to lower elevated serum IGF-I levels to within normal limits (Lakatos et al., 2000). IGF-I expression levels increase with TH treatment in osteoblasts and chondrocytes (Lakatos et al., 1993; Xing et al., 2012) as there is a TH response element in the promoter of the *IGF-I* gene which stimulates transcription when bound by a complex of TH and TH receptor- α (Xing et al., 2012). The TH effect on IGF-I may also be modulated by interaction with the sympathetic nervous system (Fonseca et al., 2014). Thus, IGF-I has been predicted to be a critical mediator of TH effects on the skeleton.

Glucocorticoids have been shown to decrease osteoblast proliferation and function, resulting in reduced trabecular bone formation (Canalis, 2005, 1993; van der Eerden et al., 2003). These changes are associated with a fall in production of both IGF-I and stimulatory IGF binding proteins (Canalis, 2005; Cheng et al., 1998; Chevalley et al., 1996) as well as decreased basal and IGF-induced chondrocyte proliferation (Jux et al., 1998). Another stress hormone, prostaglandin E2 (PGE2), can increase IGF-I production via increased cAMP synthesis (Thomas et al., 1996), while hypoxic stress can interact with stress hormones to ultimately reduce IGF-I expression (McCarthy et al., 2014).

In addition to systemic regulation of IGF expression, local regulators also play an important role. Fibroblast growth factor 2, transforming growth factor- β 1, bone morphogenetic protein

7, and interleukin-1 are locally produced growth factors which influence IGF-I production (Knutsen et al., 1995; McCarthy and Centrella, 2001; Tremollieres et al., 1991; X. Zhang et al., 2002). Moreover, the introduction of mechanical strain to bones rapidly increases IGF-I expression (Lean et al., 1996, 1995; Triplett et al., 2007; Xing et al., 2005); in fact, in one study, 10 minutes of mechanical loading was enough to increase IGF-I levels in osteocytes of the loaded bone in rats (Lean et al., 1996). Studies of mechanical strain using TE85 cells and primary human osteoblasts *in vitro* have shown that the proliferative response of osteoblasts to the combination of mechanical strain and estrogen is dependent on the estrogen receptor- α and the IGF-I receptor (IGF-IR) (Cheng et al., 2002, 1999; Rawlinson et al., 1993). *In vivo*, mice overexpressing IGF-I in osteoblasts have a several-fold greater increase in periosteal bone formation in response to low-magnitude, noninvasive loading compared to wild-type mice, which had no response to low-magnitude loading (Gross et al., 2002). Furthermore, the removal of normal loading in rats deficient in GH blocked the ability of IGF-I treatment to induce bone formation (Sakata et al., 2004, 2003). The mechanical effect on IGF-I expression is known to be mediated via an integrin-dependent process in which IGF-IR is phosphorylated and the phosphatases SHP-1 and -2 are recruited away from IGF-IR (Kapur et al., 2005; Lau et al., 2006). Additionally, the integrin-requiring resistance to IGF-I induced by skeletal unloading has been shown to be a unique feature of IGF-I signaling, over and against platelet-derived growth factor (PDGF) signaling (Long et al., 2011). More recently, the link between IGF-I upregulation and bone formation in response to mechanical loading has been more definitively demonstrated through the use of conditional IGF-I gene disruption in mouse osteoblasts expressing type 1 α collagen (Kesavan et al., 2011). Osteocyte-derived IGF-I has also been shown to be a key determinant in bone mechanosensitivity, as well as bone growth, remodeling, and regeneration (Lau et al., 2013; Sheng et al., 2014), although it is not required for bone repletion after a low-calcium challenge (Lau et al., 2015). Regulation of IGF-I expression is critically important and is accomplished a multitude of diverse systemic and local factors and their interactions.

The effects of IGF-I are mediated through binding to the IGF-IR, a transmembrane receptor tyrosine kinase arranged in an $\alpha_2\beta_2$ -configuration in which the extracellular α -subunits form a binding pocket which, upon IGF binding, causes autophosphorylation and activation of the intracellular tyrosine kinase domains of the β -subunits (LeRoith et al., 1995; Sasaki et al., 1985; Steele-Perkins et al., 1988). IGF-I and -II act physiologically in osteoblasts by binding the IGF-IR (Centrella et al., 1990; Sliotweg et al., 1990), which shares significant structural similarity with the related insulin receptor (IR). Both the IR and IGF-IR signal primarily through phosphorylation of IRS-I and -II (Kadowaki et al., 1996), both of which are necessary for proper bone metabolism; mice deficient in IRS-I display significant osteopenia (Ogata et al., 2000) and impaired bone healing (Shimoaka et al., 2004) while osteoblasts lacking IRS-2 show impaired anabolic activity and increased support of osteoclastogenesis (Akune et al., 2002). Indeed, dysregulation of some of the metabolic actions of IGF signaling in conditions such as type 2 diabetes mellitus or sarcopenia in the elderly could provide a connection between these conditions and the osteoporosis often observed in states of chronic disease. In addition, the extent to which the hepatic effects of the GH/IGF axis on bone are influenced by changes in hepatic glucose or lipid metabolism as would be seen in

diabetes remains yet to be determined. However, it is clear that IGF signaling is critical for bone development and maintenance.

2.4 IGF binding proteins

IGF-I is regulated via interaction with several binding proteins, known as IGFBP-1 through -6. Although there is a relatively large amount of IGF-I in circulation, up to 75% of IGF-I in plasma exists in a ternary complex with IGFBP-3 and an acid-labile subunit (ALS); IGFBP-3 regulates the active concentration of free IGF-I (Bagi et al., 1994; Jones and Clemmons, 1995; Rajaram et al., 1997; Rosen et al., 1994). IGFBPs can have opposing effects on IGF actions. IGFBPs-3 and -5 are generally regarded as stimulating IGF-I's effects on osteoblasts; they bind IGF-I in ternary complexes and prolong its half-life in circulation. IGFBP-5 in particular will associate with cell surface proteins, locally increasing IGF concentrations in the areas most likely to encourage binding with IGF-IRs. In addition, IGFBP-5 has been shown to act independent of IGFs (Govoni et al., 2005). By contrast, IGFBPs-1, -2, -4, and -6 are considered inhibitory; they bind IGF-I and prevent interaction with the IGF-IR (Govoni et al., 2005). For example, overexpression of IGFBP-4 in mouse osteoblasts leads to decreased bone size parameters, consistent with an increased inhibition of IGF-I activity (Zhang et al., 2003). A number of systemic agents have been shown to differentially regulate IGFBP production in varying cell types. PTH can increase expression of IGFBP-4 (Honda et al., 1996), and 1,25-(OH)₂D₃ has been shown to increase production of IGFBPs-2, -3, and -4 (Kveiborg et al., 2001; Scharla et al., 1993). Glucocorticoids increase expression of inhibitory IGFBPs-2, -4, and -6 while decreasing expression of IGFBPs-3 and -5 (Chevalley et al., 1996; Gabbitas and Canalis, 1996a; Okazaki et al., 1994), and a portion of estrogen's suppression of bone formation may be due to upregulation of IGFBP-4 expression (Denger et al., 2008; Kassem et al., 1996). Indeed, a strong interaction between IGFBP-2 and estrogen has been suggested, as *Igfbp2*^{-/-} mice show accelerated bone loss in the context of ovariectomy (DeMambro et al., 2015). Retinoic acid similarly causes an increase in inhibitory IGFBPs-4 and -6 along with a corresponding decrease in stimulatory IGFBP-5 (Gabbitas and Canalis, 1996b; Zhou et al., 1996). Local factors, including IGFs, TGF-β, BMPs, FGF, PDGF, interleukins, and IGFBP proteases, are additional regulators of IGFBP expression and activity in bone (Conover, 1995; Hayden et al., 1997; Kanzaki et al., 1994; Knutsen et al., 1995; Malpe et al., 1997; Qin et al., 2006). IGFBP actions are acutely regulated by IGFBP proteases in response to systemic and local cues. For example, studies involving transgenic overexpression and targeted knockout of pregnancy-associated plasma protein (PAPP)-A, a specific IGFBP-4 protease, have shown a key role for this protease in regulating skeletal metabolism via modulation of IGF bioavailability (Qin et al., 2006; Tanner et al., 2008). Another study showed that PAPP-A2, an IGFBP-5 protease, regulates bone size and shape (Christians et al., 2013). Thus, modulation of IGFBP protease activity may represent a therapeutic strategy to increase IGF bioavailability and to promote IGF actions in target tissues.

2.5 IGF-I action: endocrine vs. local

Although circulating IGF-I is produced largely by the liver, IGF-I is expressed by cells of all tissue types, including osteoblasts and chondrocytes. Conditional disruption of hepatic IGF-I (Sjögren et al., 1999; Yakar et al., 1999) or GHR (Fan et al., 2009) in transgenic mouse

models has shown that longitudinal bone growth was largely unaffected by up to 90% reduction in circulating IGF-I levels, although trabecular bone volume was significantly decreased in hepatic GHR-deficient mice. To further confirm the relative lack of a skeletal phenotype in mice lacking hepatic IGF-I, double and triple knockout mice were generated, which lack either total ALS or total ALS and total IGFBP-3 in addition to lacking hepatic IGF-I (Yakar et al., 2009, 2002). In these mice, circulating serum IGF-I levels were reduced by 90% and 97.5%, respectively, and although the triple knockout mice showed a significant decrease in body length, the effect was relatively small (Table 1). By contrast, the triple knockout mice exhibit a 50% reduction in cortical bone which is similar in magnitude to the reduction in cortical bone observed in total IGF-I knockout mice (Mohan et al., 2003), indicating that endocrine IGF-I plays a more significant role in regulating bone size and may act by a different mechanism in regulating bone length versus bone width/size. Indeed, cortical bone size and periosteal expansion are primary determinants of bone strength; the ability of IGF-I to increase periosteal bone formation provides an important basis for the potential use of IGF-I to treat and prevent osteoporosis and osteoporotic fractures. Furthermore, a mouse model with hepatic IGF-I knock-in in an IGF-I null background revealed a roughly 30% rescue of body size by endocrine IGF-I action (Stratikopoulos et al., 2008), and further studies in mice with hepatic disruption of the GHR (List et al., 2014) and JAK2-mediated GH signaling (Nordstrom et al., 2011) suggest that endocrine IGF-I action does indeed contribute to overall skeletal growth.

The role of local IGF-I acting in an autocrine and/or paracrine manner is also important to bone function. IGF-I is both produced by skeletal tissues and stored in mineralized matrix from which it can be released during matrix degradation and bone remodeling. Without locally produced IGFs in serum-free cultures, osteoblast proliferation was reduced by almost 50%, indicating a role for local IGF production in basal osteoblast proliferation (Mohan et al., 1989). Furthermore, mice overexpressing IGF-I only in mature osteoblasts which express osteocalcin showed augmented volume and rate of formation of trabecular bone by increasing osteoblast activity rather than number (Zhao et al., 2000). Similarly, IGF-I overexpression in osteoblasts using rat type I collagen α 1 regulatory elements showed increases in measures of bone formation and bone resorption (Jiang et al., 2006). Moreover, overexpressing other components of the IGF system which modulate local IGF bioavailability, such as IGFBPs and their corresponding proteases, also indicates that local production of IGF-I is important for bone formation (Devlin et al., 2002; Miyakoshi et al., 2001b, 1999; Qin et al., 2006; Richman et al., 1999; Zhao et al., 2000). These findings have been further confirmed both by using the osteocalcin promoter to disrupt the IGF-IR (M. Zhang et al., 2002) and by using the type I collagen α 2 promoter to disrupt IGF-I (Govoni et al., 2007b) specifically in osteoblasts; in these studies, serum IGF-I levels were unchanged while significant reductions in bone mineral density (BMD), bone size, and bone formation indices were observed (Table 1). In addition, the GH effect on osteoblast number depends on local IGF-I production (DiGirolamo et al., 2007).

Furthermore, IGF-I expression in cartilage plays a crucial role in bone development. Targeted disruption of IGF-I in chondrocytes results in decreased bone length, total body areal BMD, and bone width (Govoni et al., 2007a); the corresponding decrease in parathyroid hormone-related protein (PTHrP), *Dlx-5*, and *Sox-9* expression in the bones of

these mice indicates that locally produced IGF-I may regulate bone growth in part by modulating chondrocyte differentiation and proliferation. In addition, chondrocyte-specific IGF-I is needed for proper growth plate organization and function (Wang et al., 2015, 2011). The importance of both endocrine and locally produced IGF-I for skeletal growth is clear.

3. GH/IGF effects on bone

3.1 Bone acquisition

Though GH is largely not required for intrauterine growth (Gluckman et al., 1981; Le Roith et al., 2001), GH contributes to longitudinal bone growth and peak BMD acquisition during postnatal and pubertal growth. This effect can be seen both in mice, in which GH overexpression increases BMD (Saban et al., 1996) and lit/lit GH-deficient or GHR knockout mice have poor peak BMD (Mohan et al., 2003; Sims et al., 2000; Sjögren et al., 2000), and in humans, in which childhood onset GH deficiency leads to decreased BMD and increased fracture risk in adulthood (de Boer et al., 1994; Holmes et al., 1994; Rosén et al., 1997; Simpson et al., 2002). GH supplementation in normal human subjects increases levels of serum bone formation markers (Holloway et al., 1994), and treatment of both animals and patients deficient in GH significantly increases their reduced BMD (Bouillon and Prodonova, 2000; Cowell et al., 2000; Simpson et al., 2002). Many of these effects of GH, however, are dependent upon age and growth period; GH deficiency leads to a 4-fold greater reduction in BMD during postpubertal growth than during prepubertal growth (Mohan et al., 2003), an effect which may be explained in part by the failure of GH to appropriately activate hepatic gene transcription in prepubertal rats (Choi and Waxman, 2000). In addition, the magnitude of GH's ability to rescue BMD in GH-deficient mice depends on the age of GH administration (Kasukawa et al., 2003).

Many of the effects of GH are mediated via IGF-I. Patients with GH insensitivity, particularly Laron syndrome caused by mutations in the GHR, produce little to no IGF-I or IGFBP-3, resulting in decreased longitudinal bone growth; short body length at birth is followed by short stature during and after growth (Laron and Kauli, 2015). Mouse models of GH insensitivity indicate significant reductions in cortical bone parameters as well as measures of endosteal and periosteal bone formation (Wu et al., 2013). In GH-insensitive patients, IGF-I administration can stimulate some longitudinal bone growth, although it cannot fully compensate for the absent GH effects (Collett-Solberg et al., 2008; Laron and Kauli, 2015; Laron and Klinger, 1994; Pfäffle, 2015; Walker et al., 1992; Wu et al., 2013). Furthermore, GH and IGF synergize to produce greater effects than either would independently (Fielder et al., 1996). IGF-I has an augmented effect on osteoblasts when GH and IGFBP-3 are present (Ernst and Rodan, 1990); similarly, GH administration to mice which have been given saturating doses of IGF-I causes a further increase in growth (Hazel et al., 1994).

Although IGF-II is crucial for growth during the embryonic period, IGF-I is required for growth throughout development (Baker et al., 1993; Liu et al., 1993). Targeted *IGF-I* gene disruption in mice leads to significantly reduced femoral length, size, and BMD (Mohan et al., 2003); histomorphometric measures also show reduced bone formation and mineral apposition rates in IGF-I knockout mice (Bikle et al., 2001). These effects of IGF-I are the

result of a combination of GH-dependent and GH-independent mechanisms (Mohan et al., 2003; Mohan and Baylink, 2005; Xing et al., 2012). However, IGF-I knockout led to increased trabecular bone volume in the proximal tibia, resulting from increased trabecular connectivity, number, and spacing, and this effect was lessened in the lumbar vertebrae (Bikle et al., 2001). These conflicting data suggest a complex role for IGF-I in regulating bone structure, which may vary in cortical vs. trabecular bone compartments and in appendicular vs. axial skeletal regions. The effect of IGF-I deficiency has been confirmed in humans, in which patients with a mutant IGF-IR show growth retardation both before and after birth, and a mutant *IGF-I* gene can lead to significantly decreased BMD (Camacho-Hübner et al., 2002; Walenkamp et al., 2005; Walenkamp and Wit, 2007). Furthermore, a recent study showed a significant association between polymorphisms in the *IGF-I* gene and risk of osteoporosis in a population of Chinese women (Zhang et al., 2015). Thus, both GH and IGF-I are key regulators of peak bone mass acquisition in animals and in humans.

3.2 Bone loss

An additional IGF-independent ability of GH is to increase bone resorption and turnover (Colao et al., 1999; Thomas and Monson, 2009), as seen in the increased risk of fracture in patients with excess GH (Claessen et al., 2013; Mazziotti et al., 2013, 2008; Mormando et al., 2014; Padova et al., 2011; Wassenaar et al., 2011) and the deterioration of bone architecture and strength observed in GH-overexpressing mice (Lim et al., 2015). This increase in bone turnover may be mediated at least in part by increased expression of inflammatory cytokines such as interleukin-6 (Swolin and Ohlsson, 1996).

However, the more significant manner in which the GH/IGF system contributes to bone loss is through its overall decline with age. GH secretion is known to decrease as an individual ages (Corpas et al., 1993; Müller et al., 1999), a result of the confluence of any of a number of multiple factors including decreased GHRH stimulation, increased somatostatin inhibition, decreased sex steroid levels, lower levels of physical activity, poor sleeping patterns, inadequate nutrition, and increased negative feedback from GH and IGF-I (Bartke, 1998; Misra et al., 2003; Veldhuis and Bowers, 2003; Veldhuis and Iranmanesh, 1996). In addition, serum and bone IGF-I decrease in both concentration (Benbassat et al., 1997; Seck et al., 1999) and effectiveness at stimulating osteoprogenitor cells (Tanaka and Liang, 1996) with age, and IGF-I levels correlate well with BMD in postmenopausal women and men with or without osteoporosis (P. Gillberg et al., 2002; Muñoz-Torres et al., 2001). IGFBPs also change with age (Karasik et al., 2002; Mohan et al., 1995; Nicolas et al., 1995). In fact, a cross-sectional study of elderly women with femoral neck fractures revealed significantly decreased levels of IGF-I, IGF-II, IGFBP-3, and IGFBP-5, suggesting deficiencies in components of the IGF system may predispose elderly women to fracture (Boonen et al., 1999). However, there are also reports in the literature that show little to no effects of IGFs on fracture risk (Gillberg et al., 2001; Kassem et al., 1994b; Martini et al., 2001; Seck et al., 1999; Zofková et al., 2001). Thus, the extent to which IGF system components contribute to age-related bone loss is yet to be determined.

4. GH/IGF therapy

4.1 Therapy in children

Childhood deficiency in major components of the GH/IGF axis results in severely impaired longitudinal bone growth and failure to attain normal height in adulthood. Thus, treatment with GH or IGF-I is a logical therapy for children with such conditions. In fact, long-term administration of GH in children with GH-deficient conditions including isolated GH deficiency and multiple pituitary hormone deficiency significantly improved age-adjusted height in a manner consistent with growth approaching the children's genetic height potential (Ross et al., 2015). GH has even been effectively used to promote growth in children without proven GH deficiencies, born small for gestational age or with idiopathic short stature. However, further studies are needed to determine the safety and magnitude of increase in final height resulting from long-term GH therapy in all patient groups (Pfäffle, 2015). Furthermore, children with GH insensitivity, most commonly caused by resistance to GH due to mutations in the GHR (called Laron syndrome, described in section 3.1), have been successfully treated with IGF-I (Laron and Kauli, 2015). The inability of IGF-I to fully compensate for the lack of GH action in these patients may be attributable to the lack of IGFBP-3 carrying, stabilizing, and enhancing IGF-I in circulation as well as the lack of direct GH stimulation in bone (Pfäffle, 2015).

4.2 Therapy in adults

Since GH and IGF-I appear to be involved in age-related and osteoporotic bone loss, multiple clinical trials have been run to evaluate the possible therapeutic effects of GH and IGF-I administration in patients with and without osteoporosis. The first trial of GH therapy in patients with primary and secondary osteoporosis found increased periosteal bone formation and osteoblast activity, along with increased bone turnover (Kruse and Kuhlencordt, 1975). Since then, the effects of GH therapy have been assessed in healthy subjects (Bianda et al., 1997; Brixen et al., 1990; Holloway et al., 1994; Rudman et al., 1990), women with postmenopausal osteoporosis (Brixen et al., 1995; Clemmesen et al., 1993; Erdtsieck et al., 1995; Holloway et al., 1997, 1994; Joseph et al., 2008; Kassem et al., 1998, 1994a, 1994b; Landin-Wilhelmsen et al., 2003; Sääf et al., 1999; Sugimoto et al., 2002, 1999), and men with idiopathic osteoporosis (Peter Gillberg et al., 2002; Johansson et al., 1996). Overall, GH treatment has been well-tolerated, with few side effects beyond fluid retention and carpal tunnel syndrome. GH administration has resulted in increased bone turnover with a net anabolic effect and increased BMD (Locatelli and Bianchi, 2014). However, a minority of studies showed no net improvement with GH treatment (Clemmesen et al., 1993; Erdtsieck et al., 1995; Holloway et al., 1994; Sääf et al., 1999); in these cases, the lack of a positive GH effect is likely due to a combination of confounding factors such as poor nutrition as well as the low dosage of GH administered. In addition, dysregulation of PTH secretion patterns and peripheral PTH sensitivity are rescued by GH treatment in women with postmenopausal osteoporosis, further contributing to an increased BMD (Joseph et al., 2008; White et al., 2005). By contrast, concomitant estrogen administration attenuated GH effects on bone (Holloway et al., 1994), lending confirmation to the previous observation that oral estrogen administration decreases hepatic IGF-I production (Ho and Weissberger, 1992). Since GH treatment has been shown to increase both bone formation

and resorption, GH therapy has not been evaluated fully to treat osteoporotic patients. Promisingly, a recent study has reported that GH therapy in postmenopausal women with osteoporosis significantly improved BMD and fracture outcomes after ten years, although overall quality of life measures were unaffected (Krantz et al., 2015). What effect this report will have on the use of GH to treat osteoporosis remains to be seen.

Moreover, exogenous GH administration has been used to supplement GH-deficient adults, whose symptoms can include metabolic syndrome, osteoporosis, muscle wasting, and overall diminished quality of life (Tzanela, 2007). In general, GH replacement in GH-deficient adults has been shown to increase BMD in the context of augmented markers of bone formation and decreased markers of bone resorption (Kuřma et al., 2014). However, a recent study suggests that GH only reduces the incidence of fractures in GH-deficient adults without preexisting osteoporosis; it appears that GH therapy cannot compensate for the combined effects of GH deficiency and osteoporosis (Mo et al., 2015). A possible interpretation of these data would suggest that beginning GH administration in these GH-deficient patients before the onset of osteoporosis might prevent future fractures.

Additionally, recombinant human IGF-I (rhIGF-I) has also been investigated in several clinical studies for the treatment of osteoporosis. On the whole, IGF-I therapy resulted in significantly increased bone turnover and improved bone formation, with no significant adverse effects reported (Locatelli and Bianchi, 2014). IGF-I administration has been studied in healthy subjects (Bianda et al., 1997; Ghiron et al., 1995; Mauras et al., 1996), fasting subjects (Grinspoon et al., 1995), anorexic/osteopenic subjects (Grinspoon et al., 2002, 1996; Misra et al., 2009), postmenopausal women with and without osteoporosis (Ebeling et al., 1993; Friedlander et al., 2001), osteopenic men (Johansson et al., 1996, 1992), subjects with osteoporosis secondary to Werner syndrome (Rubin et al., 1994), and subjects with glucocorticoid-induced osteoporosis (Berneis et al., 1999). The only study which did not find any differences between IGF-I treatment and placebo (Friedlander et al., 2001) was a relatively long-term study in which negative feedback from exogenous IGF-I could have suppressed GH secretion. By contrast, other long-term studies have shown a positive IGF-I effect (Locatelli and Bianchi, 2014). In addition, treatment of patients with osteoporotic femoral fractures with an IGF-I/IGFBP-3 complex led to recovery of lost bone mass (Boonen et al., 2002). Combined treatment with IGF-I and IGFBP-3 seems to have allowed for safe and effective administration of higher doses of IGF-I than would have been possible with IGF-I alone; however, this theoretical benefit has not always been observed, and use of the combined treatment may not be indicated (Pfäffle, 2015). In summary, both short-term and long-term IGF-I treatment can increase bone turnover with a predominantly anabolic effect.

5. Conclusions

The GH/IGF axis is regulated by a host of interacting mechanisms and factors, particularly as it participates in the regulation of skeletal growth and maintenance. Age-related loss of bone mass occurs in part due to a decrease in components of the GH/IGF system with age. Therefore, supplemental anabolic GH and IGF-I therapies for the treatment of age-related bone loss and osteoporosis show great promise, especially as they demonstrate a relatively

safe adverse effect profile. Ultimately, a better understanding of the GH/IGF axis will allow greater pharmacological manipulation of the pathophysiological changes which lead to diseases of low bone mass, helping to treat and, finally, to prevent debilitating conditions which plague so many worldwide.

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Abbreviations

1,25-(OH)₂D₃	1,25-dihydroxyvitamin D ₃
ALS	acid-labile subunit
GH	growth hormone
GHR	growth hormone receptor
GHRH	growth hormone releasing hormone
GHRP	GH-releasing peptide
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGF-IR	insulin-like growth factor-I receptor
IR	insulin receptor
IRS	insulin receptor substrate
PAPP-A	pregnancy-associated plasma protein-A
PAPP-A2	pregnancy-associated plasma protein-A2
PTH	parathyroid hormone
SS	somatostatin
TH	thyroid hormone

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Highlights

- GH and IGF-I are major regulators of skeletal metabolism.
- GH affects the skeleton with and without IGF-I mediation.
- IGF-I is regulated through its synthesis, receptors, IGFBPs, and IGFBP proteases.
- Deficiency in GH/IGF-I actions contributes to the pathogenesis of osteoporosis.
- GH and IGF-I therapies have increased bone turnover with mainly anabolic effects.

Fig. 1.

Model overview of GH/IGF-I regulation of skeletal growth, including both endocrine and local actions of IGF-I. GH acts by increasing hepatic IGF-I production, by increasing local skeletal IGF-I production, and by influencing the bone directly, independent of IGF-I. Hepatic IGF-I acts in an endocrine manner, circulating in the blood primarily as a ternary complex with acid-labile subunit (ALS) and IGF binding protein (IGFBP)-3, although IGF-I can also complex with other IGFBPs. Only a small amount circulates as free IGF-I. Locally produced IGF-I acts on the bone in an autocrine/paracrine manner.

Table 1

Skeletal changes in transgenic mouse models of disrupted endocrine and local IGF-I actions.

Mode of action	Gene(s) altered	Bone length	Periosteal circumference	BMD
Endocrine + Local	Total IGF-I KO ¹	40% decrease	38% decrease	32% decrease
Endocrine	Hepatic IGF-I KO ²	6% decrease	11% decrease	9% decrease
Endocrine	Total ALS KO ²	7.5% decrease	18% decrease	9% decrease
Endocrine	Hepatic IGF-I + Total ALS KO ²	20% decrease	33% decrease	6% decrease
Local	Osteoblast IGF-I KO ³	15% decrease	24% decrease	25% decrease (DXA) ⁷ 5% decrease (pQCT)
Local	Osteocyte IGF-I KO ⁴	5% decrease	12% decrease	No change
Local	Chondrocyte IGF-I KO ⁵	6% decrease	4% decrease	5% decrease (DXA) ⁷
Local	Osteoblast IGFBP-4 OE ⁶	14% decrease	29% decrease	No change

Abbreviations: BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; KO, knock-out; OE, overexpression; pQCT, peripheral quantitative computed tomography.

¹(Mohan et al., 2003)

²(Yakar et al., 2002)

³(Govoni et al., 2007b)

⁴(Sheng et al., 2013)

⁵(Govoni et al., 2007a)

⁶(Zhang et al., 2003)

⁷BMD measured by DXA may be affected by bone size.