Growth Hormone, Insulin-Like Growth Factors, and the Skeleton

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GH and IGF-I are important regulators of bone homeostasis and are central to the achievement of normal longitudinal bone growth and bone mass. Although GH may act directly on skeletal cells, most of its effects are mediated by IGF-I, which is present in the systemic circulation and is synthesized by peripheral tissues. The availability of IGF-I is regulated by IGF binding proteins. IGF-I enhances the differentiated function of the osteoblast and bone formation. Adult GH deficiency causes low bone turnover osteoporosis with high risk of vertebral and nonvertebral fractures, and the low bone mass can be partially reversed by GH replacement. Acromegaly is characterized by high bone turnover, which can lead to bone loss and vertebral fractures, particularly in patients with coexistent hypogonadism. GH and IGF-I secretion are decreased in aging individuals, and abnormalities in the GH/IGF-I axis play a role in the pathogenesis of the osteoporosis of anorexia nervosa and after glucocorticoid exposure. (*Endocrine Reviews* 29: 535–559, 2008)

I. Introduction

GH AND IGF-I ARE IMPORTANT regulators of bone homeostasis throughout life (1). During the prepubertal period, GH and IGF-I are determinants of longitudinal bone growth, skeletal maturation, and acquisition of bone

mass, whereas in adults they are important in the maintenance of bone mass (2, 3).

Longitudinal bone growth is determined by chondrocyte proliferation and differentiation in the epiphyseal growth plate of long bones, leading to endochondral bone formation. Within the growth plate, chondrocyte proliferation, hypertrophy, and differentiation result in chondrogenesis. The newly formed cartilage is invaded by blood vessels, and it is modeled into bone trabeculae. This process, called endochondral ossification, is regulated by genetic and hormonal factors, the cellular environment, and nutrition (4). During embryonic development, IGF-I and IGF-II are key determinants of growth, acting independently of GH (5). Postnatally and throughout puberty, GH and IGF-I play a critical role in determining longitudinal skeletal growth, (6–8) and children with GH deficiency (GHD) display short stature.

In addition to the effects on longitudinal growth, GH and IGF-I are anabolic hormones and have the potential to regulate bone modeling and remodeling. Bone remodeling is a temporally regulated process of coordinated bone resorption and bone formation carried out in microscopic basic multicellular units (9, 10). There, multinucleated osteoclasts are attracted to specific sites to resorb bone. When resorption is completed, there is a reversal period and mononuclear osteoblasts are attracted to fill the cavity with newly synthesized matrix. This is followed by a resting phase. Bone remodeling is necessary to maintain calcium homeostasis and to remove potentially damaged bone. Bone modeling occurs mostly during growth. In contrast to bone remodeling, bone modeling is a process of uncoupled bone formation and bone resorption (9, 10). Often it is regulated by mechanical forces, and it serves to maintain bone shape and mass. GH and IGF-I exert their anabolic effects on trabecular and cortical bone. The latter occurs by periosteal bone apposition, a process of matrix deposition at the outer surface of bone, resulting in increased bone width, and skeletal strength (11). Effects on

periosteal apposition by GH or IGF-I may explain the characteristic bone deformities occurring in acromegaly.

The anabolic effects of GH and IGF-I in bone are important for the acquisition of bone mass during adolescence and possibly for the maintenance of skeletal architecture during adult life. Late adolescence and early adulthood are critical periods for the acquisition of bone mass, and the achievement of peak bone mass (12–14). This is a critical determinant of future risk of osteoporosis (12, 13). The precise time of the attainment of peak bone mass is not certain, and it is skeletal-site dependent. The increase in gonadal steroid synthesis at the time of puberty is an important hormonal regulator of bone accretion. Boys with constitutionally delayed puberty achieve lower peak bone mass than normal boys (15). Systemic and local skeletal IGF-I play a role in bone formation and the maintenance of bone mass (16). This review will highlight the mechanisms of GH and IGF-I actions in bone, skeletal abnormalities occurring in GHD and acromegaly, and the role of GH and IGF-I in the pathophysiology of selected forms of osteoporosis.

II. The Growth Hormone (GH)/Insulin-Like Growth Factor-I (IGF-I) Axis

GH is a single-chain peptide of 191 amino acids. GH was isolated from somatotrophs, cells of the anterior pituitary gland, in 1956 and first used for the treatment of pituitary dwarfism in 1958 (17). The synthesis of GH is under the control of central and peripheral signals (18). The synthesis and release of GH are promoted by GHRH and inhibited by somatostatin and are regulated by a negative feedback mechanism (18). IGF-I, which is secreted by the liver under GH control, inhibits GH secretion directly in somatotrophs and indirectly by stimulating the release of somatostatin (18). Ghrelin, a 28-amino acid peptide synthesized by cells of the gastrointestinal tract, is an endogenous inducer of GH release, acting on somatotrophs and the hypothalamus (19–22). Ghrelin also was reported to stimulate the proliferation of osteoblasts *in vitro* (23–28). GH secretion is under the influence of additional hormonal signals, and sex steroids and thyroid hormone stimulate, whereas glucocorticoids inhibit, GH secretion (18, 29–32).

Serum GH levels decline with age, reaching a nadir at the sixth decade (18, 33). In aged men, the daily GH secretion is 1/5 to 1/20 that observed in young adults (34). GH output decreases twice as rapidly in men as in women so that GH release remains higher in women than in men after the age of 50 (35, 36). The age-dependent decline in GH secretion is secondary to a decrease in GHRH and to an increase in somatostatin secretion (18). These changes occur at the hypothalamic level, but their cause is unknown. A reduction in central cholinergic tone leading to an increase in somatostatin release possibly explains the change in GH secretion (37). The decline in the production of sex steroids, physical activity, and the presence of aberrant sleep patterns also may contribute to the decline in GH levels during aging (38). As a consequence of the decline in GH synthesis and release, systemic IGF-I levels decline with advancing age (39). The changes in GH and IGF-I secretion that occur with aging are paralleled by a progressive loss of muscle mass and strength, a decline in physical performance, an increase in body fat, and a decrease in bone mineral density (BMD) (40–43).

GH circulates bound to a GH-binding protein, which is the extracellular domain of the GH receptor (GHR) (44, 45). The GH-binding protein is generated by proteolytic cleavage of the extracellular domain of the GHR or by mRNA splicing (46). GH-binding protein is synthesized primarily by the liver, although synthesis by extrahepatic tissues, such as muscle and adipose tissue, may contribute to the circulating level of GH-binding protein (47, 48). The serum levels of the GH-binding protein serve as a marker of GHR expression and GH responsiveness in target tissues (46). The function of the GH-binding protein is incompletely understood, although it may modulate the activity of GH either by prolonging its half-life or by reducing its availability to the GHR.

GH exerts its effects by binding to a single-chain transmembrane glycoprotein receptor. The GHR consists of an extracellular, a transmembrane, and an intracellular domain. Activation of the receptor occurs by ligand-induced dimerization and internalization of the receptor to initiate signaling, primarily by the activation of the Janus tyrosine kinase 2. This leads to autophosphorylation and to phosphorylation of the internalized GHR and recruitment and activation of intracellular proteins of which the signal transducers and activators of transcription (STAT) are the most important, although additional pathways can operate in GH signal transduction (49, 50). The GHR is highly expressed in the liver, adipose tissue, heart, kidneys, intestine, lung, pancreas, cartilage, and skeletal muscle. GH acts by inducing the synthesis of IGF-I by the liver. However, the physiology of IGF-I is complex because it acts as a circulating hormone and as a local growth factor (51). Systemic IGF-I is synthesized primarily in the liver, where its synthesis is GH dependent (51). IGF-I also is synthesized in multiple extrahepatic tissues, where it acts as a local growth factor under the control of diverse hormones. IGF-I circulates as part of a 150-kD complex formed by one molecule each of IGF-I, IGF-binding protein (IGFBP)-3, the predominant circulating binding protein, or IGFBP-5, and the acid labile subunit (ALS) (52). There are six IGFBPs, and IGFBP-1, -2, -4, and -6 also can bind IGF-I in the circulation and peripheral tissues but do not form part of the ternary complex. IGFBPs are in concentrations in excess of IGF-I. Consequently, IGF-I circulates mostly bound to the complex, and less than 1% of total serum IGF-I circulates as a free hormone. The 150-kD ternary complex stabilizes IGF-I, prolonging its circulating half-life and regulating its availability to target tissues (52). Consequently, the ternary complex plays an important role in determining the endocrine function of IGF-I. ALS is synthesized in the liver under the control of GH and circulates in excess over the other components of the complex, so that it plays a critical role in the storage and release of IGF-I (52). ALS is absolutely necessary for the accumulation and maintenance of serum IGF-I and IGFBP-3, and *als* null mice have marked reductions in serum IGF-I and IGFBP-3 levels, because the ternary complex cannot be formed (53). Despite this decrease in serum IGF-I, growth of *als* null mice is only mildly impaired, but this is consistent with the modest growth deficit found in mice with conditional deletions of *igf-1* in the liver and confirms that

systemic IGF-I is dispensable for postnatal linear growth (54). In addition to its function as a systemic hormone, IGF-I plays an important role in the autocrine and paracrine regulation of cell metabolism in a variety of tissues, including cartilage and bone. Locally, the availability and activity of IGF-I also is regulated by IGFBPs, and *in vitro* studies have demonstrated that at the tissue level most of the IGF is bound to IGFBPs, with a small fraction present in the unbound free form. However, *in vivo* binding interactions between IGF-I and IGFBPs at the tissue level have not been explored. The cellular actions of IGF-I are mediated by the IGF-I receptor (IGF-IR), a receptor tyrosine kinase that is expressed in IGF target tissues (55).

IGF-II shares biochemical and biological properties with IGF-I; it is important in skeletal development, but its function in the adult skeleton is not proven. IGF-II is synthesized by skeletal cells, but its synthesis is not GH dependent. The IGF-II/mannose 6 phosphate receptor does not play a major role in IGF signal transduction and is responsible for clearing IGF-II, regulating its levels, during fetal development (56).

III. Mechanisms of GH and IGF-I Action in Bone

The skeletal effects of GH and IGF are modulated by complex interactions between circulating IGF-I and IGFBPs and the locally produced IGF-I and IGFBPs. IGF-I and IGF-II are the most abundant growth factors present in skeletal tissue, and their synthesis and activity are regulated by systemic hormones, such as GH and PTH (57, 58). GH may act by inducing IGF-I in bone or may have direct effects on skeletal cells (Table 1).

A. GH

1. GHR and signaling. GHRs are expressed by chondrocytes and osteoblasts (59–64). The secretion of GH as well as the expression of GHRs are down-regulated by IGF-I, acting as a systemic and local feedback control mechanism (65). IGFBPs, by binding IGF-I, up-regulate GHR expression (66, 67). GH signaling in osteoblasts is mediated by a cascade of protein phosphorylation steps resulting in the activation of transcription factors. GH signals through Janus tyrosine kinase 2/STAT signal transduction pathway (68). GH utilizes

TABLE 1. Effects of GH on bone

Functions	Effects
Growth plate	
Replication of condrocytes	↑ ↑
Endochondral bone formation	↑ ↑
Bone remodeling unit	
Osteoblastogenesis	↑
Proliferation of osteoblasts	↑
Function of mature osteoblasts	↔ ↑
Production of osteoprotegerin	↑
Production of RANK-L	↔
Calcium metabolism	
Phosphate retention	↑

Effects of GH on bone. ↔ no effect; ↑ minor stimulating effect; ↑ ↑ major stimulating effect.

STAT 5 to regulate IGF-I expression in the liver, but the function of STAT 5 in bone cells is not clear because *stat 5* null mice appear to have normal bone remodeling (69, 70). *Ghr* null mice exhibit decreased bone remodeling, which is rescued by IGF-I, suggesting a role of GH/IGF-I in bone remodeling that is independent of STAT 5. Using a conditional deletion approach, STAT 5 was found to mediate the effects of GH on IGF-I expression in skeletal muscle, but similar experiments have not been conducted in skeletal cells to establish the role of STAT 5 in bone remodeling (71). GH also signals through ERK1 and -2 and MAPKs that regulate osteoblastic cell growth (72–75). Acting through STATs and ERKs, GH may modulate the activity of runt-related transcription factor-2, which is an intracellular protein required for osteoblast cell differentiation (76, 77).

2. In vitro studies. GH stimulates the proliferation of cells of the osteoblastic lineage (61, 78–80), although IGF-I is required for selected anabolic effects of GH in osteoblasts (81). GH affects the fate of mesenchymal precursors favoring osteoblastogenesis and chondrogenesis and opposing adipogenesis (82). Mesenchymal precursor cells can differentiate into adipocytes, osteoblasts, and chondrocytes in a tightly controlled process (83). Signals that enhance osteoblastogenesis often suppress adipogenesis, and adipocytes are increased in the bone marrow of patients with osteoporosis and are decreased during bone formation and chondrocyte proliferation (82, 84, 85). GH down-regulates the expression of fetal antigen-1, which is the soluble form of delta like-1 or Pref-1, and as a consequence may regulate adipogenesis (86, 87). GH also stimulates the expression of bone morphogenetic proteins, which are important for the differentiation of osteoblasts and for bone formation (88–90).

In addition to its effects on the differentiation of cells of the osteoblast lineage, GH stimulates, either directly or indirectly through IGF-I, the differentiated function of the mature osteoblast. GH *also* stimulates the carboxylation of osteocalcin, which is a marker of osteoblastic function (91, 92).

During bone remodeling, bone formation is coupled to bone resorption so that bone-forming osteoblasts fill resorbed bone surfaces with newly synthesized matrix. In addition, osteoblastic signals are necessary to initiate bone resorption so that bone resorption and formation are highly coordinated processes and agents targeting the osteoblast may influence osteoclast formation and function. Critical to these events are the receptor activator of nuclear factor κB ligand (RANK-L) and its decoy receptor osteoprotegerin (93, 94). RANK-L is synthesized by osteoblastic stromal cells and, in the presence of colony-stimulating factor-1, induces osteoclast formation. Osteoprotegerin binds RANK-L and competes with the RANK-L receptor, RANK, present on the surface of osteoclast precursors. As a consequence, osteoprotegerin impairs osteoclastogenesis. GH stimulates the production of osteoprotegerin and its accumulation in the bone matrix (95–98).

GH stimulates longitudinal bone growth, suggesting either a direct effect of GH on chondrocytes or an effect mediated by the local IGF-I (99–101) (Fig. 1). GH may act directly to stimulate the replication of cells in the germinal layer of the epiphyseal plate or indirectly through its stimulatory effect on IGF-I expression, acting at later stages of maturation (102). The growth
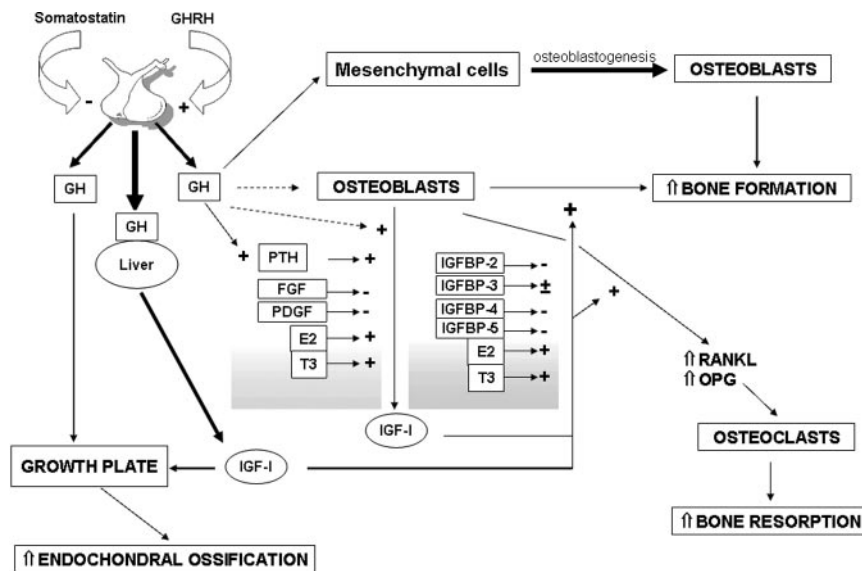
FIG. 1. Effects of GH and IGF-I on bone. ---> not consistently demonstrated effect; —> minor stimulating effect; ➡ major stimulating effect. FGF, Fibroblast growth factor; E2, estradiol; OPG, osteoprotegerin.

plate consists of three layers of chondrocytes in various stages of differentiation: the resting zone, the proliferative zone, and the hypertrophic zone (4). In the resting zone, chondrocytes replicate at a slow rate and act to replenish the pool of proliferative chondrocytes. In the proliferative zone, chondrocytes replicate at a high rate, and the resulting daughter cells line up along the long axis of the bone, where they differentiate terminally into hypertrophic chondrocytes in the hypertrophic zone. The hypertrophic zone is invaded by blood vessels and bone cells, the zone is calcified, and new endochondral bone is formed (102). Cell maturation appears to be an important factor determining the response of epiphyseal chondrocytes to GH and IGF-I (103–106).

3. In vivo studies. Mice lacking both the *ghr* and *igf-1* genes exhibit a more severe growth phenotype than either mutant alone, suggesting that GH and IGF-I have independent effects on linear growth (107). *Ghr* and *igf-1* null mice each exhibited a 25–35% reduction in the length and a 35–45% reduction in the width of tibiae, whereas double *ghr/igf-1* null mutants exhibited a 50% reduction in the length and 60% reduction in the width of tibiae at 6 months of age (107). Accordingly, *ghr* null, *igf-1* null, and dual mutants exhibited a 28, 35, and 67% reduction in body length, respectively. The phenotype was attributed to decreased proliferation and maturation of growth plate chondrocytes.

GH also influences bone metabolism indirectly by modulating PTH secretion and its circadian levels (108). The effect is mediated in part by changes in serum phosphate levels (109, 110). Serum PTH peaks around 1700 h, coinciding with the serum phosphate peak (111–113). GH favors phosphate retention by increasing the renal threshold for phosphate, an effect that is independent of PTH and vitamin D activity (114). In addition, GH and IGF-I modulate the activity of the renal 1α-hydroxylase and 24-hydroxylase, activating the former and inhibiting the latter, with an increase in the production of active 1,25 dihydroxyvitamin D_3 (115). These mechanisms may contribute to an increase in extracellular

calcium-phosphate product, and possibly bone mineralization.

B. IGF-I

1. Regulation of local IGF-I synthesis. When synthesized by peripheral tissues, IGF-I expression is controlled by diverse hormones and by growth factors. In chondrocytes, IGF-I synthesis is under GH control, whereas in osteoblasts its synthesis is fundamentally under the control of PTH (102, 116) (Fig. 1). The *igf-1* gene contains six exons and has alternate promoters in exons 1 and 2 (117, 118). The *igf-1* gene generates multiple heterogeneous transcripts due to the presence of multiple transcription initiation sites in two promoters, alternative splicing, and different polyadenylation signals. IGF-I splice variants have been reported in muscle, where their expression can be regulated by mechanical forces (119). The mature IGF-I peptide is encoded by exons 3 and 4, whereas exons 5 and 6 encode alternate carboxy-terminal extension peptides of undetermined function. Exons 1 and 2 encode mutually exclusive 5′ untranslated regions. The exon 1 promoter has four transcription initiation sites and is responsible for the regulation of IGF-I expression in most extrahepatic tissues including bone (120, 121). The IGF-I exon 2 promoter has two transcription initiation sites and is responsible for the transcriptional regulation of IGF-I by GH in the liver (120). IGF-I exon 2 is minimally expressed by osteoblasts, and GH is not a major inducer of IGF-I in these cells (116, 120). PTH and other inducers of cAMP in osteoblasts increase IGF-I expression (116). IGF-I mediates selected anabolic actions of PTH in bone *in vitro* and *in vivo* (122–127). IGF-I can reproduce selected effects of PTH on cell proliferation and survival (128). Estrogens increase and glucocorticoids decrease IGF-I transcription in osteoblasts (129–132) (Fig. 1), and selected inhibitory effects of glucocorticoids on bone metabolism can be explained by reduced IGF-I levels in the bone microenvironment. However, glucocorticoids have complex effects on osteoblastogenesis and direct effects on

osteoblastic gene expression. Glucocorticoids oppose Wnt/ β-catenin signaling and activity by destabilizing β-catenin and, as a consequence, oppose Wnt effects on osteoblastogenesis (133). Thyroid hormones are critical regulators of skeletal development and maturation, and they increase bone remodeling. T_3 increases IGF-I synthesis by osteoblasts, and IGF-I can mediate anabolic actions of T_3 in bone (134, 135). In addition to hormones, skeletal growth factors regulate IGF-I synthesis. Growth factors with mitogenic properties, such as platelet-derived growth factor (PDGF) and fibroblast growth factor decrease IGF-I transcripts and polypeptide levels in osteoblasts (136). This inhibition of IGF-I synthesis correlates with their inhibitory actions on osteoblastic differentiated function (136). In contrast, bone morphogenetic protein-2, an agent that enhances osteoblastic differentiation and function, increases IGF-I synthesis in osteoblasts (88).

2. IGF-I receptor and signaling. IGF-I signals through the IGF-IR, a transmembrane glycoprotein tetramer with ligand-activated tyrosine kinase activity. Upon ligand binding, the IGF-IR dimerizes and undergoes autophosphorylation, leading to the activation of the insulin receptor substrate (IRS)-1 and IRS-2 (55). IRS-1 and -2 mediate the effects of IGF-I in osteoblasts. IGF-I utilizes the phosphatidylinositol-3 kinase pathway, which induces the activation of Akt, and the MAPK pathway, which activates p38, Jun-N-terminal kinases, and ERK 1/2 (137–139). The usage of each pathway is dependent on culture conditions and the stage of cell differentiation. IGF-IR number and affinity are regulated by hormones and growth factors. In osteoblasts the IGF-IR is modulated by PDGF, glucocorticoids, and 1,25-dihydroxyvitamin D_3 (140–142).

3. In vitro studies. IGF-I and IGF-II are expressed by osteoblasts and exert similar biological actions, but IGF-I is more potent than IGF-II. IGF-I has modest effects on the proliferation of cells of the osteoblastic lineage, and although IGF-I does not direct the differentiation of undifferentiated stromal cells toward cells of the osteoblastic lineage, IGF-I enhances the function of the mature osteoblast (143, 144). The fundamental role of IGF-I is the stimulation of osteoblastic function and bone formation. IGF-I up-regulates type I collagen transcription and decreases the synthesis of collagenase 3 or matrix metalloproteinase 13, a collagen-degrading protease (145). This dual action, an increase in collagen synthesis and a decrease in its degradation, is important to maintain appropriate levels of bone matrix and bone mass. In accordance with the effects of IGF-I on osteoblastic function, its synthesis is regulated by factors that regulate the differentiated expression of the osteoblastic phenotype (Fig. 1). It is important to note that whereas IGF-I stimulates the differentiated function of the osteoblast, it does not have a direct effect on the differentiation of stromal cells toward mature osteoblasts (146). Indirectly, IGF-I may favor osteoblastogenesis by stabilizing β-catenin, a signaling molecule used by the Wnt canonical signaling pathway, which is essential for osteoblastogenesis (147, 148). When Wnt signals, β-catenin is stabilized and translocates to the nucleus, where it associates with members of the T cell factor/lymphoid enhancer factor

family of nuclear proteins to regulate transcription. IGF-I favors stabilization of β-catenin by inducing phosphatidylinositol-3 kinase and activating Akt, which phosphorylates and degrades glycogen synthase 3 kinase, the enzyme that phosphorylates β-catenin for its degradation by ubiquitination (147, 148). It is of interest that the IGF-I signaling molecule IRS-1 can associate with β-catenin and regulate its activity (149). The phosphatidylinositol-3 kinase/Akt pathway also is used by IGF-I to decrease osteoblast apoptosis. This effect and the modest mitogenic activity of IGF-I cause an increase in the number of osteoblasts *in vitro*. Microarray analysis of cells of the osteoblast lineage at various stages of differentiation has demonstrated down-regulation of IGF-I expression from preosteoblastic cells to fully differentiated osteoblasts (150). It would appear that a decline in IGF-I expression is necessary for cellular death to occur and to allow for the terminal differentiation of osteoblasts.

Less clear is the function of IGF-I on osteoclasts than in cells of the osteoblastic lineage. Osteoclasts express IGF-I receptors, and IGF-I has direct effects on their function (151). *In vitro*, IGF-I induces RANK-L synthesis and, as a consequence, osteoclastogenesis (152, 153). The induction of RANK-L by IGF-I may explain the stimulatory effects of IGF-I on bone resorption, whereas the induction of osteoprotegerin by GH may temper these effects. The fact that IGF-I has a dual role enhancing bone formation and bone resorption may explain why it has modest effects on bone mass *in vivo*.

4. In vivo studies. IGF-I has been tested for its effects on bone metabolism in experimental animals, where the effects on bone formation observed *in vitro* have been confirmed. Null mutations of *igf-1*, *igf-2*, or *igf-1r* in mice cause growth arrest (154–156). Data from *igf-1* and *igf-1r* gene deletions provide valuable information on the role of IGF-I during development, and conditional deletions of these genes in bone have provided information on the effects of IGF-I in the adult skeleton (154, 157). *Igf-1* null mutants exhibit impaired chondrocyte maturation and shortened femoral length, confirming the role of IGF-I in the regulation of chondrocyte differentiation (154, 156). Mice that survive exhibit reduced cortical, but not trabecular, bone, possibly due to a compensatory increase in GH secretion or due to a decrease in trabecular bone resorption (158). Severe developmental abnormalities and frequent lethality have prevented a detailed analysis of the adult skeletal phenotype of *igf-1* null mutants. *Igf-1* null mice also exhibit decreased number of functional osteoclasts, indicating that IGF-I is required for normal osteoclastogenesis (159). As a consequence, *igf-1* null mice have increased bone volume. The null mutation of the *igf-1r* also causes severe growth retardation and perinatal lethality (154). In accordance with these observations, *irs-1* null mutants display impaired chondrocyte proliferation and early closure of the growth plates, resulting in a marked reduction in growth and weight (160).

The study of genetically engineered mice has provided additional insight into the actions of systemic and locally produced IGF-I in osteoblasts *in vivo*.

a. Effect of circulating IGF-I. Mice carrying mutations of the *ghrh receptor* (lit/lit mouse) or the *gh receptor* have absent GH

secretion or action and consequently low serum IGF-I levels (70, 161, 162). These models allow for the determination of the contribution of systemic IGF-I to the skeleton, and the phenotype of either mutant is characterized by small growth plates, osteopenia, and reduced cortical bone, but normal trabecular bone. This suggests a more pronounced role of systemic IGF-I on cortical than on trabecular bone. Mice carrying a liver-specific *igf-1* deletion display a reduction in total serum IGF-I levels, normal free IGF-I levels, and a modest skeletal phenotype, characterized by a decrease in cortical volume, secondary to a reduction in periosteal bone formation (16, 54, 163). The normal serum levels of free IGF-I are attributed to IGF-I synthesis by nonhepatic sources (54). Mice carrying deletions of *igf-1* and the *als* display marked reductions in total serum IGF-I, a more significant decrease in cortical bone and a decrease in trabecular bone volume. These observations confirm the contribution of systemic IGF-I to cortical bone integrity and to a lesser extent to trabecular bone. The correlation between IGF-I and bone mass also has been documented in specific mouse strains that have allelic differences at key genomic points. The generation of congenic mice has advanced our understanding of the genetic regulation of selected phenotypes. Two inbred strains C3H/Hej (C3H) and C57BL/6J display differences in BMD, which correlate closely with differences in serum IGF-I levels. Quantitative trait locus analysis revealed the presence of one major quantitative trait locus (*Igf1sl-1*) in chromosome 6 of the C3H genome with major effects on serum IGF-I concentrations (164). Congenic mice carrying *Igf1sl-1* on a C57BL/6J genetic background display a 25% decrease in circulating IGF-I levels and decreased cortical and trabecular bone, confirming the role of circulating IGF-I in the maintenance of murine bone mass (165, 166).

b. Effects of locally produced IGF-I. Transgenic mice overexpressing IGF-I under the control of the osteoblast-specific osteocalcin promoter exhibit transient increases in trabecular bone secondary to an increase in osteoblast function and bone formation (167). Changes in osteoblast number were not observed, confirming the predominant effect of IGF-I on osteoblastic function, and not on mitogenesis.

Igf-1r null mice die shortly after birth and exhibit severe growth retardation. The expression of the Cre recombinase under the control of the osteocalcin promoter has allowed the conditional deletion of *igf-1 receptor* gene, flanked by *lox*[P] sequences, selectively in osteoblasts. Mice carrying this osteoblast-targeted conditional deletion exhibit decreased osteoblast number and function, causing reduced bone formation and trabecular bone volume (157). This observation documents the fundamental role of IGF-I in the maintenance of trabecular bone structure. Current observations suggest that systemic IGF-I is necessary to maintain cortical bone structure, whereas skeletal IGF-I appears to play a more significant role in the maintenance of trabecular bone. The function of IGF-I on skeletal homeostasis was confirmed by the study of mice carrying deletions of the signaling molecules IRS-1 and -2. *Irs-1* or *-2* null mice exhibit osteopenia (122, 168). However, their phenotypes are not identical, and *irs-1* null mice display low bone turnover osteopenia and failure to exhibit an anabolic response to PTH, whereas *irs-2*

null mice have osteopenia with increased bone resorption and display a bone anabolic response to PTH (122, 168). The stimulatory effect of PTH on bone formation *in vivo* is also not observed in *igf-1* and *igf-1r* null mice (122–124, 169). *Igf-1-* deficient mice failed to show an increase in BMD at the proximal and distal tibia after PTH administration (170). Moreover, the deletion of *igf-1r* in osteoblasts leads to impaired stimulatory effects of PTH on osteoprogenitor cell proliferation (170). These observations do not exclude the possibility of other factors mediating selected anabolic actions of PTH on the skeleton. For example, some effects of PTH in the marrow cellular niche are secondary to the induction of Jagged 1 and the activation of Notch signaling. PTH decreases the expression of the Wnt antagonist, sclerostin, potentially leading to enhanced Wnt signaling, although Wnt signaling is not required to detect anabolic effects of PTH in murine models (171, 172). PTH also increases and activates skeletal TGF β (173).

C. IGF binding proteins (IGFBPs)

IGFBPs are a family of evolutionary conserved-related proteins, which bind IGF-I and IGF-II (174, 175). IGFBPs have differential affinity for IGF-I and IGF-II and modulate the cellular effects of IGFs (174, 175). By binding IGFs, IGFBPs may sequester the growth factor and preclude its interactions with cell surface receptors. However, under selected experimental conditions, such as when IGFBPs are associated with the extracellular matrix, there may be an increase in the effective concentrations of IGF-I in the cellular environment, resulting in enhanced IGF-I effects (176, 177). IGFBPs also have IGF-independent effects.

1. Regulation of local IGFBP synthesis. IGFBP synthesis is regulated at the transcriptional, posttranscriptional, and posttranslational level. This regulation occurs by cell-specific mechanisms, and in cells of the osteoblastic lineage the pattern of IGFBP expression is dependent on the stages of osteoblast cell differentiation (178, 179). IGFBP-2 and -5 expression is highest in the proliferative phase of rat osteoblastic cell cultures, whereas IGFBP-3, -4, and -6 expression is maximal during terminal cell differentiation (180). The regulation of IGFBP expression during osteoblastic cell differentiation may be related to the relative levels of autocrine and paracrine factors present in the cellular environment. IGFs increase IGFBP-5 expression by the osteoblast, whereas growth factors with mitogenic activity inhibit IGFBP-5 and stimulate IGFBP-4 expression (181, 182). Systemic hormones also regulate IGFBP synthesis in a cell- and culture condition-dependent manner (178). GH increases IGFBP-3, and cAMP inducers increase the synthesis of IGFBP-2, -3, -4, and -5 in osteoblasts (179). The induction of binding proteins may be a mechanism to control cell exposure to IGF-I. Conversely, glucocorticoids inhibit the synthesis of IGF-I, IGFBP-3, -4, and -5 and increase the expression of IGFBP-2 in osteoblasts (132, 183). This may contribute to a reduction in levels and activity of IGF-I in the bone environment, in addition to the inhibitory effects of glucocorticoids on IGF-I synthesis. 1,25-dihydroxyvitamin D_3 increases osteoblast IGFBP-3 and -4 expression (184).

2. In vitro and in vivo studies. The ubiquitous overexpression of IGFBP-1 in mice causes hyperglycemia, suggesting a role for free IGF-I in glucose homeostasis, but the function of IGFBP-1 in skeletal cells has not been defined (185). IGFBP-2 is important to transport IGFs, and IGFBP-2 serum levels correlate with BMD and bone turnover in humans (186). *In vitro*, IGFBP-2 prevents the effects of IGF-I on osteoblast function, and the constitutive and indiscriminate overexpression of IGFBP-2 causes impaired growth, decreased bone mass, and failure to respond to the anabolic effects of GH in murine bone (187). However, the effects of IGFBP-2 are complex, and *igfbp-2* null mice exhibit gender-specific changes in bone turnover. Female *igfbp-2* null mice have increased cortical bone, whereas male null mice display decreased cortical and trabecular bone secondary to decreased bone formation (188). These observations suggest that IGFBP-2 is required for normal bone formation in male mice and are in agreement with clinical observations indicating a correlation between serum IGFBP-2 levels and bone remodeling and an anabolic effect of the administration of IGF-II/IGFBP-2 in disuse osteoporosis (189, 190). It is also of interest that mechanical loading up-regulates the expression of IGF-I and IGFBP-2 transcripts in osteocytes (191, 192). IGFBP-3 is a major component of the circulating IGF complex, and its concentrations are GH dependent (174, 175). *In vitro*, IGFBP-3 can inhibit or stimulate IGF activity, the latter by up-regulating IGF-I delivery to cell surface receptors (193). However, the constitutive and ubiquitous overexpression of IGFBP-3 *in vivo* causes growth retardation and osteopenia (194). IGFBP-4 and IG-FBP-5 are inhibitors of IGF-I, but under certain experimental conditions they can simulate bone cell function independently of their interactions with IGFs (195). It is important to note that transgenic mice overexpressing either IGFBP-4 or IGFBP-5, under the control of the osteoblast-specific osteocalcin promoter, exhibit osteopenia secondary to decreased bone formation (196, 197). The osteopenia is explained by the sequestration of IGF-I present in the bone environment and confirms the inhibitory function of IGFBP-4 and -5 in the skeleton. It is possible that the differential activity of IGFBP-4 and -5 depends on interactions with extracellular matrix proteins or on the levels of the IGFBP present in a specific tissue. The inhibitory effects of IGFBP-5 were documented *in vitro* using retroviral vectors to overexpress IGFBP-5 in osteoblastic cells. Constitutive overexpression of IGFBP-5 inhibited osteoblastic cell function, whereas the expression of IGFBP-5 fragments had no stimulatory or inhibitory activity (198). The function of IGFBP-6 in skeletal tissue is not known.

3. Regulation of IGFBP action. The abundance of IGFBPs is regulated by IGFBP proteases. Osteoblasts secrete matrix metalloproteinases and serine proteases, and these cleave IGFBPs (199). Pregnancy-associated plasma protein-A (PAPP-A) is a metalloproteinase expressed by skeletal cells that plays a critical role in osteoblastic function by modulating IGF-I bioavailability (200). PAPP-A cleaves the inhibitory IGFBP-4 in an IGF-dependent manner, and as a consequence the bioavailability of IGF-I is increased (201). IGFBP-2 and -5 also are substrates for PAPP-A. Addition of PAPP-A to osteoblast cultures promotes cell proliferation by increasing IGF-I bioavailability, and transgenic mice over-expressing PAPP-A under the control of the type I collagen promoter exhibit increased bone formation and bone area indicating a bone anabolic effect *in vivo* (202). *Papp-a* null mice lack IGFBP-4 proteolytic activity, and at birth they exhibit dwarfism secondary to the sequestration of IGF-II by IGFBP-4 (203). Postnatally, *papp-a* null mice exhibit decreased trabecular bone volume, low bone turnover osteopenia, and delayed fracture healing (204, 205). These observations are consistent with a decrease in bioavailable IGF-I in the bone environment due to IGFBP-4 in excess and confirm the fundamental role of PAPP-A in the bioavailability of IGF pre- and postnatally. The expression of PAPP-A in osteoblasts is enhanced by TGFβ, agents that induce cAMP, such as PTH, and cytokines such as IL-1 and -4 and TNFα (206, 207). The synthesis and activity of other metalloproteinases, such as collagenase 3, in osteoblasts also are regulated by cytokines and hormones, such as IL-6 and glucocorticoids (208, 209). Limited proteolysis of IGFBPs by a variety of serine proteases control the bioavailability of IGF-I in the circulation and at the cellular level, particularly by cleaving IGFBP-3 (174). In addition, IGFBP-3 is cleaved by plasmin, and the bioavailability of IGF-I in the bone microenvironment can be regulated by activators and inhibitors of plasminogen (210).

IV. Skeletal Manifestations of GH Deficiency and Excess in Humans

A. Adult growth hormone deficiency (GHD)

Adult GHD is a recognized and treatable clinical entity (211–213). Severe GHD in adults is associated with adverse changes in body composition, lipid metabolism, insulin sensitivity, and exercise capacity (211, 212, 214). Adult patients with GHD suffer from low bone turnover osteoporosis leading to increased fracture risk, which may contribute to the increased risk of mortality observed in GHD (1, 215).

1. Bone turnover and calcium metabolism in untreated GHD. GH and IGF-I play an important role in modulating bone remodeling (216). Patients with GHD have a marked reduction in bone turnover. Bone biopsies from male adult patients with GHD reveal decreased osteoid and mineralizing surfaces and decreased bone formation rate (217). Serum levels of osteocalcin and bone resorption markers are decreased, confirming the state of low bone turnover (1, 109, 218–222). Patients with GHD exhibit renal, skeletal, and intestinal cell insensitivity to PTH, leading to a mild state of PTH resistance and increased serum PTH levels (109, 223). Consistent with a decrease in end-organ sensitivity, the calcemic response to PTH is delayed (223). GHD also is accompanied by abnormalities in the circadian rhythm of PTH, which may affect bone remodeling (109, 224, 225).

2. BMD and fractures in untreated GHD. Decreased BMD is reported in patients with GHD, either isolated or combined with other pituitary hormone deficiencies (1, 215, 226, 227). Patients with GHD exhibit greater losses of cortical than trabecular bone, and this observation is in accordance with the greater effect of systemic IGF-I on cortical than on trabecular bone (228, 229). The degree of bone loss is related to the duration and age of onset of GHD, the severity of the

disease, and the age of the patients (230–233). About half of the patients have normal vertebral BMD (234).

GHD is a heterogeneous disorder, and the clinical manifestations in patients developing GHD in their childhood are different than in patients developing the disease after puberty (235). The etiology of childhood-onset GHD is frequently unknown (*i.e.*, idiopathic GHD); however, it may be secondary to cranial irradiation, neurosurgical removal of craniopharyngioma, or other hypothalamic-pituitary masses and infiltrative diseases (*e.g.*, histiocytosis X). Adult-onset GHD is often secondary to neurosurgery and/or radiotherapy for a pituitary adenoma, but may be also observed in other clinical conditions (*e.g.*, empty sella) (236, 237). Patients with childhood-onset GHD are smaller and have a more pronounced decrease in muscle and bone mass and lower IGF-I and IGFBP-3 serum concentrations than subjects with adult-onset GHD (235, 236). In childhood-onset GHD, vertebral BMD is markedly reduced with T-scores often between −1 and −2; about one third of the patients have T-scores of −2.5 or less (238). In contrast, patients with adult-onset GHD often have vertebral T-scores of −1 or above (222, 225, 238–253). The reason for the different degrees of bone loss may be because childhood-onset GHD occurs before the achievement of peak bone mass, and because the duration of the disease is longer. In childhood-onset GHD, there may be impaired acquisition of bone mass during childhood and adolescence and during the transition period, a period of life occurring between the closure of the epiphyseal plate and the attainment of peak bone mass (13, 254–260).

Potential confounding factors in the determination of BMD in childhood-onset GHD are the presence of decreased muscle mass and of short stature. The latter occurs in 40% of patients with childhood-onset GHD, even after replacement therapy with GH (261). Differences in BMD between childhood-onset and adult-onset GHD also are found after correction for height (262, 263). Consequently, short stature probably plays a minor role in the lower BMD observed in childhood-onset GHD, whereas changes in body composition may play a more significant role (264–266).

The degree of bone loss in adult-onset GHD correlates with the age of the patients and the duration and the severity of the disease (222, 228, 230, 239, 252, 267). Patients with severe GHD, as defined by a GH response in the range of 3.1 to 9.0 μg/liter after GHRH and arginine, or very severe GHD, as defined by a GH response of 3.0 μg/liter or less after GHRH and arginine, display significant reductions in BMD (222, 230).

The increased prevalence of osteopenia/osteoporosis in isolated GHD suggests that GHD *per se* is the cause of bone loss. However, in subjects with multiple hormone deficiencies, one cannot exclude the possibility that the deficiency of other pituitary hormones and hormonal replacement may contribute to the bone loss. In fact, hypogonadism and the use of replacement therapy with thyroid hormone and glucocorticoids in excess may influence the reduction of BMD in patients with multiple pituitary hormone deficiencies (268–270). TSH deficiency may also contribute to the bone loss (271, 272).

Although untreated GHD can lead to a decrease in bone mass, data demonstrating an increase in the risk of fractures

are limited. The risk of nonvertebral fractures is increased about 3-fold in untreated GHD patients (227, 273, 274). Fractures in GHD are frequently localized to the radius, suggesting a loss of cortical bone (227, 274). Patients with GHD also have an increased incidence of vertebral deformities, suggesting an increased incidence of vertebral fractures (234). The prevalence of bone fractures is related to the degree of GHD and seems not to be affected by the presence of other pituitary hormone deficiencies or by hormonal replacement therapy (227, 268, 275). The different impact of other pituitary hormone deficiencies or hormonal replacement therapy on BMD and fractures is consistent with the finding that fractures do not correlate with BMD in GHD (Fig. 2A) (234, 268). This is also the case in other forms of secondary osteoporosis (276–278).
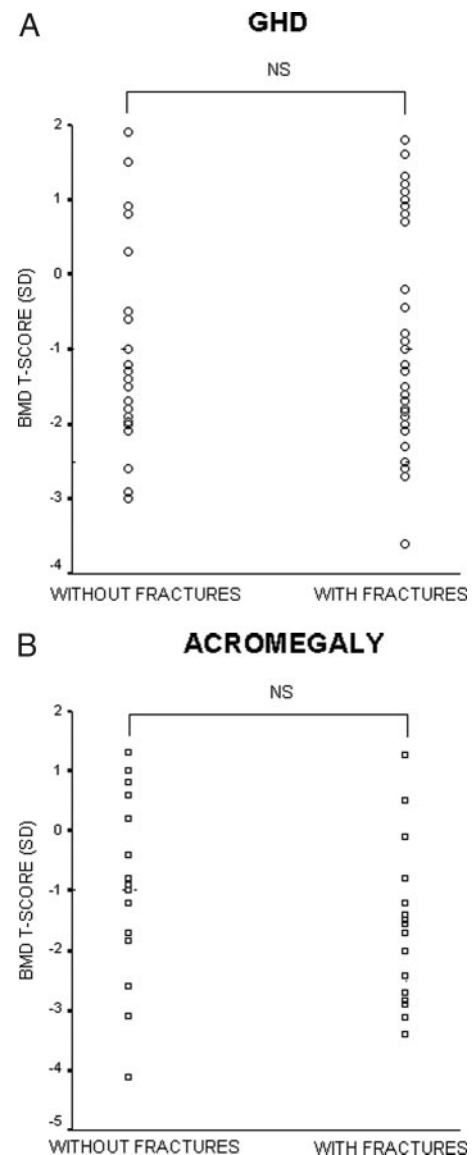

FIG. 2. Scatter plots of BMD T-scores in patients with GHD (A) or acromegaly (B), with and without fractures. NS, Not significant. [Adapted from G. Mazziotti *et al.*: *J Bone Miner Res* 21:520–528, 2006 (234); and S. Bonadonna *et al.*: *J Bone Miner Res* 20:1837–1844, 2005 (276), with permission of the American Society for Bone and Mineral Research.]

An additional risk of fractures in adult-onset GHD is a decrease in muscular strength and, when GHD is secondary to pituitary tumors, impaired vision. Both factors can lead to falls and fractures (227, 279).

B. Skeletal effects of recombinant human GH (rhGH) in adult-onset GHD

1. Bone turnover and calcium metabolism in treated GHD. Replacement therapy with rhGH leads to an increase in bone turnover, as determined by changes in biochemical markers of bone resorption and bone formation (280). The effect of rhGH on bone remodeling is biphasic; rhGH causes a maximal effect on bone resorption after 3 months and on bone formation after 6 months. The effect on bone formation is sustained for prolonged periods of time (1, 97, 246, 280–283). The effect of rhGH on biochemical markers of bone turnover is dose dependent (221, 232, 243, 284), but not influenced by the modality of administration (*i.e.*, continuous *vs.* daily administration, and daily *vs.* administration three times a week) (285, 286). rhGH causes an increase in serum and urinary calcium after 3 to 6 months, an effect caused by calcium mobilization from the skeleton, an increase in intestinal calcium absorption and in the renal reabsorption of calcium due to increased sensitivity to PTH (287–290). rhGH is antiphosphaturic and increases the intestinal absorption of phosphate leading to an increase in serum phosphate levels (115, 291–293). rhGH may normalize the circadian rhythm of PTH secretion (294).

2. BMD and fractures in treated GHD. The effect of rhGH on BMD in adult-onset GHD is variable and depends on the duration of the treatment. Short-term studies failed to show an increase in BMD, and a decline in BMD may occur in the initial months of therapy due to an increase in bone resorption occurring after 3 months without an increase in bone formation, which occurs after 6 months (240, 250, 295–301). Subsequently, when bone formation is increased, bone mass increases, but an increase in BMD can be documented only after 6 to 12 months in children and after 18 to 24 months in adults receiving rhGH therapy (1, 302–311). The increase in BMD is observed for periods of up to 10 yr in patients receiving continuous rhGH therapy (244, 308). Moreover, BMD may even continue to increase 18 months after rhGH discontinuation (312, 313). In contrast, the effects on body composition are lost after the discontinuation of rhGH (312, 313). rhGH increases bone mineral content to a greater extent than BMD because rhGH also increases bone area (246, 299). This is supported by histomorphometric findings demonstrating

an increase in periosteal bone formation during rhGH treatment (301). The effects of rhGH on bone may be potentiated by antiresorptive drugs, such as bisphosphonates. The addition of pamidronate or alendronate at the start of rhGH treatment has been shown to prevent the initial decline of BMD induced by rhGH treatment (314). The benefits of alendronate are also observed when it is started in patients receiving long-term rhGH treatment (306, 315). It is unknown whether bisphosphonates alone can correct the low-turnover osteoporosis in GHD patients not replaced with rhGH, as they do in other forms of secondary osteoporosis (278).

Because measurement of BMD in GHD is not a reliable predictor of fracture risk (234), a direct evaluation of fractures by a radiological morphometric approach is desirable in GHD. A concern is the lack of prospective data documenting a reduction in fractures after rhGH therapy because most studies have used BMD as primary end-point (304). Cross-sectional studies have suggested that rhGH treatment reduces the risk of vertebral and nonvertebral fractures in GHD (Table 2). The effect seems to occur even in patients with untreated hypogonadism (268). The efficacy of rhGH in reducing bone fractures is closely related to the lag-time spanning between the diagnosis of GHD and initiation of therapy, and GH is beneficial only in patients receiving rhGH shortly after the diagnosis is made (Fig. 3) (234).

3. Predictors of rhGH response in bone

a. Sex. Male and female patients with GHD may display different responses to rhGH in terms of changes in bone turnover and BMD. In men, bone formation and resorption are increased within 1 month of rhGH treatment, whereas in women the increase occurs after 3 months. The increase in markers of bone resorption precedes the change in bone formation markers by about 9 months (316). The early change of bone remodeling may lead to a greater increase in BMD in males than in females, in whom only stabilization of BMD is achieved (253, 281, 282, 305, 309, 311, 317–319). These gender-dependent differences may also reflect the different impact of hypogonadism in male and female subjects with GHD (320–322). Recently, a decreased incidence of clinical fractures has been reported only in males with treated adult-onset GHD as compared with the normal population (323).

b. Age of onset of GHD. The effect of rhGH on bone turnover and BMD is different between childhood-onset and adult-onset GHD. In adult-onset GHD, bone formation increases after 6 months of treatment, with little change thereafter (232, 324). In contrast, in childhood-onset GHD there is a progres-

TABLE 2. Risk of fractures in adult-onset GHD patients untreated and treated with rhGH

| Study (Ref.)	No. of patients	Type of fractures	Fracture Risk	
			Untreated	rhGH treated
Rosen *et al.* (273)	107	Clinical nonvertebral	Increased	N.D.
Wuster *et al.* (227)	2084	Clinical nonvertebral	Increased	Decreased
Vestergaard *et al.* (275)	199	Clinical nonvertebral	Increased	Unchanged
Bouillon *et al.* (274)	66	Clinical nonvertebral	Increased	N.D.
Mazziotti *et al.* (234)	107	Morphometric vertebral	Increased	Decreased

N.D., not determined.
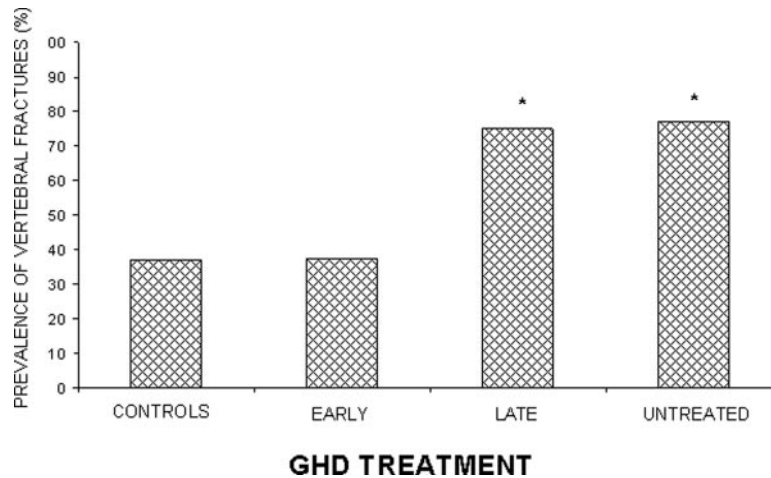
FIG. 3. Prevalence of fractures in adult-onset GHD, untreated and treated (early and late in the course of the disease) with rhGH. *, $P < 0.05$ untreated *vs.* late treated or untreated GHD. [Adapted from G. Mazziotti *et al.*: *J Bone Miner Res* 21:520–528, 2006 (234) with permission of the American Society for Bone and Mineral Research.]

sive increase in bone formation for up to 12 months after rhGH, which is followed by a return to baseline values after 18 months of therapy (233). Patients with childhood-onset GHD generally display an increase in BMD after 6 to 12 months of rhGH therapy, whereas patients with adult-onset GHD require 18 to 24 months of rhGH to exhibit a change in BMD (246, 305, 324, 325).

c. Dose of GH. In childhood-onset GHD, there is a linear correlation between the dose of rhGH and the increase in BMD (326), indicating a need for optimization of the dose of rhGH in children. In contrast, in adult-onset GHD, low of rhGH have an optimal effect on BMD, whereas at high doses rhGH may cause an initial decline in BMD, probably due to an increase in bone resorption (243, 324, 327–329).

d. Concomitant diseases. The underlying pituitary disease may alter the effects of rhGH on bone mass. A delayed effect of rhGH replacement therapy on BMD is observed in patients with Cushing's disease and in patients with hyperprolactinemia and hypogonadism, when compared with patients bearing nonsecreting pituitary adenomas (330).

4. Monitoring patients with GHD and the response to GH. Patients with GHD are at high risk for osteoporosis and fractures (212, 213, 238). Therefore, it is important to follow the patients and monitor changes in skeletal metabolism. Osteoporosis, when complicated by fractures, contributes to an increased mortality (331). Current guidelines from The Endocrine Society recommend obtaining BMD before initiating therapy with rhGH (238). An independent assessment of fractures also could be useful because BMD is not a good predictor of fractures in GHD. In fact, an early diagnosis of vertebral fractures is important to assess the future risk of osteoporotic fractures (332). After the initiation of rhGH, the measurement of serum calcium, phosphate, alkaline phosphatase activity, and osteocalcin levels may be useful to ensure that a therapeutic response was achieved. After 18 to 24 months of treatment and then every 2 yr, BMD measurements should be considered (238). The measurement of BMD is important in severe GHD patients during the so-called

transition period, to monitor the achievement of the normal peak bone mass. The European Society for Pediatric Endocrinology recommends measuring BMD at baseline and at 2- to 5-yr intervals, and the attainment of a normal peak bone mass is defined in the presence of T-score as greater than −1 SD (333).

C. Skeletal manifestations of acromegaly

Acromegaly is a disease caused by the excessive secretion of GH, and in more than 90% of the cases its etiology is a benign monoclonal pituitary adenoma (334). The incidence of acromegaly is approximately three cases per 1 million persons per year, and its prevalence is about 60 cases per million (335). The clinical manifestations of acromegaly range from subtle signs of GH/IGF-I excess, such as acral overgrowth and coarsening of facial features, to significant metabolic, cardiovascular, and respiratory manifestations, leading to an increase in morbidity and mortality (336–338).

1. Bone turnover and calcium metabolism in untreated acromegaly. Patients with acromegaly have increased bone turnover, as determined by changes in biochemical markers, calcium kinetics, and bone histomorphometry (339–348). Biochemical markers of bone formation and bone resorption are increased, but markers of bone resorption are disproportionately increased in relation to markers of bone formation, and their increase could reflect the degree of bone loss observed. Serum GH and IGF-I levels correlate with markers of bone resorption, whereas only circulating levels of IGF-I correlate with markers of formation (349).

Serum concentrations of PTH, 1,25-dihydroxyvitamin D_3, calcium, and phosphorus are increased in active acromegaly (343, 350, 351). GH stimulates the parathyroid gland and may affect PTH pulsatility, but the contribution of PTH to the skeletal effects observed in acromegaly is not clear (108, 351, 352).

2. BMD and fractures in untreated acromegaly. The effects of GH excess on BMD are variable in relation to the skeletal site possibly due to different sensitivity to GH excess of trabec-

ular and cortical bone (1). Decreased BMD has been reported in acromegaly almost exclusively at the lumbar spine, a site rich in trabecular bone, whereas increases in BMD may be observed in the forearm, a site rich in cortical bone (339, 340, 347, 348, 353–358). The variability of data on BMD in acromegaly may also be explained by the diversity of densitometric techniques used. However, peripheral quantitative computerized tomography and bone biopsies consistently demonstrated differential responses of trabecular and cortical bone to excess GH/IGF-I in humans (359).

Age, gender, and the presence or absence of hypogonadism influence vertebral BMD in acromegaly (346, 348, 349, 355, 356, 360–362); vertebral BMD is inversely correlated with the duration of the hypogonadism (361).

Studies on the prevalence of bone fractures in acromegaly are limited (276, 363, 364). However, a higher incidence of radiological vertebral deformities is observed in postmenopausal women with active acromegaly than in nonacromegalic postmenopausal women (276). This suggests that acromegaly is associated with an increased risk of osteoporotic vertebral fractures, although they are often not diagnosed or detected (332). The occurrence of vertebral deformities in acromegaly correlates with the duration of the active disease and with serum levels of IGF-I, but not with BMD, and they are found in patients with normal or minimally decreased BMD (Fig. 2B) (276). The mechanisms underlying the metabolic bone disease of acromegaly are multifactorial and possibly include an increase in bone resorption secondary to IGF-I excess and to sex hormone deficiency.

3. Effects of treatment on skeletal abnormalities in acromegaly. There are limited data on the effects of treatment of acromegaly in bone metabolism, BMD, and fractures. Metabolic abnormalities observed in acromegaly improve after transphenoidal pituitary surgery or pharmacological therapy (365–368). The effects of the normalization of GH/IGF-I secretion on PTH levels and activity are variable (367, 369–371). After hormonal control (372), acromegalic patients develop a reduction in PTH target organ sensitivity and a reduced nocturnal rise in PTH (351). Postmenopausal women with controlled acromegaly have reduced risk of radiological vertebral fractures compared with patients with active acromegaly. This reduced risk occurs in the absence of a significant increase in BMD (276) (Fig. 4). It is of interest that after

therapy, fractures are observed more often in patients with low levels of serum IGF-I (276).

A sustained normalization of bone turnover occurs after treatment of acromegaly with the GH antagonist pegvisomant, a selective antagonist of the GHR, which controls IGF-I secretion (371, 373).

4. Monitoring patients with acromegaly. A consensus statement from the Pituitary Society and European NeuroEndocrine Association for the diagnosis, treatment, and follow-up of acromegaly and its complications was published in 2003 (336). The determination of BMD was recommended only in hypogonadal acromegalic patients. Consequently, hypogonadal and postmenopausal patients should be screened with BMD. Radiological evaluation of the spine is advisable to exclude morphometric prevalent fractures (276). As recommended in the cited consensus statement, patients with uncontrolled active acromegaly or with established osteoporosis should be monitored with periodic BMD measurements, particularly when they are hypogonadal.

V. Skeletal Manifestations of Selected Diseases with Abnormal GH/IGF-I Axis

A. Postmenopausal and senile osteoporosis

Postmenopausal osteoporosis occurs in approximately 35% of postmenopausal white women and 19% of white elderly men (374). Estrogen deficiency plays a role in the pathogenesis of postmenopausal osteoporosis and possibly male osteoporosis (375, 376). A number of other factors have been implicated in the etiology of bone loss, including secondary hyperparathyroidism, vitamin D deficiency, and decreased IGF-I levels, which could lead to impaired osteoblastic function in an elderly population (377, 378).

There are some analogies between the clinical features of adult GHD and of advancing age, and a relative GH-deficient state, the somatopause, may occur during aging. A decline in GH and IGF-I secretion may play a role in the pathogenesis of osteoporosis (379–381). There is a correlation between serum IGF-I levels and BMD in postmenopausal women, and *igf-1* promoter polymorphisms have been linked to bone mass (382–386). The content of IGF-I in human cortical bone decreases with age, a decline that parallels the one observed
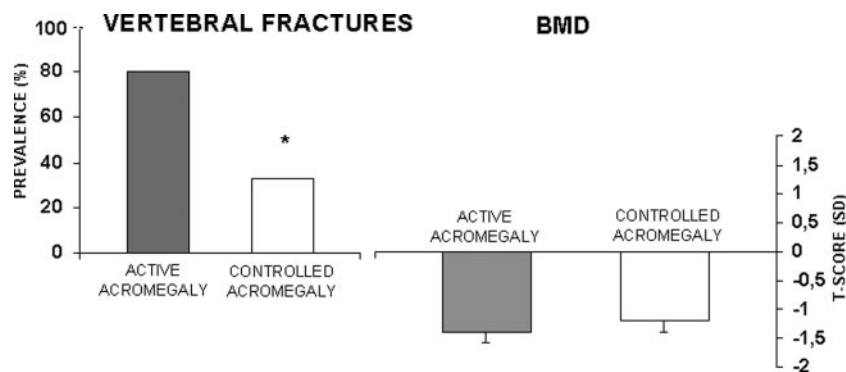

FIG. 4. Differences in fracture rates and BMD between patients with active acromegaly and those with controlled acromegaly. *, $P < 0.05$ controlled *vs.* active acromegaly. [Modified from S. Bonadonna *et al.*: *J Bone Miner Res* 20:1837–1844, 2005 (276), with permission of the American Society for Bone and Mineral Research.]

in serum concentrations of IGF-I (387). Consequently, changes in IGF-I content in the cortical bone may be due to a decrease in skeletal IGF-I accumulation from the systemic circulation, or due to a decrease in the synthesis of IGF-I by the aging skeleton.

rhGH increases bone turnover in normal subjects and improves bone mineral metabolism in postmenopausal females, males with idiopathic osteoporosis and elderly patients, but the effect of rhGH on BMD is controversial (388–399). Some studies have reported an increase in BMD after rhGH treatment of normal subjects and osteoporotic patients, whereas some have shown a lack of an effect (395–400). The lack of a response is observed despite increases in serum IGF-I. A recent meta-analysis has demonstrated no beneficial skeletal effect of rhGH in older subjects. This finding may be influenced by the scarcity of controlled trials suitable for the meta-analysis, as well as by the heterogeneity of the subjects examined (401).

An orally active GH secretagogue (MK677) combined with alendronate determined an improvement of BMD only at femoral neck in postmenopausal osteoporosis (402). Recombinant human IGF-I can influence bone metabolism in humans, but the lack of skeletal specificity and potential side effects would limit its possible use in osteoporosis (391, 403–408).

B. Anorexia nervosa

Anorexia nervosa is a severe eating disorder that leads to progressive malnutrition (409). The prevalence of the disease in adolescents and young adults is between 0.5 and 1%, with an incidence of five to 10 new cases per 100,000 women between 15 and 19 yr of age per year (410). Osteopenia and osteoporosis are important features of anorexia nervosa, occurring in about 92 and 38% of the patients, respectively (411–414). Anorexia nervosa is associated with an increased prevalence of fractures (415, 416). The bone loss is severe and rapid with an average annual loss of BMD of 2.5% (412). Bone loss in anorexia nervosa is due to a reduction in bone formation and an increase in bone resorption (406, 412, 416, 417). The pathogenesis of the bone loss is multifactorial, and decreased body weight is the most important predictor of osteoporosis (418). Peak bone mass is not achieved during adolescence (417–423). Body weight recovery leads to skeletal improvement, and lean body mass is the most important component determining skeletal recovery (412, 417). Anorexia nervosa is associated with profound metabolic abnormalities, including amenorrhea, GH resistance with decreased IGF-I synthesis, hypercortisolism, and decreased serum leptin levels. These abnormalities may play a role in the pathogenesis of the bone loss, which occurs mostly at sites rich in trabecular bone (424). Although hypogonadism may be a factor in the development of osteoporosis, estrogen deficiency alone does not explain the extreme degree of bone loss observed in anorexia nervosa (412, 425, 426). In fact, bone loss is more severe in young women with anorexia nervosa than in age-matched women of normal weight suffering from hypothalamic amenorrhea and with an equivalent degree of estrogen deficiency (427). Preservation of gonadal function does not protect against the bone loss, although the resump-

tion of menstrual function is an important predictor of vertebral BMD recovery in amenorrheic women with anorexia nervosa (412, 425). Replacement therapy with estrogen/progestin in women with anorexia nervosa does not increase BMD, confirming that factors other than hypogonadism are important determinants of the bone loss (428, 429).

A state of GH resistance characterized by increased GH and decreased IGF-I serum levels is present in anorexia nervosa and may be related to the nutritional state of the patient (424, 430–447). Serum IGF-I, IGFBP-2 and -3 levels are useful nutritional indicators, and in anorexia nervosa, serum levels of IGF-I and IGFBP-3 are low, whereas serum IGFBP-2 is elevated (448–456). The decreased serum levels of IGF-I and IGFBP-3 and the increased levels of IGFBP-2 may contribute to the bone loss observed in anorexia nervosa. In fact, these parameters correlate with markers of bone formation, and IGFBP-3 is an independent determinant of hip BMD (457–460). Moreover, an improvement in the nutritional status of patients with anorexia nervosa leads to an increase in serum IGF-I levels, followed by a progressive increase in markers of bone formation (457, 460–462).

IGF-I was assessed for its effect on bone turnover and bone mass in anorexia nervosa. Short-term rhIGF-I treatment increases bone turnover in a dose-dependent fashion (407). Interestingly, low doses of rhIGF-I induced an increase in bone formation without a stimulation of bone resorption (407). Low doses of rhIGF-I in combination with estrogens increase BMD (429). These results are encouraging, but appropriate trials to establish the role of rhIGF-I in the treatment of anorexia nervosa are needed.

C. Glucocorticoid-induced osteoporosis

Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis (278). Glucocorticoids have direct and indirect effects on the skeleton. Glucocorticoids impair the replication, differentiation and function of osteoblasts and induce the apoptosis of mature osteoblasts and osteocytes (278). These effects lead to a suppression of bone formation, a central feature in the pathogenesis of glucocorticoid-induced osteoporosis. Glucocorticoids inhibit GH secretion by increasing the somatostatin tone in the hypothalamus, and glucocorticoids reduce the GH response to GHRH (18, 463–466). This also is observed in asthmatic patients using inhaled glucocorticoids, although serum levels of IGF-I and biochemical markers of bone remodeling are not altered by inhaled glucocorticoids (467). *In vitro*, glucocorticoids suppress IGF-I transcription in bone cells (468).

The abnormal GH secretion in glucocorticoid-induced osteoporosis is associated with abnormal bone turnover and ultrasonometric data (469). An increase in serum osteocalcin, carboxy-terminal propeptide of type I procollagen, and carboxy-terminal telopeptide of type I collagen *was* observed after short-term rhGH treatment in a selected group of patients on chronic corticosteroid therapy (470). Combined rhGH and rhIGF-I therapy counteract selective negative effects of short-term glucocorticoid therapy in healthy volunteers (471). Theoretically, due to the kinetics of bone markers in subjects treated with glucocorticoids and GH, a window of opportunity exists for GH treatment in patients with glu-

cocorticoid-induced low bone turnover osteoporosis. However, bone cells are resistant to the effects of GH and IGF-I in the presence of glucocorticoids (133, 472), and there is little evidence for a beneficial effect of GH or IGF-I in glucocorticoid-induced osteoporosis (473). Observational and controlled studies in children receiving glucocorticoid therapy for juvenile idiopathic arthritis showed that rhGH restores normal height velocity with a concomitant improvement in bone mineralization (474–476). Potential side effects of chronic rhGH administration in long-term glucocorticoid users are hyperglycemia and hypertension, although additional benefits on body composition by GH administration are possible (214).

VI. Conclusions

GH and IGF-I are anabolic hormones with an important role in the regulation of bone remodeling. GH and IGF-I are necessary to achieve and maintain bone mass throughout life. IGF-I mediates most of the effects of GH on skeletal metabolism, IGF-I increases bone formation by regulating the differentiated function of the osteoblast, and as a consequence GH and IGF-I increase bone remodeling. Diseases affecting the GH/IGF-I axis are frequently associated with significant alterations in bone metabolism that often lead to bone loss.

Acknowledgments

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