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Growth Hormone and Bone*

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I. Introduction

GH AFFECTS several tissues including liver, muscle, kidney, and bone. GH effects on muscle and kidney have recently been extensively reviewed in *Endocrine Reviews* by Florini *et al.* (1) and Feld and Hirschberg (2). Since GH has important effects on skeletal tissues, our focus in this article will be on our current understanding of GH effects on bone. A large increase in bone mass occurs during childhood and puberty via endochondral bone formation. A gradual increase in bone mass is then seen until peak bone mass is reached at 20–30 yr of age. Subsequently, bone mass decreases with an accelerated bone loss seen in females after menopause. Bone remodeling is regulated by a balance between bone resorption and bone formation. In this process GH is known to play a role (3–7). A net gain of skeletal mass due to new bone formation caused by GH was first shown in adult mongrel dogs (8). After treatment with GH for 3 months a 2% increase in cortical bone mass, as assessed by histomorphometry, was found.

Due to limitations in the supply of GH, a limited number of animal and clinical studies were performed until the mid-1980s when recombinant human GH became available. The initial use of recombinant human GH was restricted to treatment of growth-retarded GH-deficient (GHD) children. However, it is now well established that GH also exerts important effects in adults, and GH treatment of GHD adults is now approved in several countries. Recent studies, in both animals and humans, have demonstrated that GH exerts potent effects on bone remodeling.

In this article we will discuss the role of GH in the process of bone growth until peak bone mass is achieved and present evidence that an increased endogenous production of GH or treatment with GH might increase bone mass in adults. Recent studies of the cellular mechanism of action for GH in the regulation of bone growth are given in *Section II*. It is proposed that GH stimulates longitudinal bone growth directly by stimulating prechondrocytes in the growth plate followed by a clonal expansion caused both by the GH-induced local production of insulin-like growth factor I (IGF-I) and by a GH-induced increase in circulating levels of IGF-I. However, the main purpose of this article is to present recent data indicating that GH is important in the regulation of bone remodeling. Finally we will present a hypothetical model for the mechanism of action of GH in the regulation of bone remodeling and bone mass.

II. Effects of GH on Longitudinal Bone Growth

A. GH and regulation of postnatal longitudinal bone growth

During the process of longitudinal bone growth, prechondrocytes in the germinal cell layer differentiate and thereafter undergo limited clonal expansion in individual chondrocyte columns in the growth plate. Subsequently, cells in the hy-

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pertrophic zone mature and degenerate and are eventually incorporated into bone (9–12).

Several hormones are important for normal postnatal longitudinal bone growth, but it is generally accepted that GH is the most important hormone in this respect. Furthermore, it has been demonstrated that GH stimulates growth of cartilage and other tissues by increasing the number of cells rather than by increasing cell size (11–14). A widely discussed question during the last two decades has been whether GH acts on tissues directly, or whether the effect is mediated by a liver-derived growth factor, initially called sulfation factor, but later renamed somatomedin, and subsequently shown to be identical to IGF-I. According to the original somatomedin hypothesis, GH stimulates skeletal growth by stimulating liver production of somatomedin which, in turn, stimulates longitudinal bone growth in an endocrine manner (15–17).

In the early 1980s the somatomedin hypothesis was challenged by a study demonstrating that injection of GH directly into the rat tibia growth plate stimulated longitudinal bone growth at the site of injection (18). This initial observation has subsequently been confirmed and extended, and it is now well documented that GH stimulates growth of many different tissues directly (19–34).

By studying the effects of GH and IGF-I in 3T3 preadipocytes, Green and co-workers (35, 36) made the observation that GH and IGF-I act on cells at different stages of maturation. Thus, GH was found to stimulate young preadipocytes, whereas IGF-I stimulated cells at a later stage of development. The hypothesis by Green and co-workers (35), that GH acts on progenitor cells and that IGF-I stimulates the subsequent clonal expansion, was named the "dual effector theory." The finding that GH stimulates longitudinal bone growth directly (18) and increases the local production of IGF-I by stimulating transcription of the IGF-I gene (37) led to the proposal that the dual effector theory of GH action is valid for the regulation of longitudinal bone growth as well (10). Subsequent *in vitro* studies using cultured epiphyseal chondrocytes in suspension revealed that GH and IGF-I stimulate cells at different stages of maturation. Thus, GH stimulates the colony formation of young prechondrocytes, whereas IGF-I stimulates cells at a later stage of maturation, giving support to the hypothesis that cell maturation is indeed an important factor determining responsiveness of epipyseal chondrocytes to GH and IGF-I (38–42). The hypothesis that GH preferentially acts on chondrocyte progenitor cells *in vivo* is directly supported by another study from our laboratory. By labeling slowly cycling prechondrocytes with radioactive thymidine and studying the subsequent labeling pattern in sagittal sections of the tibia growth plate by means of autoradiography, the observation was made that local injection of GH increased the number of labeled cells in the prechondrocyte layer of the growth plate. In contrast, IGF-I did not stimulate incorporation of radioactive thymi-

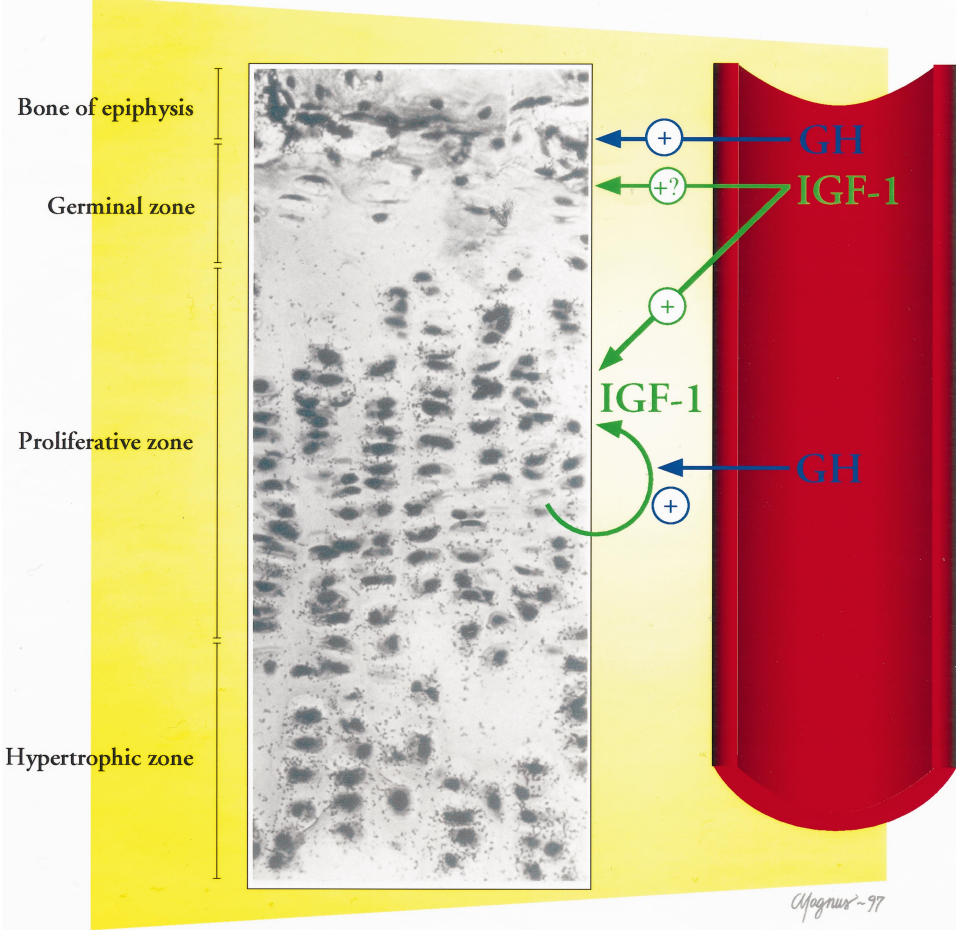

FIG. 1. The authors' proposed mechanism of action for GH and IGF-I in stimulating longitudinal bone growth. The different zones of the rat tibial growth plate are indicated. GH stimulates longitudinal bone growth directly by stimulating prechondrocytes in the growth plate followed by a clonal expansion caused both by the GH-induced local production of IGF-I, and by a GH-induced increase in circulating levels of IGF-I. GH is the major determinant for the stimulation of progenitor cells although it is possible that IGF-I might stimulate progenitor cells to some extent.

dine in cells in the same layer (43). Using a histomorphometric technique, it was found that GH as well as IGF-I has the capacity to stimulate prechondrocytes, as both GH and IGF-I reduced the cell cycle time of prechondrocytes. However, it was found in the same study that growth plate prechondrocytes from GH-treated animals had a 50% shorter cell cycle time compared with IGF-I-treated animals (44). These data indicate that at least some of the growth-promoting effect of GH is exerted via direct stimulation of prechondrocytes.

B. Results supporting an important physiological role of IGF-I for bone growth

Studies performed during the last 20 yr involving systemic administration of IGF-I to GH-deficient animals and man suggest that both IGF-I and GH have the capacity to stimulate longitudinal bone growth *in vivo* (45–54). Elimination of IGF-I and the IGF-I receptor by homologous gene recombination have demonstrated that the IGF-I-signaling pathway is very important for tissue development and growth. Thus, mice with IGF-I deficiency show severe retardation of statural growth that first becomes apparent at day 12 in embryonic life, and subsequent postnatal growth is severely retarded (55–58). IGF-I receptor "knock-out" mice are affected more profoundly and die of respiratory failure early postnatally due to poor development of respiratory muscles (57). Furthermore, a patient with a deletion of the IGF-I gene demonstrated intrauterine growth retardation and postnatal growth failure (59). These experimental studies and the clinical observation clearly demonstrate that a normal expression of IGF-I, as well as its receptor, plays a critical role for normal growth and tissue development. However, these experiments are unable to answer the question of whether locally produced (autocrine/paracrine acting) IGF-I is more important for normal tissue growth and development than circulating (endocrine acting) IGF-I.

Several studies have shown that systemic administration of recombinant IGF-I stimulates longitudinal bone growth as well as body weight gain in hypophysectomized rats, giving support to the theory that IGF-I has endocrine actions on statural growth. Interestingly, administration of IGF-I particularly promoted the growth of nonskeletal tissues. Thus, the effect of IGF-I on kidney, spleen, and thymus growth was larger in magnitude compared with other tissues (46, 47, 60). The quantitative difference in tissue response to IGF-I has also been found in transgenic mice overexpressing IGF-I, suggesting that IGF-I has particularly important functions in nonskeletal tissues (61, 62). These data demonstrate that systemic delivery of IGF-I has the capacity to increase growth in animals.

C. Evaluation of the somatomedin theory vs. the dual effector theory

The fact that both GH and IGF-I stimulate tissue growth makes an analysis of the relative importance of the peptides for this effect, in terms of spatial and temporal patterns, quite complex. It seems justified to critically analyze available experimental and clinical data and find out how available data fit into the two different theories, the somatomedin theory and the dual effector theory. The effect of systemic administration of GH and IGF-I to hypophysectomized rats has shown that GH and IGF-I have independent and differential functions (45, 46, 63). When the two compounds are given together, they exert additive or synergistic effects (45, 60, 64). Also, administration of GH to animals treated with maximal doses of IGF-I stimulates growth further (63). Furthermore, the differences between GH and IGF-I are quite obvious in transgenic animals overexpressing either GH or IGF-I. Thus, GH-transgenic animals grow to approximately twice the size of their normal littermates (62, 65). In contrast, mice generated from a cross of mice overexpressing IGF-I and mice lacking GH-expressing cells demonstrate an increase in longitudinal bone growth and body weight when compared with their GH-deficient controls. However, IGF-I transgenic mice do not grow more than their nontransgenic siblings (61, 66), demonstrating that overexpression of GH, but not IGF-I, causes supranormal growth. Local administration of GH, but not IGF-I, stimulates the local production of IGF-I by stimulating the transcription of the IGF-I gene (22, 37), giving direct experimental support to the notion that there is an interplay between GH and IGF-I. Administration of antibodies to IGF-I abolishes the stimulatory effect of locally administered GH (31), supporting the theory that the locally produced IGF-I has an important functional role in the expression of the effect of GH at the site of the local tissue level (10, 67).

Treatment of GH insensitivity syndrome (GHIS) patients with recombinant IGF-I has shown that IGF-I is quite effective in stimulating statural growth for 1–2 yr (49–51, 53, 54, 68–72), supporting the somatomedin theory. However, available clinical data suggest that the effect of IGF-I subsequently becomes less effective, perhaps due to a decreased rate of stimulation of prechondrocytes, a lack of GH- induced IGF-binding protein 3 (IGFBP-3) and/or a suboptimal IGF-I administration. However, from these clinical studies it is difficult to make a general conclusion whether IGF-I stimulates tissue growth by endocrine or autocrine/paracrine mechanisms under physiological circumstances in the intact organism. The question whether autocrine/paracrine or endocrine IGF-I is the more important factor for the stimulation of tissue growth will probably not be solved until tissue growth can be studied in transgenic animals with tissue-specific gene deletions of IGF-I or the IGF-I receptor.

Taken together, available data suggest that GH stimulates longitudinal bone growth directly by stimulating prechondrocytes in the growth plate followed by a clonal expansion caused both by the GH-induced local production of IGF-I, and by a GH-induced increase in circulating levels of IGF-I. GH is the major determinant for the stimulation of progenitor cells, although it is possible that IGF-I might stimulate progenitor cells to some extent (Fig. 1).

III. Effect of GH *in Vitro*

A. Effects of GH in bone tissue cultures

Several *in vitro* models, developed to study the effects of hormones and growth factors on bone remodeling, have been presented, and some of these will be discussed in this

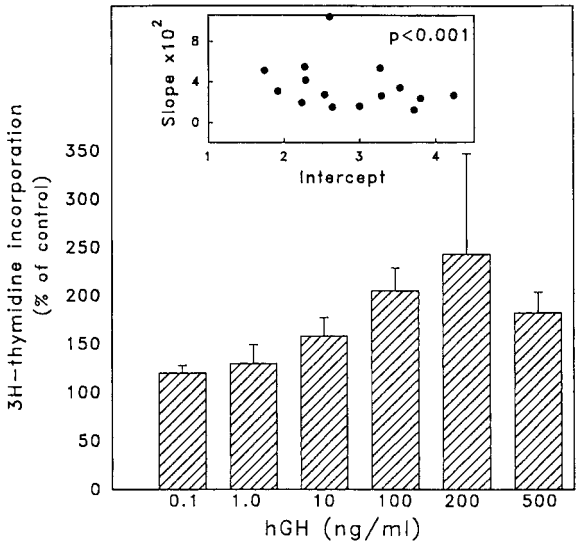

FIG. 2. Effects of GH on [^{3}H]thymidine incorporation in DNA of human osteoblast-like cells. Data are shown as mean ± SEM (n = 15). Absolute value of the control cultures is 1950 dpm/ml. To illustrate the statistical method used to analyze dose-response relationship, an *inset* is included in this figure. For each cell strain tested, the log dose-response relationship was described by a regression line. *Inset* shows the slopes obtained in the 15 independent cell strains studied plotted against their intercepts. Variations in slopes (along the y-axis) represent differences in responsiveness to GH among various cell strains. In hypothetical testing these slopes were tested for their deviation from zero by Student's paired *t* test. [Reproduced with permission from M. Kassem *et al.*: *Calcif Tissue Int* 52:222–226, 1993 (24).]

section. They include different types of tissue cultures, osteoblastic cell lines, and primary cells. Bone tissue cultures offer the advantage of preserved intercellular interactions, thus presenting a more *in vivo*-like experiment compared with isolated cell systems. In 1984 Stracke *et al.* (33) reported that GH increased alkaline phosphatase (AP) activity in the culture medium from embryonal rat tibias in tissue culture. Furthermore, IGF-I was increased in the culture medium after addition of GH to cultured tibias, indicating that GH stimulated IGF-I production in the bone specimen. These observations were later confirmed and extended by studies of Maor *et al.* (26, 27). Pieces of cartilage isolated from the rat mandibula were cultured on the top of collagen sponges in the absence or presence of GH. Three-day incubation with GH caused a marked increase in DNA synthesis and in the size of the cartilage specimen. The effect of GH was even more pronounced after 6 days in culture, at which time a distinct network of trabeculae was noted throughout the extracellular matrix. The trabeculae contained osteocyte-like cells and were in close contact with both osteoblast-like and osteoclast-like cells. Positive staining with antibodies against bone-specific antigens, *i.e.*, osteocalcin and osteopontin, provided further support for the notion that the newly formed trabecular formation was comprised of bone matrix components. Untreated control cultures lacked bone-like structures, demonstrating that GH directly induced bone formation *in vitro* (27).

B. Effects of GH on osteoblasts

1. GH directly stimulates osteoblasts. The effect of GH has been studied in a number of osteoblastic cell lines and primary isolated cells of various origin, including human, chicken, rat, and mouse primary cells, and the SaOS-2 human and UMR 106.01 rat osteosarcoma cell line. GH induces proliferation of primary isolated rat (21, 73), mouse (74), chicken (32), human (24, 75–78), and rat osteosarcoma cells (19, 79), as well as cells from a rat osteoblast-like cell line (80) and human osteosarcoma cells (75, 81) (Fig. 2). The effective concentrations of GH are in the physiological range (half-maximal stimulation at 10–50 ng/ml), suggesting that GH exerts direct actions on osteoblasts. Not only does GH stimulate the proliferation of osteoblasts, but, in some studies, it also stimulates differentiated functions of these cells. Thus, typical phenotypic functions of osteoblasts such as AP, osteocalcin, and type I collagen are stimulated by GH (4, 21, 24, 74, 80, 82). For osteoblasts it is difficult to find a good model system for the identification of the actual target cell of GH action. However, bone marrow- derived precursors of human bone cells are responsive to GH (76, 77), suggesting, in analogy to the actions of GH in early progenitor cells in adipose tissue and cartilage, that GH interacts with progenitor cells.

2. The role of IGF-I for GH action in osteoblasts. IGFs exert anabolic effects on osteoblasts. IGF-II is expressed in both rodent and human osteoblasts (83–86). IGF-I is produced by rodent (87–90) osteoblasts while contradictory results have been presented for human osteoblasts. Chenu and co-workers (82), but not Kassem *et al.* (24), were able to detect significant amounts of IGF-I in the culture medium from human osteoblast-like (hOB) cells. Studies both detecting (85, 86, 91) and not detecting (92) IGF-I mRNA transcripts in human osteoblasts have been presented. Furthermore, an *in situ* hybridization study demonstrated that osteoblasts in adult human osteophyte tissue express the IGF-I mRNA transcript (93).

Whereas circulating levels of IGF-I are GH dependent, GH may not be the chief determinant of local IGF-I production in bone. Thus, *in vitro* regulatory effects of estrogen, PTH, and cortisol, as well as a variety of local growth factors, on IGF I production have been demonstrated (86, 88, 94–102). The regulation of local IGF-I by growth factors and hormones is of potential clinical importance, but in this article only GH-modulated effects in bone are discussed. A stimulation of osteoblastic IGF production by GH has been demonstrated by some authors (80, 82) but not by others (24, 83). To examine the importance of IGF-I as a mediator of GH action, endogenous IGF-I was sequestered by an antiserum to IGF in bone cell cultures. As a result, the proliferative action of GH was abolished (21), indicating that local IGF-I is important for GH-induced cell proliferation. In another study, by Scheven *et al.* (75), it was demonstrated that GH induced osteosarcoma growth but not growth of human osteoblast-like cells when the cells were cultured in the presence of IGF-I antibodies. In summary, GH induces IGF-I expression in rodent osteoblasts, while the induction of IGF-I by GH in human osteoblasts is uncertain.

The bioactivity of IGFs in bone tissue is modulated by several IGFBPs, mainly IGFBP-3, -4, and -5 (103). Therefore,

some of the GH effect may be mediated via a regulation of the local production of IGFBPs in osteoblasts. It is well known that GH treatment increases serum levels of IGFBP-3 (104–109), and the complex of IGF-I and IGFBP-3 is more effective in stimulating cortical thickness in ovariectomized (OVX) rats than IGF-I alone (110). IGFBP-3 is produced by osteoblasts. GH increases IGFBP-3 production in rat cells (73, 96, 111, 112), while no effect of GH is seen on IGFBP-3 expression in human cells (24, 83, 113). IGFBP-4 was originally isolated from bone as the inhibitory IGFBP (114), while IGFBP-5 is regarded as a stimulatory IGFBP for osteoblastic proliferation (115–117). In rat, as well as in human osteoblasts, it was found that GH decreases IGFBP-4, as determined by ligand blotting (113, 118), while no effect of GH was seen on IGFBP-4 protease activity in human osteoblast-like cells (119). In primary rat osteoblasts, IGFBP-5 mRNA levels were increased 2-fold after GH treatment (96). Interestingly, a recent clinical study has demonstrated that GH treatment increases serum levels of IGFBP-5 in GHD children (109). Whether this effect of GH is a direct effect on osteoblastic IGFBP-5 production remains to be shown. In conclusion, there are some indications of a GH-induced regulation of IGFBPs that potentially might have a regulatory role in bone metabolism.

Many functions of GH can be exerted without prior synthesis of IGFs. Thus, the expression of the protooncogenes c-*fos*, c-*jun*, jun B, and c-*myc* are expressed in the presence of protein synthesis inhibitors (120). Recently, using human osteoblast-like cells, Melhus and Ljunghall (121) demonstrated that different sets of genes were induced by IGF in some cases and GH in others, indicating that these factors have separate actions. In conclusion, it appears that some of the effects of GH on osteoblasts are mediated by IGFs, but others are not.

3. Regulation of GH receptor (GHR) expression. Barnard and colleagues (19) were the first to show specific high-affinity GHRs on osteoblast-like cells (UMR 106.06 cells). The presence of these receptors has been confirmed in primary isolated cultured human (78) and mouse (122) osteoblasts. Serum decreases the number of GHRs (79). In a search for the factors in serum responsible for this reduction in GHR expression, it was found that IGF-I and -II decrease the number of GHR in a dose- and time-dependent manner (123). This decrease was accompanied by a decrease in the levels of mRNA encoding the GHR. Similarly, the action of GH on osteoblastic proliferation was decreased after preincubation of the cells with IGFs (124). Conversely, it was found that IGFBPs up-regulated GHR number and activity, possibly through inhibition of IGF activity (123, 124) (Fig. 3). These findings suggest a local feedback of the GH/IGF axis at the tissue level. Thus, hypothetically, IGF decreases GHR number and activity, fine-tuned by the presence of IGFBPs (123, 125). Leung *et al.* have suggested that the negative feedback of the GH/IGF-I axis in skeletal tissue might involve three different mechanisms: a) liver-derived IGF-I inhibits pituitary GH secretion, b) bone-derived IGF-I inhibits pituitary GH secretion, and c) bone-derived IGF-I inhibits local action of GH by reducing GHR availability (125) (Fig. 4).

Retinoic acid is another modifying factor for GHR expression. Similar to what was shown previously in embryonal stem cells (126), it induces an increase in GHR number in mouse osteoblasts (122). Estrogen is known to exert important effects on bone tissue, and a recent study indicates that estrogen interacts with GH action at the cellular level. In rat and human osteoblasts, 17β-estradiol promoted GH-stimulated proliferation and increased [^{125}I]GH binding and GHR mRNA levels (127). High levels of glucocorticoids induce an increase in [^{125}I]GH binding and GHR mRNA levels in rat osteosarcoma cells and GHR mRNA levels in human osteoblasts (79, 128). In contrast, corticosteroids reduce [^{125}I]GH binding and GHR mRNA levels in primary isolated rat growth plate chondrocytes (our unpublished data). In both rat osteoblasts and in rat growth plate chondrocytes the effect of GH was reduced by high levels of glucocorticoids.

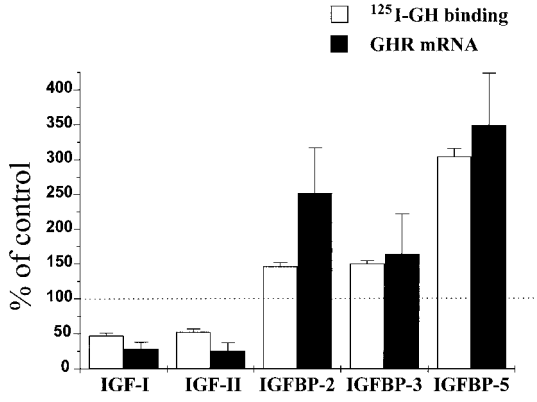

FIG. 3. Regulation of GHR expression in rat osteosarcoma cells. Effects of IGF-I (100 ng/ml), IGF-II (100 ng/ml), IGFBP-2 (3000 ng/ml), IGFBP-3 (3000 ng/ml), and IGFBP-5 (2000 ng/ml) on [^{125}I]GH binding and GHR mRNA expression in rat osteosarcoma cells. Values are given as percent of control culture. [Figure is derived from Slootweg *et al.* (123, 124).]

In conclusion, a regulation of GHR expression in osteoblasts may be important for 1) a local autocrine feedback loop in the GH/IGF-I axis and 2) for sex steroids, glucocorticoids, and other factors modulating the effect of GH in osteoblasts.

4. GH signal transduction in osteoblasts. The GHR is a member of the cytokine/hemopoietic growth factor receptor family (129). GH signaling via its receptor has now been shown to be mediated through cascades of protein phosphorylation resulting in activation of nuclear proteins and transcription factors. The GHR itself is not a tyrosine kinase. Instead, after binding of GH to its receptor, an association with a protein, JAK2, occurs. JAK2 is then phosphorylated and in turn phosphorylates the GHR (130–132). A number of signaling pathways may transduce the signal from this complex to the nucleus. The first cascade is via STAT proteins (133–135), which upon phosphorylation are translocated to the nucleus and bind to DNA. Also, the Ras-Raf signaling pathway plays a role in the GH-induced signaling (136, 137). IRS-1 and -2 are also proteins functioning as signal transducers for the GHR after they have been phosphorylated on tyrosine residues (138–140).

In mouse osteoblasts, it has been shown that GH induces the nuclear protooncogenes c-*fos*, c-*myc*, c-*jun*, and Jun-B (120, 141). The formation of diacylglycerol was induced, and

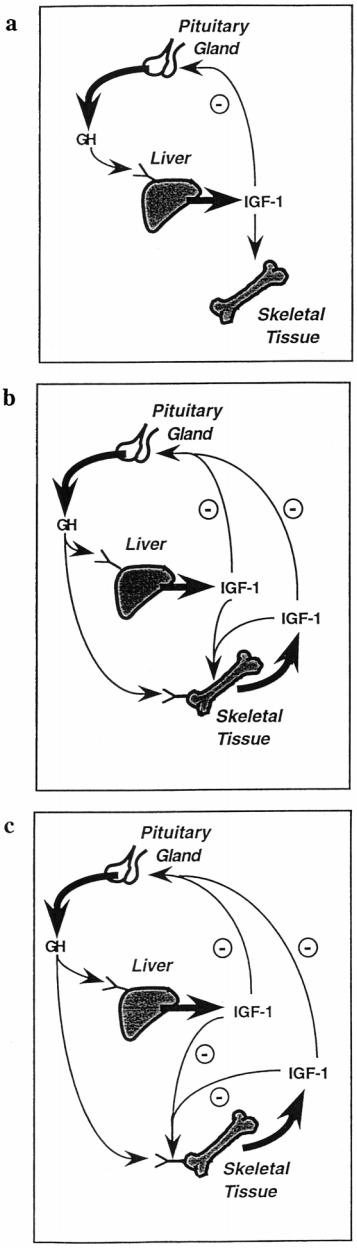

FIG. 4. Schematic representation of regulation of skeletal tissue by GH and IGF-I. Y depicts GHRs. a, Classic negative feedback mechanism. In accordance with the somatomedin hypothesis, circulating IGF-I derived from the liver in response to GH stimulates skeletal tissue growth and feeds back centrally to inhibit pituitary GH secretion. b, Modified classic negative feedback mechanism. In addition to the endocrine effects of IGF-I derived from the liver, GH is able to directly stimulate skeletal tissue growth through local production of IGF-I. Hepatic and extrahepatic sources of IGF-I contribute to feedback inhibition of GH release. c, Proposed peripheral negative feedback loop. IGF-I produced by skeletal tissue in response to GH feeds back to inhibit the local action of GH by reducing GHR availability. [Figure is adapted from Leung *et al.* (125).]

the signaling was found to be dependent on a form of protein kinase C. In these cells, the involvement of the phorbol ester-sensitive transregulating transcription factor AP1 in GH-induced gene transcription was demonstrated for the first time (141).

In rat osteosarcoma cells, another signaling protein, annexin 1, was detected recently as being tyrosine phosphorylated upon stimulation of the cells with GH (142). The tyrosine phosphorylation of this protein also occurs after stimulation of cells with epidermal growth factor, $pp60^{v\text{-}scr}$, angiotensin II, and insulin (143). Although it appears as if this is a general mechanism of signal transduction in cells, the exact function of this protein is unknown, as is its place in the already known signaling cascades (143).

C. Effects of GH on osteoclasts

GH increases the number of osteoclasts in the metaphysial bone of the proximal tibia of hypophysectomized rats (25). However, the mechanism for this effect is less clear. GHR mRNA has been detected in mouse marrow cultures (144) and in mouse hemopoietic blast cells (145). In a recent study by Nishiyama *et al.* (145), using mouse stromal cells and hemopoietic blast cells, it was found that GH stimulates osteoclastic bone resorption through both direct and indirect actions on osteoclast differentiation and indirect activation of mature osteoclasts. Factors that may mediate the indirect GH-regulated osteoclast formation include IGF-I and IL-6, both of which are involved in osteoclast formation and have been shown to be regulated by GH (33, 81, 145–149). It has earlier been demonstrated that IGF-I supports activation and formation of osteoclasts in cultures of unfractionated mouse bone cells (146, 149) and that osteoblasts mediate IGF-I-stimulated formation of osteoclasts in mouse marrow cultures and activation of isolated rat osteoclasts (150). Furthermore, human osteoclasts express functional IGF-I receptors (151). In another study by Ransjö *et al.* (144), using mouse marrow cultures, GH caused an inhibition of osteoclast formation by an IGF-I-independent mechanism. In summary, available data suggest that GH regulates osteoclast formation but both stimulatory and inhibitory mechanisms have been presented, probably due to differences in culture conditions. Future studies are necessary to improve the understanding of the physiology of GH-induced effect on osteoclast.

IV. Effects of GH on Bone Metabolism in Animals

In vivo animal models are useful when evaluating the influence of GH treatment on changes in bone mass, bone metabolism, and mechanical strength of bones. For histological analyses the models are excellent because it is possible to perform static and dynamic histomorphometry and to evaluate differences in regional response. Systemic GH administration increases circulating levels of other hormones that influence bone such as IGF-I and the active vitamin D metabolite ($1,25\text{-}(OH)_2D_3$) (152, 153). Until now, systemic GH administration has been used in nearly all animal experiments, and it has been impossible to elucidate whether the measured changes are caused by local or systemic (via circulating IGF-I) GH stimulation. GHRs have been detected in rat femur epiphyseal and calvarial osteoblasts using immunoreactive and mRNA techniques (80, 154). The effect of local delivery of GH to bone has been studied in rats, and the effects of local expression of GH in bone tissue in GH-transgenic mice have been presented. These recent studies, *in vivo*,

show that GH is able to stimulate bone formation via a direct interaction with bone tissue (155–158).

A. Effects of GH deficiency and GH replacement on bone parameters

Hypophysectomy (HX) of rats with subsequent replacement with T_4 and glucocorticoid is followed by a rapid and pronounced decrease in the amount of metaphyseal and vertebral body cancellous bone. Bone volume, trabecular number, and trabecular thickness are decreased, and bone formation is minimal (159–162). The cancellous bone resorption is also enhanced. Using tetracycline labeling, dynamic histomorphometry demonstrates that bone resorption is enhanced after HX (161). The result is in accordance with the previously observed decline in bone mass but perhaps unexpected because static histomorphometric investigations have clearly shown that HX decreases the number of osteoclasts and bone surface area covered by osteoclasts (25, 162). Biochemical markers for bone formation are also decreased after HX. Thus, circulating osteocalcin declines, and the mRNA levels of osteocalcin and α1(I)-procollagen in the bone are decreased (163, 164).

When GH is given to HX rats, increases in both bone formation and the number of osteoclasts are seen (25, 161). Correspondingly, an increase in serum osteocalcin and bone mRNA levels of osteocalcin and α1(I)-procollagen is observed (163–165). Furthermore, GH, but not IGF-II, increases incorporation of radioactive thymidine and proline in femur and tibia of HX rats (165). In bone from HX rats a decrease in mRNA levels of IGF-I is found, and the levels are restored after GH replacement (163). This observation strongly suggests that GH has a direct effect on bone cells. However, the bone content of IGF-I protein was not influenced by HX. In summary, HX of rats results in a decreased bone formation with a concomitant decrease in bone mass.

GH replacement therapy restores bone formation and bone mass. Conflicting results have been presented regarding the specific effect of GH on bone resorption after HX, probably due to the fact that these animals are also lacking gonadotropins and are sex steroid deficient. Thus, the dwarf rat (dw/dw) with a normal pituitary function, except for GH deficiency, is probably more appropriate for studying the specific effect of GH deficiency. This animal model was recently used in bone mass and metabolic experiments (166–170). Cancellous bone volume, bone mineral density (BMD), and serum AP are decreased in the dwarf rats, compared with normal rats fed *ad libitum* and food-restricted animals, although the food restriction caused growth retardation that was similar to that in dwarf rats (169). Dwarf rats treated with GH showed no difference in bone volume when compared with normal animals, while BMD was decreased and serum AP increased in these animals (169). In cortical bone from these dwarf rats, GH treatment caused increased periosteal bone formation and collagen deposition and a slight decrease in BMD (170). Taken together, these studies support the earlier observations in HX rats, *i.e.*, that GH increases bone formation and bone mass in GHD animals.

B. Effects of GH treatment on bone parameters of animals with normal GH secretion

Normal rats have been used widely for studying the influence of GH on intact bone, and experiments have been performed in young, adult, and old rats. However, in almost all of these experiments GH administration has induced linear bone growth because the growth plates do not close until the rats are very old (171). Therefore, the data have to be evaluated in relation to both growth/modeling and remodeling (172, 173). The response pattern in rats should be compared with the situation in primates (monkeys, humans), which will be discussed later on in this section. In primates the growth plates are closed after sexual maturation and confounding factors, due to stimulation of bone growth and bone modeling, are of less importance.

1. Effects of GH in rodents. GH administration increases cortical bone mass in normal rats. Tetracycline labeling of the mineralization front demonstrates that GH induces subperiosteal bone formation without influencing the endosteal bone surface (174–176) (Fig. 5). The new bone is organized in a manner similar to that of adjacent bone that was formed before the start of GH injection, *i.e.*, in concentric lamellae and with the same direction of the collagen fibers. After withdrawal of GH administration, the subperiosteal bone formation ceases quickly in areas with minimal bone formation before the start of GH treatment. A remaining effect of GH, however, was found in areas where active bone formation occurred before the start of treatment. The new bone formed during GH administration is preserved after discontinuation of the treatment (176). Corresponding to the increased bone mass, there is also an increase in mechanical strength of the whole bone, and the mechanical quality of the bone itself is almost the same in GH-injected animals as in controls (175–177). The GH-induced subperiosteal bone formation also shows regional differences. At the outer surface around the lumbar vertebrae, new bone deposition is seen whereas no effect of GH is observed at the surface of the vertebrae toward the vertebral canal (178). GH also causes formation of cavities inside the cortical shell of the vertebral body in contrast to diaphyseal cortical bone in rats (178, 179), suggesting that GH exerts site-specific effects on bone.

In all these experiments the GH-treated rats gained weight. When GH was given during spaceflight, an increase in subperiosteal bone formation was seen in rats under weightless condition, and the amount of added bone was similar to that obtained by GH administration on the ground (180). This observation demonstrates that the increased bone formation caused by GH treatment was not caused by the increased mechanical stress due to the weight gain in the rats.

Cancellous bone mass of the vertebral body does not seem to be affected by GH administration in normal old rats as no differences in bone volume and bone surface/bone volume have been found (178). Apart from increasing cortical bone mass, GH also increases bone turnover. GH administration increases serum osteocalcin and increases formation of bone collagen in both cancellous and cortical bone as determined by *in vivo* labeling with radioactive proline (181–183). Bone resorption is also augmented, as shown by measuring excretion of pyridinolines and the specific marker [^{3}H]tetra-

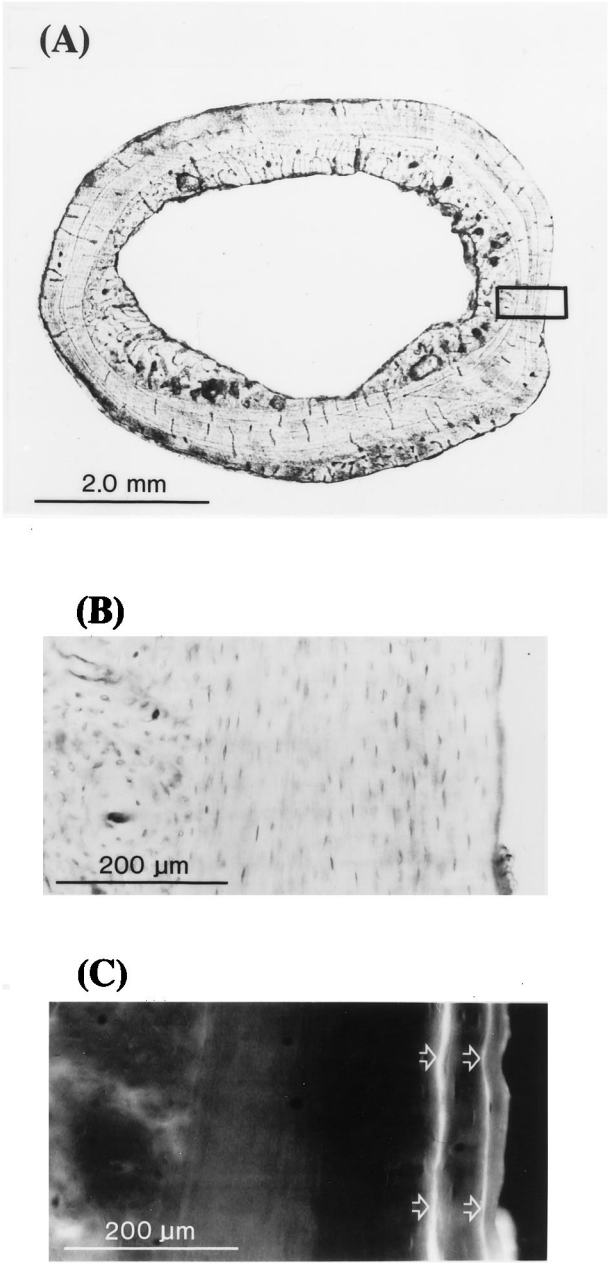

FIG. 5. GH increases periosteal bone formation in old male rats with a normal GH secretion. The rats were given GH (2.7 mg/kg/day) for 80 days. All animals were labeled with tetracycline on days 41 and 69. Only in the GH-treated group was subperiosteal tetracycline double labeling seen. Cross-sectional appearance of the femur diaphysis from a rat given GH for 80 days. A, The unstained cross-sectional appearance using light microscopy. The area inside the frame is shown using light microscopy (B) and epifluorescence microscopy (C). The two tetracycline-labeling lines (days 41 and 69) are marked by *arrows*. Bone formation also takes place from day 69 until animals are killed (distance between labeling line day 69 and periosteal border). [Reproduced with permission from T. T. Andreassen *et al.*: *J Bone Miner Res* 10:1057–1067, 1995 (176).]

cycline in rats labeled with [^{3}H]tetracycline before GH treatment (184). Because bone matrix is a major reservoir for IGF-I, Yeh *et al.* measured the content of bone matrix IGF-I after 9 weeks of GH treatment. However, these investigators found no increase in bone matrix content of IGF-I in the GH-treated animals (181).

Intermittent PTH injection to rats increases bone mass primarily by inducing endosteal and cancellous bone deposition, whereas the subperiosteal bone deposition is modest (185, 186). When GH and PTH are given simultaneously, a substantial increase in bone mass of vertebral bodies is seen because the GH-induced subperiosteal bone deposition takes place together with the PTH-induced endosteal and cancellous bone deposition (187), suggesting that different treatment protocols using combinations of PTH and GH might be clinically useful.

In summary, these results from GH-treated rats with a normal GH secretion clearly demonstrate that GH increases cortical bone mass by inducing subperiosteal bone formation while no large effect on cancellous bone mass is seen.

2. Effect of GH in transgenic mice. The creation of the first giant GH-transgenic mouse in 1982 attracted considerable attention from scientists as well as the popular press (65, 188). The extent of GH expression and tissue distribution of GH in the transgenic mice depend on which promoter is attached to the GH gene. In most bone metabolic studies, the metallothionein promoter (MT) fused to the GH gene has been used, resulting in very high serum levels of GH (188–194). However, two new GH-transgenic lines with a tissue-specific expression resulting in high local concentrations of GH without affecting serum concentrations of GH have recently been described: 1) Baker *et al.* (158) used the osteocalcin promoter, resulting in GH expression in osteoblasts; 2) Saban *et al.* (156) used GH driven by β-globin regulatory-elements, resulting in an erythroid expression with an "adult" expression in the bone marrow.

The femora of MT-GH-transgenic mice with very high serum concentrations of GH demonstrate an increased bone growth, an increased BMC, no change in BMD (BMC/vol), and an increased mechanical strength (188, 194). The increase in mechanical strength was due to an increased cortical width and not due to an improved quality of the bone. Rather, one of the parameters measuring the quality of the cortical bone, the E-module, was decreased in GH transgenic mice (194). It should be emphasized that these mice have been exposed to supraphysiological serum levels of GH (more than 10 times increased) from late prenatal life (194). Interestingly, disproportionate skeletal gigantism has been found in adult MT-GH-transgenic mice, suggesting that supraphysiological GH levels exert differential effects on different parts of the skeleton (189). These studies in GH-transgenic mice with increased serum concentrations of GH give support for the fact that GH increases cortical bone formation, resulting in an increased mechanical strength of the bone. However, the net result on bone mass in old MT-GH-transgenic mice is also highly dependent on bone growth and bone modeling.

The erythroid-specific GH-transgenic mice had increased cortical bone thickness, and the authors suggested that the local effect of GH from erythroid cells in the bone marrow is a major contributor to the increased bone deposition in these GH-transgenic mice (156). However, a slight increase in serum levels of GH was seen, indicating that some of the effect of GH may have been systemic. In the osteoblast promoter-

driven GH-transgenic mice the femora demonstrated an increased growth, increased cortical width, and an increased mechanical strength (157, 158). Similar to the situation in the MT-GH-transgenic mice the quality of the bone, as measured with the E module, was decreased (157, 194). As the serum levels of GH were not increased in these GH-transgenic mice, it was concluded that the stimulatory effect on bone formation was caused by local effect of GH.

GH-transgenic mice have also been used as a model to study the functional interaction between male and female sex steroids with increased expression of GH. It was found that preserved gonadal function was a prerequisite for the increase in bone mass caused by overexpression of GH (192, 193).

3. Effects of GH in primates. As discussed above, GH exerts potent effects in rodents, resulting in an increased bone formation. However, it is possible that some of these effects are due to bone growth and bone modeling, as rodents close the epiphyseal plate late in life. The monkey is a primate and the bone metabolism in these animals is more similar to that of humans. The effect of GH has been studied in hypogonadal female monkeys. The monkeys were made hypogonadal by treatment with a GnRH agonist for 10 months, resulting in a 12% decrease in BMD (BMC/area). GH supplementation (100 μg/kg/day) reduced the decline of BMD in GnRH agonist-treated monkeys (195). A recent study, using old female monkeys, demonstrated that GH (100 μg/kg/day), but not IGF-I (120 μg/kg/day), given for 7 weeks increased bone formation as measured with mineral apposition and bone formation rates (196) (Fig. 6). No additional effect was seen when IGF-I was given together with GH. The effect was seen both in the tibia and in the femur whereas no significant effect was seen in the vertebrae. The difference between the long bones (predominantly cortical bone) and the vertebrae (predominantly cancellous bone) in terms of GH responsiveness is similar to what has been described earlier in old rats. These experimental studies of primates are promising for future human clinical studies. However, further long-term studies with GH treatment of primates are needed to elucidate whether the increased bone formation results in an increased bone mass and mechanical strength.

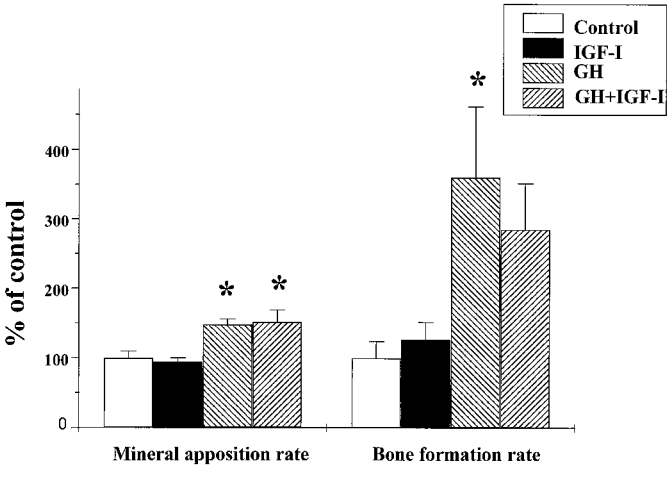

FIG. 6. Histomorphometric study of effect of GH and/or IGF-I treatment in old female monkeys. GH (100 μg/kg/day) but not IGF-I (120 μg/kg/day) given for 7 weeks increased bone formation in the tibia as measured with mineral apposition rate and bone formation rate. No additional effect was seen when IGF-I was given together with GH. Mineral apposition rate was measured as micrometers per day, and bone formation rate was measured as cubic micrometers per μm^2/yr with a surface referent. Values are means $\pm$ SEM of control. *, $P < 0.05$ *vs.* control. [Figure is derived from Ref. 196.]

4. Effects of GH in OVX animals. Ovariectomy in rats results in a substantial loss of cancellous bone and an increase in bone turnover rate (197–199). In cortical bone, ovariectomy results in enhanced resorption at the endosteal surface, whereas bone formation at the periosteal surface initially increases, but thereafter reacts as seen in intact animals (200–202). OVX rats have been accepted as an animal model of postmenopausal bone loss and the current FDA "Guidelines for preclinical and clinical evaluation of agents used in the treatment or prevention of postmenopausal osteoporosis (1994)" recommend that new potential agents first should be evaluated in the OVX rat model (203–205). As GH is a potential anabolic agent, there have recently been a number of studies in which GH was given systemically to OVX rats (187, 206–209). GH increases cortical bone mass by inducing subperiosteal bone formation (187, 207). In the OVX model, where cancellous bone mass is normally measured in the tibial metaphyses or inside the vertebral body shell, the results show an increase in bone volume, bone surface/bone volume, mineralizing surface, osteoid surface, and osteoclastic surface in response to GH treatment (187, 206, 208). In addition, the mechanical strength of the vertebral body is increased and correlates well with the increase in bone mass (187, 210). The results of GH treatment on cancellous bone seems rather promising, but it must be emphasized that GH also stimulated linear bone growth, which makes the interpretation of the results difficult. At present, it is not known whether there is any relationship between linear growth and an increased cancellous bone volume in this model. GH was unable to augment cancellous bone volume in old rats that do not show linear growth, although GH increased cortical bone mass considerably in these animals (176, 178). In OVX rats the bone metabolism is enhanced, and no further increase in pyridinolines excretion, circulating osteocalcin and cancellous bone osteoid, or mineralizing surfaces has been observed after treatment with a low dose of GH (209).

These studies, using OVX rats, indicate that GH alone or in combination with another hormone may be useful in the treatment of postmenopausal osteoporosis. However, further studies need to be performed in old OVX rats and primates with closed growth plates.

5. Effects of GH in animals treated with glucocorticoids. In rats, rabbits, and dogs, glucocorticoid treatment has been shown to decrease bone formation and bone mass (211–214). The effects, however, vary with species and in the rat model low doses of glucocorticoids increase bone mass and mechanical strength of bone whereas higher doses decrease bone formation, bone mass, and bone strength (215–217). In mice, simultaneous administration of GH and glucocorticoids prevents the catabolic effect of glucocorticoids whereas this does not seem to be the case in rats. However, the number of

experiments is still very limited, which is why the interpretation should be cautious. Using mice, Altman *et al.* (218) showed that glucocorticoids caused a decline in linear bone growth, trabecular bone volume, cortical bone width, mineral bone content, and bone alkaline- and acid-phosphatase activity. The observed declines in different bone parameters were inhibited when glucocorticoid and GH were given simultaneously. The results correspond well with histological data obtained in a trial in children when GH and glucocorticoid were given either separately or simultaneously (219). In rats a short dose-response study showed that GH is able to prevent glucocorticoid-induced growth inhibition (220). In long-term experiments, however, GH does not seem to counteract the glucocorticoid-induced decline in linear growth, bone formation, and bone mass, although GH alone increases these parameters (179, 221).

C. Effects of GH on fracture healing

When movements between the ends of a fractured bone are possible, bone healing is initiated by formation of a thick periosteal callus of woven bone with a central area of cartilage. Through endochondral ossification the cartilage is subsequently replaced by woven bone. Later in the healing phase, a marked modeling takes place and hereby the callus volume declines and the density is enhanced (222, 223). As GH stimulates both periosteal bone formation and linear growth where bone formation takes place by endochondral ossification, it has been natural to examine the effect of GH treatment on healing bones.

In rats, GH administration increases callus formation and mechanical strength of healing fractures (224–229). The enhanced rate of healing continues after withdrawal of GH (230, 231). A considerable delay in mechanical strength development of healing fractures is seen in old rats and GH treatment partly prevents this delay (232, 233). Augmented callus formation is found in the rat bone defect model, when GH is administered both systemically and locally (155). GH has not previously been applied locally either to intact bone or healing fractures, and the data imply that GH exerts a direct, non-liver-mediated effect on bone tissue (155). In studies in which rats were used, only a few papers show no effect of GH on healing bone defects and fractures (234, 235), and GH has not been able to stimulate formation of new bone in titanium bone conduction chambers (236).

In rabbits, GH has not been able to increase callus formation or mechanical strength in healing fractures and bone defects (234, 237–239). However, when subperiosteal bone formation was induced by applying a cerclage band around the femur, GH was able to enhance bone formation in rabbits (240).

In dogs, GH administration augments callus formation in bone defects, and in human trials GH treatment stimulates healing of fractures and pseudoarthroses, when evaluated by radiographs and clinical examination (241–244).

In summary, GH treatment in rats obviously increases callus formation and the mechanical strength of healing bones, whereas the response in the rabbit model seems to be much weaker. At present, it is not possible to evaluate whether GH treatment has any role in human fracture healing because only a few clinical trials and experiments in higher animals have been performed.

V. Effects of GH on Bone Metabolism in Humans

A. Bone metabolism in patients with acromegaly and GH deficiency

1. Acromegaly. Active acromegaly has consistently been associated with increased bone turnover (245–252). In a study of 16 acromegalic subjects, osteocalcin concentration was increased 2-fold, and urinary excretion of hydroxyprolin increased 3-fold compared with control subjects (251). The serum concentration of osteocalcin is positively correlated to GH and/or IGF-I concentration (252). Successful treatment of these patients normalized serum osteocalcin and the urinary excretion of hydroxyproline (252, 253). Octreotide treatment reduced osteocalcin concentration but not type I procollagen (PICP) (254). The net effect of the increased bone metabolism in untreated acromegaly has been obscured in some studies by confounding factors such as hypogonadism (255). In fact, many years ago acromegaly was seen as a cause of osteoporosis (256) whereas later studies revealed normal or more often increased bone mass in patients with acromegaly without gonadal insufficiency (251, 255, 257–261). The trabecular bone density of the lumbar spine in patients with acromegaly was decreased in one study, as determined by quantitative computerized tomography, while it was increased in another study using dual energy x-ray absorptiometry (DEXA) (246, 262). In contrast, bone histomorphometric investigations in patients with acromegaly disclosed a significant increase in both cortical and trabecular bone mass in iliac crest. In trabecular bone, resorption surfaces and active and total formation surfaces were increased (249). BMD assessed with DEXA was increased in the proximal femur whereas the BMD of the lumbar spine was similar to that of healthy controls (251). In summary, most studies suggest that cortical bone mass is increased in acromegaly (4, 5, 249, 251, 255, 260, 262) whereas trabecular bone seems largely unaffected.

2. GH deficiency (GHD). There is no conclusive data on the effects of GHD on bone remodeling in adults. Serum levels of osteocalcin, reflecting osteoblast activity and bone formation, have been found to be decreased (263–267), increased (268), or unchanged (269). Most studies have shown that there is no difference in resorption markers between controls and adult GHD patients (264, 268, 270).

a. Bone mass in adult patients with childhood-onset GHD. In children with GH deficiency, a relative osteopenia is found before the start of exogenous GH treatment, an effect that might be due to a delay in skeletal maturation (271–273). Several studies have shown low bone mass in adults with childhood-onset GHD (268, 273–280). In a cross-sectional study of 30 young adult males, with childhood-onset GHD, Kaufman *et al.* (273) found decreased bone mineral content in the lumbar spine and forearm compared with age- and height- matched controls. The BMC in the lumbar spine was shown to be between 9 and 19%, and in the forearm 20 and 30% lower compared with controls, using dual- and single-photon absorptiometry, respectively. A similar decrease in

BMC was observed in patients with multiple pituitary deficiencies and isolated GHD. These observations were recently confirmed by de Boer *et al.* (275) who performed a similar cross-sectional study in 70 adult men with childhood-onset GHD. This investigations found that the BMD area (BMC/bone area) in GHD patients was significantly reduced at the lumbar spine as well as the nondominant hip. In fact, in 33% of the patients the lumbar spine BMD area was at least 2 SD lower than normal. They also observed a positive relationship between body height and BMD area. Patients and controls differed in body height, which partly explained the difference in BMD area. However, also after correction for bone size, the difference in BMD area between patients and controls still remained. Similar results were obtained in patients with multiple pituitary deficiencies and isolated GHD.

The similar results observed both by Kaufman *et al.* and de Boer *et al.* in patients with multiple pituitary deficiencies and isolated GHD suggest that lack of GH is the most important factor behind the observed low bone mass in childhood onset GHD (273, 275). A reported reduction in vertebral trabecular bone density assessed by CT technique in 10 males with childhood-onset isolated GHD further supports this conclusion (274). There is no evidence suggesting that bone loss is enhanced after cessation of GH treatment in young adults (273). Thus, it is conceivable that insufficient acquisition of bone mass during childhood and thus reduced peak bone mass explain the reduced BMC and BMD observed in these patients. The cause of the reduced bone mass is probably suboptimal GH therapy in these patients. Patients included in the cited studies were mainly treated with GH when the supply of GH was limited, and the doses used were lower and cessation of treatment occurred earlier than current pediatric practice. In patients with hypopituitarism of childhood onset, the induction and timing of puberty are also important in reaching the optimal peak bone mass. Boys with constitutionally retarded puberty will achieve a lower peak bone mass than boys with puberty of normal onset (281). At present there are no studies showing that GH replacement during childhood results in a normalization of BMD when peak bone mass is reached, suggesting that GH also is important for the additional increase of bone mass that occurs after completion of linear growth. It has been suggested that GH treatment should be continued until the attainment of peak bone mass, irrespective of the height achieved (282).

b. Bone mass in adult patients with adult onset GHD. An increased prevalence of osteoporosis has been found in several recent studies of patients with adult-onset GHD (283–289). In a population of 122 hypopituitary patients, Wüster *et al.* (283) observed that 57% of the patients had low bone mass of lumbar spine as assessed with dual photon absorptiometry, and 73% of the patients had low bone mass of the proximal forearm as assessed with single photon absorptiometry. Johansson *et al.* (284) studied 17 adult GHD men and found that total, but not spinal, BMD, measured with DEXA, was lower in the patients compared with controls. In a study by Rosén *et al.* (286) of 95 (55 males and 40 women) patients with adult-onset GHD with a mean age of 54 yr, BMC was assessed in the third lumbar vertebra with dual-photon absorptiometry. The control population comprised 214 women aged 35–80 yr and 199 men between 16 and 79 yr of age. BMC was found to be lower in all males and in females with untreated as well as treated gonadal deficiency. BMC was lower in patients below 55 yr of age and normal in patients above 55 yr of age. Holmes *et al.* (287) measured vertebral trabecular BMD with quantitative computed tomography (QCT), total BMD and BMD in lumbar spine and hip with DEXA, and BMC in the forearm with single photon absorptiometry (SPA) in adult patients with GHD. There was a highly significant reduction in QCT and in DEXA of the lumbar spine and in SPA of the forearm in these patients. Similarly, as has been shown in patients with childhood-onset GHD, there was no difference in Z-scores between those patients with isolated GH deficiency and those with GH and gonadotropin deficiency. In a subgroup analysis of patients with an estimated age above 30 yr at the onset of the disease, a reduction was still present in QCT and in DEXA of the lumbar spine, and in SPA of the forearm. Interestingly, older adults had less reduction in bone mass than younger, confirming the observation by Rosén *et al.* (286, 287). In a cross-sectional study comprising 64 hypo-pituitary patients, Beshyah *et al.* (288) demonstrated a significant reduction in lumbar spine BMD and BMC in both male and female patients compared with controls. The area and the width of the vertebra were similar in patients and controls. In contrast, Degerblad *et al.* (289) observed normal total, spine, and hip BMD in males with adult onset GHD and Kaji *et al.* (290) found a normal BMD in the spine and midradius of patients with adult onset GHD. However, Degerblad *et al.* (289) found low total, spine, and hip BMD in women with adult onset GHD. A recent study in elderly patients (over 60 yr old) with adult-onset GHD demonstrated normal BMD in the hip and the lumbar spine (291). In summary, most studies demonstrate that patients 55 yr of age or less with adult-onset GHD have decreased bone mass.

c. Fracture rate in GHD patients. Few studies have investigated whether or not GHD patients have an increased fracture rate. The reasons for this are probably that a huge number of GHD patients are required for a meaningful study and/or that additional pituitary hormone deficits may confound the results. However, an increased risk of osteoporotic vertebral fractures has been suggested in hypopituitary patients (283). The consequences of low BMD in GHD adults have only recently been delineated by Rosén *et al.* (292) who found a higher fracture rate in patients with adult-onset GHD compared with that in healthy controls. The fracture rate was studied in 107 patients with adult-onset GHD, and a subsample of the Göteborg WHO MONICA Study was used as a reference population. The total fracture frequency was 2- to 3 times higher in the patients compared with the controls. Confounding factors such as longstanding untreated hypogonadism might have contributed to the low bone mass in some subjects, since most of the studied patients also had other pituitary deficiencies. On the other hand, Holmes *et al.* (287) found a similar reduction in bone mass in patients with isolated GHD and in those with multiple pituitary deficiency. Since peak bone mass may not be reached until the third or fourth decade of life (293), failure of accretion of bone mass may also be partly responsible for the reduced BMD in adult-onset GHD. Again, since patients who acquired their GHD after the age of 30 also have reduced

bone mass, it is likely that GH *per se* is important for the maintenance of the adult bone mass (287).

3. Treatment of GHD patients with GH. GH treatment of GHD adults has consistently been shown to have marked effects on markers for bone formation [serum osteocalcin, serum levels of C-terminal propeptide of PICP, and AP] and bone resorption [urinary hydroxyproline, collagen cross-links, and serum concentrations of collagen type I telopeptide (CITP)] and serum IGF-I levels (263, 266–270, 279, 289, 294–309). There is a dose-dependent increase of bone markers during GH treatment (266, 309), and the increase in resorption- and formation markers was maximal after 3 and 6 months, respectively (300, 304). The similarity in time courses of the bone markers supports the concept of a temporal coupling between bone resorption and bone formation, with resorption preceding formation during bone remodeling (300). After 2 yr of GH treatment these markers were still elevated, suggesting that the increased rate of bone remodeling was sustained (304). An increased bone turnover and an increased cortical thickness, as studied by histomorphometric indices, was found after GH treatment in a study of GHD men (310). Similarly, increased bone turnover has been observed in patients with long-standing acromegaly (251), indicating that bone turnover can be elevated for many years as a result of high plasma levels of GH. Furthermore, indices for bone formation remain elevated for several weeks after short-term treatment with GH, suggesting that the half-life for processes reflecting bone resorption/formation is quite long (302, 311). Administration of GH to healthy volunteers for 7 days produced no discernible effect on serum calcium concentration, but urinary calcium excretion increased. Serum PTH concentrations increased, as did the concentrations of phosphate and 1,25-dihydroxyvitamin D (312). In contrast, in other double blind, placebo-controlled trials (6 months duration) of adult GHD patients, no effect on 1,25-dihydroxyvitamin D concentrations (313) and no effect (313) or a decrease (296) in intact PTH concentrations was found. These changes were accompanied by a concomitant increase in total serum calcium concentrations (296, 313). A similar increase in serum calcium concentrations has been observed by others (297, 299, 300). Still, after 2 yr of GH treatment, serum calcium concentration was elevated compared with baseline (304). GH may enhance 1α-hydroxylase activity (314), thus increasing the concentration (315) or availability (316) of vitamin D_3, which is conceivably the mechanism behind the sustained increase in serum calcium concentration. An alternative hypothesis is enhanced mobilization of skeletal calcium due to increased bone turnover.

Trials involving adults with childhood onset GHD have yielded conflicting results regarding the effect of GH on bone mass. Several short-term placebo-controlled (306–308) and short-term open trials (268, 278, 294, 299) have failed to show any increase in BMD or BMC during GH treatment. In fact, some of these studies (299, 306, 307) reported a slight decrease of BMD or BMC after 3–6 months of treatment. In contrast, O'Halloran (274) reported an increase in vertebral BMD assessed with QCT after 6 months of GH treatment but no changes in proximal or distal forearm BMC. After more prolonged treatment periods (12–30 months), several studies have disclosed more encouraging results (274, 305, 307, 317). Degerblad *et al.* (317) showed an increase in distal and proximal forearm BMD in six patients by 12 and 3.8%, respectively, after 24 months of treatment. Similarly, Vandeweghe *et al.* (307) reported a significant and progressive increase in BMC above pretreatment values, reaching 7.8% for total BMC at the lumbar spine and 9.9% for total BMC at the forearm, after 30 months of GH administration.

Short-term trials of 6–18 months in adults with adult onset GHD (263, 289, 297, 298, 300) failed to show any increase in BMC or BMD. In analogy with the findings observed in adults, with childhood onset GHD, several studies have shown a decrease in BMD and or BMC after 6–12 months of treatment. Holmes *et al.* (263) observed a decrease in BMD after 6 months of treatment at several skeletal sites. After 12 months of treatment, however, there was only a significant reduction in lumbar spine BMD. Similarly, Degerblad *et al.* (289) showed a decrease in total body and lumbar spine BMD after 6 months of GH treatment, but after 12 months of GH treatment there were no differences compared with baseline values. Furthermore, Hansen *et al.* (300) showed that in a placebo-controlled trial of 12 months, a decline occurred in forearm BMC and BMD by 4.2 and 3.5%, respectively. In contrast, in the longest placebo-controlled trial reported so far (18 months), Baum *et al.* (295) reported a significant increase in BMD in lumbar spine and femoral neck of 5.1 and 2.4%, respectively, using a daily dose of GH of only 4 μg/kg. Surprisingly, BMD increased at sites mostly composed of trabecular bone but not at sites composed of cortical bone. Since bone absorptiometry only detects the mineralized component of the bone, the reduction in BMD observed after short periods of GH treatment is best explained by the increased remodeling activity, with an increased remodeling space and an increased proportion of new unmineralized bone. Interestingly, the addition of a bisphosphonate to GH therapy in GHD adults reduced the GH-induced bone turnover and prevented the initial decrease in bone mineral content seen during GH treatment alone (318). Therefore, bisphosphonates might perhaps be an important adjunct to GH replacement therapy in adults with GHD and severe osteopenia during the early phase of GH treatment. However, if bone resorption is a prerequisite for bone formation, it is possible that an initial GH-induced bone resorption is crucial for the following GH-promoted bone formation.

Johannsson *et al.* (304) recently demonstrated that 2 yr of GH treatment in 24 men and 20 women with adult-onset GHD induced a sustained increase in overall bone remodeling activity and a net gain in BMD in several weight-bearing skeletal locations (Fig. 7). A significant increase in BMD first became apparent after 18 months, which might explain why previous trials of shorter duration were unable to demonstrate an increase in BMD. The study also demonstrates the importance of an adequate duration of treatment to include a sufficient number of remodeling cycles and sufficient time for mineralization to occur before a net gain in BMD can be detected with bone absorptiometry. After 2 yr of GH treatment, the total body BMC increased but not the total body BMD. Furthermore, the increment in BMC was slightly more marked than the increment in BMD at the different skeletal loci, suggesting that there was an increase

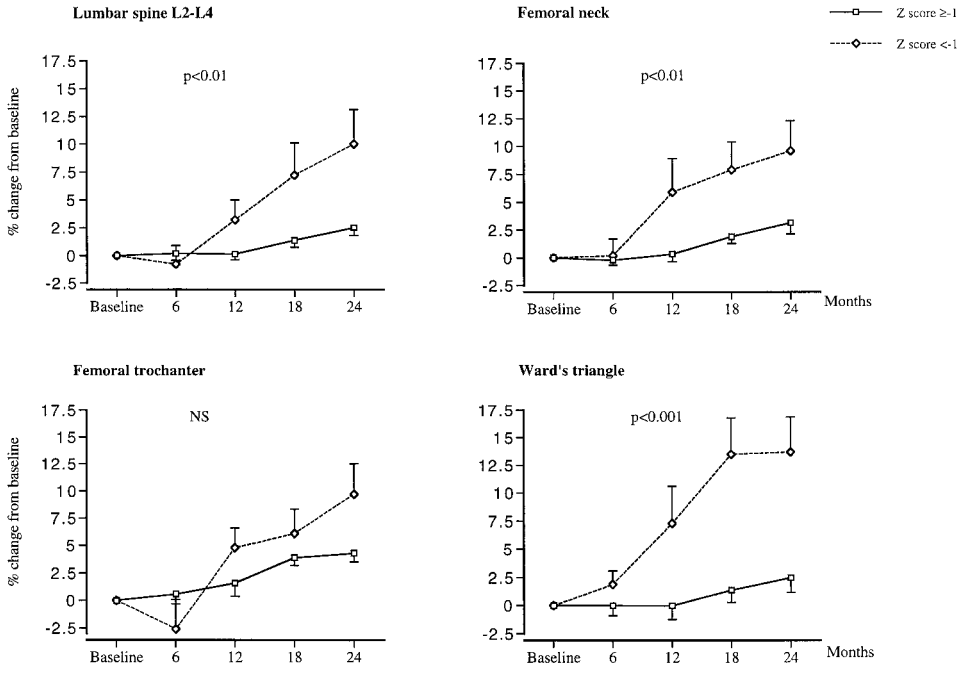

FIG. 7. BMD (BMC/area) in response to 2 yr of GH treatment in two subgroups of patients with adult-onset GH deficiency. One group comprised 13 patients with a baseline z-score of less than −1 SD (*broken line*), and the second group comprised 31 patients with a baseline z-score of −1 SD or more (*solid line*). Values are given as means ± SEM. *P* values denote the difference between the percent changes from baseline in the two groups of patients by two-way ANOVA. [Reproduced with permission from G. Johannsson *et al.*: *J Clin Endocrinol Metab* 81:2865–2873, 1996 (304). © The Endocrine Society.]

in the bone area. A similar increase in bone area after GH treatment of rats has been described in detail in *Section IV*. Interestingly, patients with a z-score of less than −1 SD demonstrated the most pronounced increase in BMD (Fig. 7), reducing the calculated number of patients with the greatest fracture risk by 40–50%, dependent on skeletal loci. However, this calculation is based on the assumption that the changes in quality of bone in GHD adults are similar to what was earlier described in postmenopausal women. Furthermore, it should be emphasized that half of the patients in the study by Johannsson *et al.* (304) were given a supraphysiological GH dosage, which resulted in abnormally elevated IGF-I levels (304). This study suggests that the remodeling balance during GH treatment in GHD adults is positive, particularly in those with a low pretreatment BMD. This is supported by a study demonstrating a continuous increment in forearm cortical BMC 13 months after the discontinuation of GH treatment (319).

A gender difference has also been observed in response to GH. Thus, males responded with a higher increase in serum osteocalcin, PICP, and CITP concentrations, whereas women increased more in total BMC and BMD. This suggests that the interaction between GH and estrogens induces a more positive remodeling balance with less increment in bone-remodeling activity than the interaction between GH and androgens (304).

B. Effects of the GH/IGF-I axis on bone metabolism and bone mass in patients with normal GH secretion

1. Osteoporosis. The causes of osteoporosis are complex and multifactorial. Bone mass decreases with aging, but the mechanisms behind this decrease are unclear. Aging is associated with a decrease in GH secretion (320, 321) and serum IGF-I concentration (322). The GH/IGF-I axis is also influenced by lifestyle factors. For example, smoking decreases IGF-I while physical activity increases GH secretion (322). It has been suggested that the GH/IGF-I axis is one of the major determinants of adult bone mass (323, 324). Thus, a positive relationship between BMD and serum concentrations of IGF-I and IGFBP-3 was observed in healthy men (325). Furthermore, in a study of 245 healthy elderly women, serum IGF-I concentration was found to be an independent predictor of total BMC (326). Circulating levels of IGF-I have been reported to be significantly lower in men (327) and women (328) with osteoporosis. In addition, low plasma levels of IGF-I and IGFBP-3 were found in both male and females with osteoporosis (329), and a relationship has been shown between baseline IGF-I and femoral bone density in women over 70 yr of age (326). Furthermore, IGF-I concentrations are decreased in the skeletons of elderly patients, suggesting that the normal age-dependent decrease in bone mass may be due to a local IGF-I deficiency in the skeleton (330). In contrast, no differences in serum levels of IGF-I, IGF-2, or IGFBP-3 were observed between women with osteoporosis and normal age-matched controls (331, 332).

Several studies have demonstrated that GH increases markers for bone resorption as well as bone formation in subjects with a normal GH secretion. GH increases bone turnover in young healthy male volunteers (311) (Fig. 8) as well as in osteopenic postmenopausal women (333). Osteoporotic patients display similar responsiveness to GH as healthy subjects in the regulation of bone markers for bone formation and bone resorption (334). In a small study in which three patients with primary and secondary osteoporosis were treated with GH, an increase of periosteal new bone formation, as determined with bone histomorphometry, was seen (335). This increase in periosteal bone formation is interesting as it is similar to what has been found in experimental studies (176). However, larger clinical studies with bone histomorphometric analyses are needed to con-

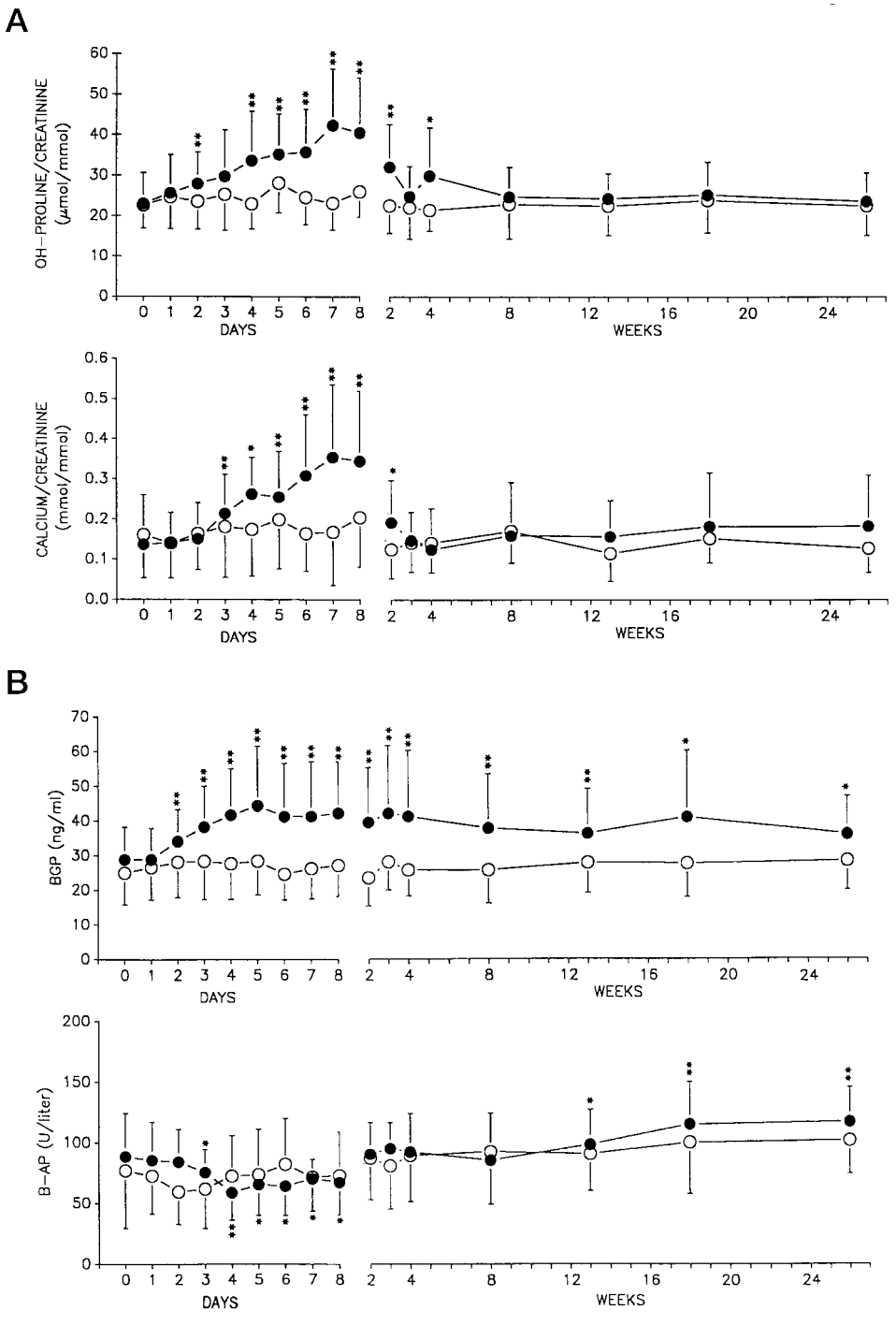

FIG. 8. Effect of 7 days treatment with GH (*filled circles*, n = 10) and placebo (*open circle*) on biochemical markers of bone resorption (panel A) and bone formation (panel B) (mean ± SD) in normal male volunteers. The GH dosage was 0.1 IU/kg twice a day administered subcutaneously. *, $P < 0.05$ and **, $P < 0{,}01$ difference from pretreatment values. [Reproduced with permission from K. Brixen *et al.*: *J Bone Miner Res* 5:609–618, 1990 (311).]

firm that GH induces periosteal bone formation in patients with osteoporosis.

Present treatment modalities of osteoporosis rely almost exclusively on agents aiming at reducing bone resorption. In contrast, GH has a stimulatory effect on bone formation as well as bone resorption, as measured with biochemical parameters, and the gain in BMC/BMD that was observed after long-term GH treatment in adult GHD occurred after the first remodeling cycle (304). There are no GH treatment trials yet of adequate duration (>18 months) in patients with postmenopausal osteoporosis. Aloia *et al.* (336) demonstrated no net gain of total body calcium in osteoporotic women after 12 months of GH treatment. In a 2-yr study of postmenopausal women, addition of GH to continuous, combined, or sequential calcitonin treatment had no additional effects on total body calcium (337, 338). No additional effect of GH treatment was found on bone mineral mass in postmenopausal women treated with pamidronate during 12 months and with the addition of GH for 6 months. On the contrary, the beneficial effect of pamidronate on bone mass and the reduction of biochemical markers for bone turnover was blunted by the addition of GH (339). Holloway *et al.* (340)

recently conducted a study in which postmenopausal women were given cyclic GH treatment for 7 days every 56th day. This cyclic treatment was repeated 12 times and resulted in a small but statistically significant increase in BMD of the lumbar spine and of the hip (1–2%). A cyclic block of bone resorption with calcitonin did not alter the effect of GH in this study. In summary, decreased serum levels of IGF-I/IGFBP-3 are associated with osteoporosis, indicating that the GH/IGF axis is involved in the pathogenesis of osteoporosis. It is not yet shown whether GH increases bone mass in patients with osteoporosis. However, most of the reported studies have been short-term studies, and future long-term studies are needed to determine whether prolonged GH treatment increases bone mass.

The present treatment of postmenopausal osteoporosis includes calcium, vitamin D, calcitonin, bisphosphonates, and estrogen replacement therapy. Ho *et al.* (341, 342) presented data indicating that oral estrogen treatment decreases serum levels of IGF-I and increases GH secretion. The authors claim that oral estrogen results in an impaired hepatic IGF-I production with a concomitant reduced feedback inhibition of GH secretion. In contrast, transdermal estrogen treatment resulted in a slight increase of IGF-I and had no effect on GH secretion. However, Friend *et al.* (343) demonstrated more recently that both transdermal and oral estrogen increase GH secretion and decrease serum levels of IGF-I. These results indicate that the GH/IGF axis also may be involved in the pathophysiology of postmenopausal osteoporosis, and it should be considered in the choice of treatment in patients with postmenopausal osteoporosis.

Deficiency in bone mineral has been widely reported in Turner's syndrome (344, 345). It is generally accepted that osteopenia in Turner's syndrome is believed to be due to estrogen deficiency and not skeletal dysplasia *per se*. Twelve months treatment with GH did not increase spinal BMD in these patients (346). However, long-term treatment of Turner's patients with GH resulted in a normal bone mineral status (347, 348).

2. Effects of GH in elderly. Studies in healthy subjects have not shown any impressive effect of GH on bone mass. Rudman *et al.* (349) studied elderly men above 60 yr of age and reported a slight (1.6%) increase in lumbar BMD after 6 months of treatment. BMD did not change during a 6-month placebo-controlled trial with GH in healthy elderly women, whereas a slight decrease was observed in the placebo group. Marked increases during GH treatment were observed in hydroxyproline and pyridinoline excretion (107, 312). However, serum osteocalcin did not change in women receiving estrogen therapy and increased only in those without estrogen treatment (107) suggesting, in concordance with the data by Ho and Weissberger (342) described above, that hepatic IGF-I generation was suppressed due to oral delivery of estrogen. A positive effect of GH on bone mass in GHD patients is not seen until 18 months of treatment. Thus, long-term studies (>18 months) to explore whether GH increases bone mass in elderly patients are needed before further conclusions regarding the effect of GH on bone mass in these subjects can be made.

3. Corticoid-induced osteoporosis. Glucocorticoid-induced osteoporosis is characterized by a concomitant decrease in bone formation and increase in bone resorption (350). Short-term GH treatment for 7 days in patients receiving chronic glucocorticoid treatment for autoimmune disorders resulted in a significant increase in serum osteocalcin, PICP, and CITP concentrations (351). Further long-term studies are needed to clarify whether or not GH is useful on glucocorticoid effects in bone.

4. Effect of IGF-I. Regarding longitudinal bone growth, IGF has been suggested to be a mediator of some of the GH effect in its regulation of bone remodeling. Because of its potent mitogenic propensity on osteoblasts, IGF-I has been thought to have potential as a formation-stimulating drug in the treatment of osteoporosis. The first clinical study, in which IGF-I (160 μg/kg/day) was given for 7 days to one male with idiopathic osteoporosis, indicated that IGF-I increases biochemical markers of both bone formation and bone resorption (352). Normal women were treated for 6 days with different doses of IGF-I: 30, 60, 120, and 180 μg/kg body weight each day. Numerous side-effects were observed with the two highest treatment doses but none with the lowest dose (353). A dose-dependent increase in PICP (an index of collagen synthesis) and of urinary excretion of deoxypyridinoline were observed, confirming that IGF-I influences both biochemical markers for bone formation and bone resorption. In contrast, Ghiron *et al.* (354) observed that lower doses of IGF-I (15 μg/kg/day) exerted no effect on resorption markers while osteocalcin and PICP increased progressively in elderly women. The authors claimed that a low dose of IGF-I is independently capable of stimulating bone formation without inducing bone resorption. Higher doses of IGF-I gave similar results on markers for bone formation and bone resorption as did GH. In a study (302) of men with idiopathic osteoporosis, the effects of GH and IGF-I on bone metabolism were compared after 7 days of treatment. Both treatment regimens gave a similar increase in osteocalcin concentration and increase in urinary excretion of deoxypyridinoline. However, IGF-I treatment increased PICP more than GH did. Urinary excretion of calcium increased during GH treatment whereas no changes occurred during IGF-I treatment. The authors concluded that the differences between GH and IGF-I might be dose dependent, but could also indicate separate mechanisms of actions of the two peptides at the cellular level. This notion is supported by a recent study in short-term fasting women. Thus, in these subjects, IGF-I administration increased biochemical markers for bone formation but not for bone resorption (355). These data suggest a novel use of IGF-I to selectively stimulate bone formation in states of undernutrition and low bone turnover.

In cortical bone of rats, IGF-I treatment results in augmented subperiosteal bone formation, whereas both increased and decreased bone formation has been reported in the cancellous bone (356–358). In primates, however, GH but not IGF-I increased bone formation, as determined by mineral apposition rate (196), and it is not yet clear whether IGF-I promotes bone formation as determined by histomorphometry or bone mineral measurements.

In summary, both GH and IGF-I treatment increases bio-

chemical markers for both bone formation and bone resorption. GH treatment increases bone mass in GHD patients, but it is not yet clear whether IGF-I also has the capacity to increase bone mass in humans. *In vitro* data have demonstrated that GH exerts direct anabolic effects on osteoblasts (see *Section III*), and some of these direct effects of GH may not be achieved by a systemic IGF-I treatment. Future clinical studies will clarify whether IGF-I treatment is as efficient as GH in increasing bone mass.

VI. Summary and Conclusions

It is well known that GH is important in the regulation of longitudinal bone growth. Its role in the regulation of bone metabolism in man has not been understood until recently. Several *in vivo* and *in vitro* studies have demonstrated that GH is important in the regulation of both bone formation and bone resorption. In Figure 9 a simplified model for the cellular effects of GH in the regulation of bone remodeling is presented (Fig. 9). GH increases bone formation in two ways: via a direct interaction with GHRs on osteoblasts and via an induction of endocrine and autocrine/paracrine IGF-I. It is difficult to say how much of the GH effect is mediated by IGFs and how much is IGF-independent. GH treatment also results in increased bone resorption. It is still unknown whether osteoclasts express functional GHRs, but recent *in vitro* studies indicate that GH regulates osteoclast formation in bone marrow cultures. Possible modulations of the GH/IGF axis by glucocorticoids and estrogens are also included in Fig. 9.

GH deficiency results in a decreased bone mass in both man and experimental animals. Long-term treatment (>18 months) of GHD patients with GH results in an increased bone mass. GH treatment also increases bone mass and the total mechanical strength of bones in rats with a normal GH secretion. Recent clinical studies demonstrate that GH treatment of patients with normal GH secretion increases biochemical markers for both bone formation and bone resorption. Because of the short duration of GH treatment in man with normal GH secretion, the effect on bone mass is still inconclusive.

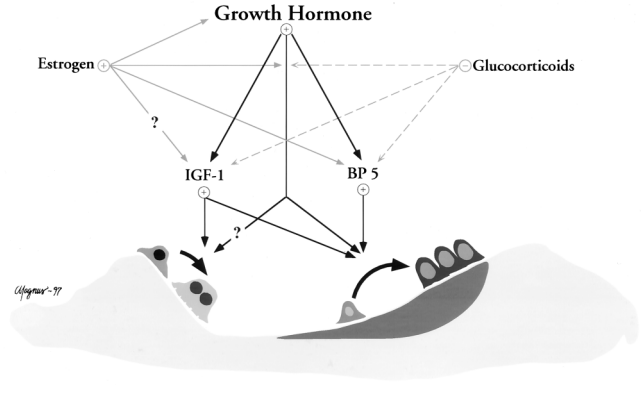

FIG. 9. The authors' proposed mechanism of action at the cellular level for GH in regulation of bone remodeling. The *left part* of the figure represents osteoclast-mediated bone resorption, while the *right part* represents osteoblast-mediated bone formation. ? indicates that both stimulatory and inhibitory effects have been shown.

Interestingly, GH treatment to GHD adults initially results in increased bone resorption with an increased number of bone-remodeling units and more newly produced unmineralized bone, resulting in an apparent low or unchanged bone mass. However, GH treatment for more than 18 months gives increased bone formation and bone mineralization of newly produced bone and a concomitant increase in bone mass as determined with DEXA. Thus, the action of GH on bone metabolism in GHD adults is 2-fold: it stimulates both bone resorption and bone formation. We therefore propose "the biphasic model" of GH action in bone remodeling (Fig. 10). According to this model, GH initially increases bone resorption with a concomitant bone loss that is followed by a phase of increased bone formation. After the moment when bone formation is stimulated more than bone resorption (transition point), bone mass is increased. However, a net gain of bone mass caused by GH may take some time as the initial decrease in bone mass must first be replaced (Fig. 10). When all clinical studies of GH treatment of GHD adults are taken into account, it appears that the "transition point" occurs after approximately 6 months and that a net increase of bone mass will be seen after 12–18 months of GH treatment. It should be emphasized that the biphasic model of GH action in bone remodeling is based on findings in GHD adults. It remains to be clarified whether or not it is valid for subjects with normal GH secretion.

A treatment intended to increase the effects of the GH/IGF-I axis on bone metabolism might include: 1) GH, 2) IGF, 3) other hormones/factors increasing the local IGF-I production in bone, and 4) GH-releasing factors. Other hormones/growth factors increasing local IGF may be important but are not discussed in this article. IGF-I has been shown to increase bone mass in animal models and biochemical bone markers in humans. However, no effect on bone mass has yet been presented in humans. Because the financial costs for GH treatment is high it has been suggested that GH-releasing factors might be used to stimulate the GH/IGF-I axis. The advantage of GH-releasing factors over GH is that some of them can be administered orally and that they may induce a more physiological GH secretion. Clinical

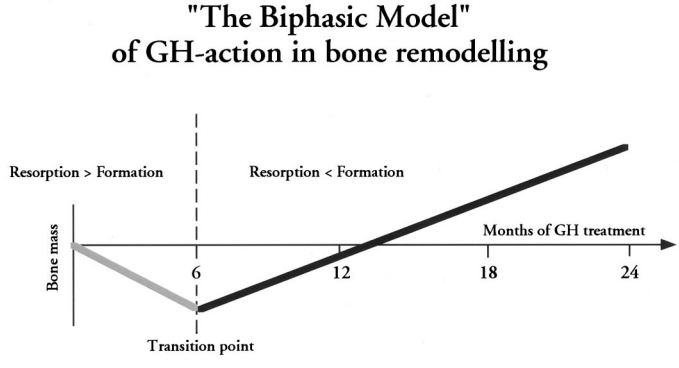

FIG. 10. The biphasic model of GH action in bone remodeling. According to this model, GH results initially in an increased bone resorption with a concomitant bone loss followed by a later increased bone formation. After the moment when bone formation is stimulated more than bone resorption (transition point), bone mass is increased. However, a net gain of bone mass of GH may take some time as the initial decrease in bone mass must first be replaced.

studies and initial experimental studies in dogs have demonstrated that GH-releasing factors increase GH secretion and regulate biochemical bone markers (Ref. 359 and our unpublished results).

We conclude that GH treatment increases bone mass in GHD adults, and future clinical studies will determine whether some patients with decreased bone mass of other origins will benefit from treatment with GH alone or in combination with other treatments.

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