MINIREVIEW

Insulin-like Growth Factors and Bone: The Osteoporosis Connection (43726)

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Abstract. **In the last five years significant progress has been made defining the role of insulin-like growth factors (IGFs) in the process of bone remodeling. In this paper, we present critical evidence that IGF-I and IGF-II are produced by bone cells and regulate specific osteoblastic and osteoclastic functions. In addition, we review work from several laboratories establishing the role of the skeletal IGF binding proteins as an integral component of a unique IGF regulatory system. Data presented suggest that the calciotropic hormones active in the bone remodeling process may exert their effects through the IGF regulatory system. In contrast to the well-defined local action of IGF-I and IGF-II on the skeleton, the relationship between circulating IGF-I and bone remodeling is less certain. Newer data are presented which suggest the potential utility of serum growth factor measurements in certain clinical states. Finally, this paper presents an overview of the most current efforts to stimulate bone formation using recombinant IGFs. However, work on the beneficial aspects of IGFs for the skeleton remains preliminary at best with the eventual therapeutic role of IGF-I in osteoporosis yet to be defined.** [P.S.E.B.M. 1994, Vol 206]

The importance of the insulin-like growth factor (IGF) regulatory system in the acquisition and maintenance of skeletal integrity has recently been recognized. Isolation of a human IGF-I cDNA in 1983 and successful expression of recombinant IGF-I in 1985 have made it possible to consider growth factors as therapeutic agents for metabolic bone diseases (1, 2). Clinical trials with recombinant human insulin-like growth factor-I (rhIGF-I) in patients with osteoporosis have already started, while animal studies employing IGF analog and IGFs coupled to IGF binding proteins (IGFBPs) are now being planned.

Insulin-like growth factors have an important role in maintaining bone mass. The skeleton is a tremendous repository for IGF-I/-II, IGFBPs, and IGFBP-specific proteases. As aging progresses, changes in systemic hormones affect this skeletal IGF network. Growth hormone (GH) and IGF-I concentrations decline, while parathyroid hormone (PTH) levels rise. Each of these hormones uniquely modulates the IGF regulatory system which in turn regulates bone remodeling (3–10). Low levels of circulating IGF-I may impact directly on bone mass. Like serum, cortical bone from elders contains considerably less IGF-I than bone from younger individuals. This relative deficiency is related to a generalized reduction in mean wall thickness (11). The near-absence of this important anabolic growth factor could contribute to lower bone formation rates in senescent bone. Since age is one of

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the strongest predictors of fractures, changes in the IGF regulatory system over time could be an important pathophysiologic component of the osteoporotic syndrome.

The mechanisms which lead to the development of osteoporosis remain to be elucidated. This makes development of newer therapeutic agents more difficult. Currently, therapy for this disabling disorder is aimed at preventing further bone loss, primarily by inhibiting bone resorption. With the advent of recombinant technology, it may be possible to restore depleted growth factors and thereby augment bone mass. hIGF-I may be the first of this new class of site-directed growth factors targeted solely for the purpose of enhancing skeletal mass.

This review will summarize current information about the bone IGF regulatory system, its relationship to physiologic bone remodeling, and the potential usefulness of IGFs in both the diagnosis and the treatment of patients with osteoporosis.

Historical Perspective

The identification of IGF-I and IGF-II resulted from the convergence of research involving investigation of three different types of biological activities in serum. First, in 1957 Salmon and Daughaday (12) attributed sulfation factor activity to a GH-dependent substance present in serum from normal, but not hypophysectomized, rats. In the absence of exogenous GH, this factor was capable of stimulating uptake of sulfate into cartilage (12). Second, in 1963 Froesch *et al.* (13) identified the nonsuppressible insulin-like activity of a serum factor that continued to exert insulin-like action on muscle and adipose tissue in the presence of anti-insulin antibodies. Third, in 1973 Dulak and Temin (14) reported multiplication-stimulating activity in serum-free medium conditioned by BRL-3A rat hepatoma cells. In 1972, it was agreed that all three types of biological activities represented a similar group of factors that could be unified under the term *somatomedin* (15).

The original somatomedin hypothesis maintained that pituitary GH exerted its somatogenic effects in target tissues by stimulating release of a direct acting intermediary growth factor from liver (12). In 1978, Rinderknecht and Humbel (16–18) purified two distinct somatomedins which were designated insulin-like growth factors I and II (IGF-I and IGF-II). Subsequent chemical and molecular studies of somatomedins revealed only peptides identical to IGF-I or IGF-II, and physiological studies determined that only IGF-I was directly regulated by GH. Consequently, the term somatomedin was abandoned and the term IGF was adopted for this family of peptides (19).

In the last decade tremendous strides have been made in defining the missing pieces of the IGF regulatory system. Characterization of IGF receptors, isolation and sequencing of six human IGFBPs, and classification of IGFBP-specific proteases have allowed investigators to more clearly define the precise role of the IGFs in pre- and postnatal development, growth, and maintenance of body tissues.

Bone and the IGFs

The importance of the IGF skeletal regulatory system to bone can best be appreciated by understanding the physiology of bone turnover. The bone remodeling cycle is a complex yet orderly process occurring at all skeletal sites after puberty. Sequential replacement of old bone with new occurs within a defined quantum of bone, the basic multicellular unit (BMU) (20). This well-orchestrated cycle begins with multinucleated osteoclasts which originate from hematopoietic stem cells. These cells cause bone dissolution by release of protons and proteases. Five to seven days of resorption by the osteoclast produce a resorption lacuna. After a short quiescent phase, bone formation in the resorptive area proceeds over a span of approximately 100 days. Mesenchymal-derived osteoblasts synthesize new bone to fill the resorption lacuna with an organic matrix which is eventually mineralized. The complete remodeling cycle requires an intricate balance between the amount of bone resorbed and formed within the BMU.

The frequency of BMU activation is dependent on the nature of the activating signal (e.g., hormones, lack of gonadal steroids, cytokines, and growth factors) and the skeletal architecture (trabecular bone undergoes more frequent remodeling than cortical bone) (20). Although osteoclasts appear early in the remodeling cycle, osteoblasts, by virtue of their calciotropic hormone receptors, prime the remodeling cycle. Osteoblasts also produce IGFs and are the principal target for hormonal and pharmacological regulation of the remodeling unit (21) (Fig. 1).

When bone resorption exceeds formation in the remodeling cycle, bone loss occurs. Over time this leads to reduced bone mass, decreased bone strength, and osteoporotic fractures. Current therapy for osteoporosis centers on manipulating the BMU to restore a new balance between resorption and formation. Often this means pharmacologic intervention to slow bone dissolution and permit *de novo* bone synthesis to catch up. Treatment allows coupling to be reestablished. This results in a lower frequency of remodeling but a new steady state within the BMU where bone mass is preserved (20, 21). Theoretically, future treatments (e.g., growth factors) could be site-directed to enhance formation without affecting resorption, thereby increasing (rather than preserving) bone mass. However, to accomplish that feat would require another type of uncoupling, i.e., blocking local signals that ac-

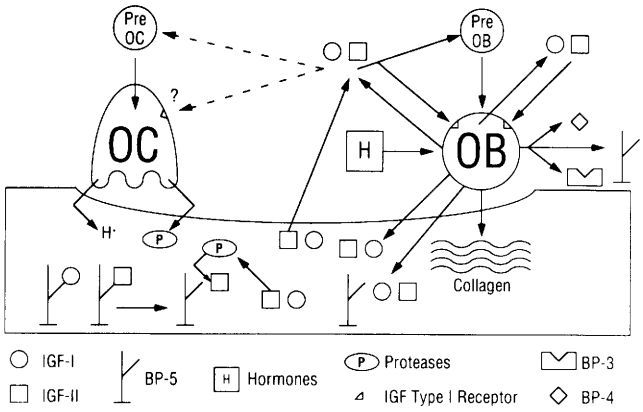

Figure 1. This schematic represents the basic multicellular unit (BMU) and its relationship to the IGF regulatory system. Each component of this system plays an integral role in the process of bone remodeling as illustrated above. Osteoblasts (OB) produce IGF-I, IGF-II, and at least three IGFBPs (-3, -4, -5). The IGFs are liberated from bone matrix (after being bound to IGFBP-5) during the process of bone resorption as osteoclasts (OC) release H^+ and proteolytic enzymes (P). These proteases serve to break down the organic bone matrix but, under the influence of growth factors (including IGF-I/-II), could promote dissociation of the IGFBPs from their IGFs. Both IGFs can act in a paracrine fashion to enhance further production of IGF-I/-II or the IGFBPs from OBs. IGFs can also contribute to the circulating pool of growth factors or become dissociated from their respective IGFBPs and act on bone cells. The effects of IGFs on OCs remain somewhat controversial, although it seems likely that some form of OC recruitment can occur under the influence of IGF-I. Systemic hormones (H) (e.g., PTH, 17β-estradiol, vitamin D, thyroid hormone, etc.) can regulate OB synthesis and release of IGFs and IGFBPs. The IGFBPs serve to promote or antagonize the action of the IGFs. In addition, both the Type 1 and the Type 2 IGF receptor (Type 2 not shown) are present on OBs and possible OCs. The IGFs can promote mitogenesis of OB precursors as well as differentiated functions of mature OBs (collagen synthesis). Therefore, it seems likely that the IGFs are another set of unique polypeptides which may couple the processes of resorption with formation. The reader should be cautioned that the picture is overly simplistic and that many other cytokines and growth factors are involved in the intercellular communication network necessary for the bone remodeling process to occur.

tivate resorption without affecting formation. Therein rests the challenge for scientists and clinicians treating osteoporosis.

Although systemic factors such as estrogens, growth hormone, parathyroid hormone, and vitamin D exert control over remodeling, it is certain that intercellular signals represent the final common pathway necessary for coupling. IGFs are an integral part of a network which includes pluripotent cytokines (IL-1, IL-6, IL-11, TNF, TGF-β, PDGF), a group of bone morphogenetic proteins (BMPs), and several osteoinductive factors (22). In this review, evidence will be presented that IGFs are critical to each aspect of the remodeling cycle. However, the reader should be cautioned that bone remodeling is an extremely complex process with multiple regulatory circuits and IGFs are just one component in this network. Therefore, attempts to link cell coupling to a single molecule are both unrealistic and oversimplified.

Proof that IGFs are critical for the BMU is derived from three lines of evidence: (i) IGFs (and their binding proteins) are found in very high concentration in bone matrix; (ii) osteoblasts synthesize both IGFs and possess Type 1 and Type 2 IGF receptors; and (iii) IGFs act as mitogenic and differentiative stimuli for bone cells.

IGF-I and IGF-II are single chain polypeptide growth factors (7.6 and 7.5 kD, respectively) with three intrachain disulfide bridges (16, 18). Although these proteins share 60% amino acid sequence homology and have similar biological effects on growth and glucose transport, IGF-I and IGF-II are products of different genes. Human IGF-I is encoded by a single gene 90 kb in length located on Chromosome 12, while human IGF-II is the product of one 30-kb gene situated on Chromosome 11 (23).

IGF-I and IGF-II differ in their ability to promote growth (24). Functional variability may relate to the presence of two distinct IGF receptors. The Type 1 dimeric IGF receptor is structurally and functionally homologous to the insulin receptor but binds IGF-I with higher affinity than IGF-II or insulin (25). The monomeric Type 2 IGF receptor bears no resemblance to the insulin or Type 1 receptor, has a higher affinity for IGF-II than IGF-I and cannot bind insulin. Also, in contrast to the insulin or Type 1 receptor, the Type 2 receptor exhibits no intrinsic kinase activity. Rather, it is structurally similar to the mannose 6-phosphate receptor (25). Although data exist that the mannose 6-phosphate receptor is involved in targeting lysosomal enzymes, the precise function of the Type 2 IGF receptor has not been defined.

The original somatomedin hypothesis proposed that insulin-like growth factors were hepatically derived circulating mediators of growth hormone (GH) (15). Indeed, liver is the major source of IGF-I in serum, but skeletal IGFs also enter the total circulating pool. In fact, the proportion of IGF-II:IGF-I in human skeletal tissue and adult rat tissue is nearly identical to the serum ratio (4). Whether serum concentrations of growth factors reflect local tissue levels remains an open question. It seems likely that circulating IGF-I provides an integrated index of IGF-I expression throughout the body but does not necessarily measure the IGF-I that promotes growth in a given tissue (26).

In general, local IGFs are regulated by a myriad of autocrine, endocrine and paracrine factors. In particular, skeletal IGFs are modulated by both systemic calciotropic hormones and local growth factors. Across species from cartilaginous fish to mammals, IGFs are highly conserved. It is the regulation of these growth factors that confers species and tissue specificity (4).

IGFs in skeletal tissue originate from three sites: (i) the circulation, (ii) the osteoblast, and (iii) the bone

marrow stromal cell. As previously mentioned, human bone contains IGFs in relative concentrations that are comparable to adult serum (10^{7}–10^{-9} *M*) (4). Calcified tissue may act as a reservoir to trap IGFs from the circulation or extraskeletal sites. The nature of the hydroxyapatite crystal, the resorptive process which leads to bone matrix exposure, and the recent discovery of IGFBP-5 which anchors IGFs to the crystalline matrix support this contention (5). However, these observations do not preclude significant local synthesis of IGFs by osteoblasts or bone marrow cells.

Osteoblasts synthesize and secrete significant amounts of both IGF-I and IGF-II (27). Rodent calvariae, human tumor cell lines, and long bone explants express IGF transcripts and secrete IGFs into conditioned media (22). Likewise, human bone cells of the osteoblast phenotype (HBCs) produce IGFs, although these cells make greater amounts of IGF-II than IGF-I (27). In contrast to the liver where GH is the principal regulator of IGF-I production, osteoblastic IGF-I gene transcription is influenced by both paracrine and endocrine factors (see Regulation of IGFs in Bone). Similarly, IGF-II production in bone is regulated by systemic factors other than GH. In addition to synthesizing IGFs, osteoblasts also possess Type 1 and Type 2 IGF receptors (28). The presence of growth factor receptors on osteoblasts is strong evidence for autocrine and paracrine regulation of skeletal IGF synthesis.

In contrast to osteoblasts, osteoclasts do not synthesize IGFs or IGFBPs, although IGFs may promote osteoclast differentiation (29) (see IGF Actions on Bone Cells). However, bone marrow stromal cells, which are rich sources of cytokines for hematopoietic progenitors, actively synthesize IGF-I and various IGFBPs (30, 31). These cells mediate the actions of GH on granulocytic precursors through production of IGF-I. It is likely that these stromal cells can contribute IGFs to the overall skeletal pool. Regardless of their origin, the IGFs are bound to bone matrix. During resorption, the IGFs are released and act in concert with other cytokines to stimulate osteoblast proliferation and differentiation (Fig. 1).

IGF Actions on Bone Cells

Since the discovery of IGFs, their GH-independent mitogenic properties have been the subject of numerous studies. In general, the IGFs are relatively weak mitogens that permit competent cells to progress through the S-phase of the cell cycle (32). This is probably true for skeletal IGFs although species differences clearly exist. The proliferative characteristics originally attributed to circulating IGFs rested on observations that serum from GH intact animals promoted cartilage growth *in vivo* by stimulating proteoglycan synthesis and sulfation incorporation (12). The mitogenic properties of skeletal IGFs are ambiguous, in part due to the complex nature of bone formation and its relation to sequential osteoblast proliferation and differentiation. For example, in transgenic mice that overexpress IGF-1, proliferation is principally related to selective organomegaly without apparent increases in skeletal growth (33). Administration of IGF-I to male rats stimulates epiphyseal growth only in the proliferative zone, a limited area in the proximal tibia (34). Green *et al.* (35) have suggested that IGF-I acts as a progression factor for clonal expansion of only a small portion of previously differentiated GH-primed chondrocytes. Their contention is supported by Nilson *et al.* (131), who have shown that GH enhances the number of mitotically active cells and increases chondrocyte sensitivity to IGF-I.

IGF-I is a potent mitogen for bone cells in fetal rat calvaria. After exposure to IGF-I or *des* IGF-I (an amino-truncated derivative which binds very weakly to IGFBPs), calvarial osteoblasts exhibit enhanced [^{3}H]thymidine incorporation and increased mitotic indices (36, 37). In this model, IGF-I is four to seven times more potent in stimulating DNA synthesis than IGF-II. In murine osteoblast-like cells (MC3T3-E1), physiological concentrations of IGF-I and IGF-II rapidly increase mRNA expression of the proto-oncogene, *c-fos,* 20- to 40-fold in 15–30 min (38). This growth-related gene encodes transactivation factors which are expressed primarily during the proliferative phase of the osteoblast development sequence (39). Similarly, in human osteoblast-like cells (HOB), IGF-I, IGF-II, and GH stimulate cell growth in a dose-dependent manner (40). Also, GH-induced HOB cell growth can be blocked by simultaneous addition of a specific monoclonal antibody to IGF-I. The degree of mitogenic activity induced by IGF-I may vary with cell type and environmental conditions.

IGF-II is a potent mitogen for cultured HBCs (41). In chick calvarial osteoblasts, IGF-II stimulates mitogenesis even when administered with maximal doses of IGF-I, suggesting that the Type 2 receptor regulates osteoblastic proliferation (42). However, preliminary studies suggest that certain osteoblasts may be more responsive than others to both IGF-I and IGF-II. Changes in binding affinity of the IGF receptors and the stage of osteoblast differentiation, most likely determine bone cell responsiveness to IGFs (43).

Besides their proliferative actions on bone, IGFs have pre- and post-translational effects on collagen and noncollagenous proteins. In osteoblast-enriched rat parietal bone, [^{3}H]thymidine incorporation is unaffected by either IGF, but the cells respond to IGF-I by increasing [^{3}H]proline incorporation into collagen (44). Physiologic concentrations of IGF-I (10^{-8} *M*) enhance Type 1 collagen production and bone matrix biosyn-

thesis in rat calvariae, independent of cell replication (37, 45). In addition, IGF-II stimulates Type 1 collagen synthesis in HBCs (46).

IGF-I increases collagen mRNA to a greater extent than general protein synthesis (45). This relatively rapid effect can be duplicated with IGF-II although significantly higher amounts are required (37, 46). Since insulin also stimulates collagen synthesis *in vitro,* it is conceivable that IGF-II affects bone collagen synthesis by activating the Type 1 IGF receptor (47). The same scenario holds for cartilage where IGF-I has a greater stimulatory effect on proteoglycan synthesis in bovine chondrocytes than IGF-II, even though both are equipotent in inhibiting proteoglycan catabolism (48).

IGFs have pretranslational effects on expression of the differentiated osteoblast phenotype. Both IGF-I and IGF-II stimulate alkaline phosphatase activity (49). In rat calvarial cultures, IGF-I increases production of osteocalcin, an osteoblast-specific protein (50). The IGFs also act synergistically with 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$), a potent differentiation factor, to up-regulate production of osteocalcin (51). In the human osteosarcoma cell line, OHS-4, neither IGF-I nor IGF-II alone induced osteocalcin synthesis but both IGFs acted synergistically with $1,25(OH)_2D_3$ to increase osteocalcin production (52). Additionally, IGF-I infusions without $1,25(OH)_2D_3$, into spontaneously diabetic BB rats (a model for low bone turnover since baseline osteocalcin concentrations and bone formation rates are low), positively affect the bone mineral apposition rate but do not raise serum osteocalcin (53).

Other differentiated functions in the osteoblast may be affected by IGFs. In one study, IGF-I preferentially maintained rat calvarial cells which expressed not only alkaline phosphatase activity but also PTH responsiveness (54). IGFs can inhibit expression of specific skeletal derived collagenases (19). In addition, IGFs have considerable post-translational effects including nonspecific inhibition of protein degradation, promoting decreased Type 1 collagen breakdown and inhibition of cytokine-mediated proteoglycan catabolism (55, 56). These changes are probably mediated through the IGF Type 2 receptor (57). Kovacina *et al.* (58) studied CHO cells which overexpress the Type 2 IGF receptor and found those cells exquisitely more sensitive to IGF-I inhibition of protein degradation than control cells. In general (although there are species differences), the Type 1 receptor appears essential for phenotypic expression of the differentiated osteoblast, the Type 1 and 2 receptors are important in cell proliferation, and the Type 2 receptor has an integral role in preventing collagen degradation.

Most growth factors (e.g., TGF-β) and cytokines (e.g., IL-1) in the remodeling unit stimulate both formation and resorption. However, surprisingly little work has focused on the putative role of IGFs in regulating osteoclast recruitment and activity. Scheven *et al.* (59) reported that addition of rhIGF-I to rat bone marrow cells stimulated macrophage growth in a dose-dependent manner. IGF-I also induced formation of multinucleated cells some of which were tartrate-resistant acid phosphatase (TRAP) positive, an indicator of differentiated osteoclast function. Slootweg *et al.* (60) were unable to demonstrate any effect of IGF-I on fetal mouse radii/ulnae which possessed a large population of mature osteoclasts. However, when IGF-I was added to fetal mouse metacarpals/metatarsals which contained significantly greater numbers of osteoclast precursors and progenitors, bone resorption was substantially increased. This response could have been mediated through IL-6, since addition of IGF-I to UMR-106 osteogenic cells stimulates release of IL-6, a pluripotent osteoclast-recruiting cytokine (60). More recently, Mochizuki *et al.* (29) reported that IGF-I (but not IGF-II) increased both the number and the activity of murine osteoclasts. IGF-I, in the presence of $1,25(OH)_2D_3$, also increased the number of TRAP-positive multinucleated cells and promoted survival of blast cells in a manner similar to GM-CSF. High-affinity binding sites for IGF-I on osteoclasts were also demonstrated.

Although only three studies support a role for IGF-I osteoclast activation, the IGFs may still be important in the bone resorption process. During the resorptive phase of remodeling, IGFs bound to IGFBPs in the skeletal matrix are liberated (46). Theoretically these IGFs are destined for osteoblasts (and osteoblast precursors). However, the presence of an IGF receptor on osteoclasts suggests that "free" IGF-I or IGF-II could act at some point in the differentiation process (Fig. 1). Though there is minimal support for this hypothesis currently, there is no reason to doubt that IGF-I could act in a manner similar to other bone-active cytokines and growth factors (i.e., activating both osteoblasts and osteoclasts). In fact, *in vivo* studies using GH and IGF-I suggest that both these growth factors simultaneously activate resorption and formation, a critical point which could limit the clinical application of growth factors to enhance bone mass (159, 162, 163, 183).

Regulation of IGFs in Bone

The frequency and activation of BMU activation determines bone turnover and subsequent bone mass. Although local factors couple resorption to formation, various hormonal signals are responsible for initiating the remodeling sequence. Since IGFs are essential components to the BMU, it is logical to conclude that

activation signals trigger growth factor synthesis in osteoblasts. Skeletal IGFs are regulated by systemic hormones, by paracrine growth factors (bone-related cytokines), and by autocrine effectors (IGF-I, IGF-II). Each class of compounds acts genomically to regulate IGF synthesis. But, in all likelihood, it is the combination of hormones and local growth factors that works in synergy to stimulate or suppress production of skeletal IGFs.

Growth hormone has been considered the prototypic hormonal regulator of IGF-I synthesis. In humans, GH excess is associated with increased serum concentrations of IGF-I and enhanced skeletal formation (61). Similarly, GH deficient adults with panhypopituitarism have subnormal bone mineral densities which correlate with serum IGF-I (62). In hypophysectomized rats, GH infusions into the growth plate increase expression of IGF-I mRNA and promote longitudinal growth (63). GH also increases IGF-I production in cultured osteoblasts and calvarial explants although not nearly to the extent observed with physiologic concentrations of PTH or triiodothyronine (T^3) (32, 64).

In vivo studies with GH have yielded conflicting results. GH administration to *lit/lit* (GH deficient) mice does not increase bone ash weight (Donahue LR, personal communication). When elders are administered GH, bone mineral density changes are minimal, possibly because of sequential increases in bone resorption (see Diagnostic and Therapeutic Aspects of Skeletal IGFs) (65). In addition to activating the entire remodeling sequence, GH also enhances IGFBP-3 production in liver and bone (66). IGFBP-3 can alter IGF bioavailability and depending on local circumstances may affect IGF action. Furthermore, osteoblast responsiveness to GH depends on the current GH status of the organism and the stage of osteoblast differentiation. Hence, anabolic skeletal responses to GH are a function of whether treatment is aimed at physiologic replacement (GH deficient patients, some elders) or pharmacologic augmentation (some elders and most osteoporotic subjects).

Estrogens induce IGF-I mRNA expression in reproductive tissues (67). In several cell lines of osteoblasts, low concentrations of estrogen receptors have been reported (68). In immortalized rat calvarial osteoblasts (RCT-1) and UMR-106 cells, 17β-estradiol stimulates steady state expression of IGF-I mRNA (69). The bone-forming effect of 17β-estradiol is blocked by anti-IGF-I antibodies and is dependent on the number of estrogen receptors per osteoblast (70, 71). However, administration of estrogens to humans *in vivo* leads to inhibition of bone resorption with no apparent increase in bone formation (72). This antiresorptive effect may be mediated through cytokines produced by osteoblasts and bone marrow stromal cells (73). Despite estradiol-induced changes in skeletal IGFs in some species, the principal skeletal response to estrogen treatment in humans is inhibition of bone resorption, not stimulation of osteoblastic IGFs.

PTH has a pronounced effect on the skeletal IGF system. Increased bone resorption and reduced cortical bone mass were described more than thirty years ago in patients with hyperparathyroidism (74). However, clear and consistent anabolic skeletal effects from PTH administration have recently been reported (75–77). For example, in patients with primary hyperparathyroidism, trabecular bone mass may be increased (78). Also, intermittent subcutaneous administration of human PTH to osteoporotic men and women produces a significant increase in trabecular bone density with little change in cortical bone mass (75, 76). Newer studies have started to decipher the mechanisms responsible for the skeletal response to PTH.

The seminal observation that PTH stimulated skeletal IGF-I was first reported in rats by McCarthy *et al.* (79) and in mice by Linkhart and Mohan (80). In osteoblast-enriched cultures from fetal rat parietal bones, IGF-I mRNA transcripts and immunoreactive IGF-I increase 100%–200% after 1–10 n*M* PTH (79). These changes are dose- and time-dependent, are associated with enhanced Type 1 collagen biosynthesis, and are independent of cell replication (37). In addition, the PTH effects on skeletal IGFs can be duplicated using cyclic AMP analogues (81). In rat calvaria, intermittent low-dose PTH stimulates *de novo* bone synthesis while continuous PTH administration prevents Type 1 collagen synthesis (21). The anabolic effects of PTH in this system can be blocked by anti-IGF-I antibodies (81).

PTH-induced changes in bone IGFs are detectable in other model systems. In chick osteoblasts, IGF-I increases [^{3}H]thymidine incorporation, while PTH has virtually no effect (82). However, the combination of PTH and IGF-I potentiates the mitogenic actions of IGF-I. In neonatal mouse calvaria, PTH in a dose-dependent manner significantly increases both IGF-I and IGF-II, irrespective of bone resorption (80). Steer *et al.* (83) analyzed tibial IGF-I by immunocytochemistry and IGF-I mRNA by *in situ* hybridization following 4 weeks of human PTH(1–34) treatment of 12-week-old oophorectomized rats. Cancellous bone volume and the IGF-I mRNA hybridization signal in tibia were significantly enhanced in oophorectomized rats treated with PTH. Recently, Goad and Tashjian (84) postulated that a feedback modulation of agonist-stimulated cAMP production can be produced by IGF-I either locally generated or released from resorbed bone matrix. They showed that IGF-I inhibited

PTH-stimulated cAMP production and enhanced PGE_2-stimulated cAMP production in human osteoblast-like SaOS-2 cells.

The anabolic action of PTH on bone may be mediated entirely through growth factor synthesis in the osteoblast. However, skeletal IGFs also originate from the circulation. The effects of PTH on serum IGF-I were examined in two studies. Coxam *et al.* (85) measured hepatic blood flow in calves after bovine PTH administration and found a significant increase in hepatic vein IGF-I without changes in serum GH. Rosen *et al.* noted that 24 hr after intravenous or subcutaneous human PTH(1–34), serum IGF-I (and GH-dependent IGFBP-3) fell significantly, as did GH (86). This effect persisted during 30 days of intermittent PTH administration and the drop in GH was inversely related to the increase in ionized serum calcium. However, these changes were barely significant and the number of subjects was small. Thus, it still remains problematic whether PTH has a significant indirect effect on bone IGFs through modulation of circulating growth hormone.

Other calciotropic hormones affect skeletal IGF production. Glucocorticoids alter the bone IGF regulatory system and adversely affect mineral density. In fetal rat calvariae, cortisol up-regulates the number of Type 1 IGF receptors in osteoblasts and briefly stimulates collagen synthesis (87). Physiologic concentrations of cortisol can potentiate IGF-I enhancement of collagen biosynthesis and procollagen mRNA levels although pharmacologic doses of cortisol dramatically suppress production of IGF-II (88). In primary cultures of osteoblast-enriched rat cells, cortisol reduces IGF-I transcript and immunoreactive levels by 50%–70%, while dexamethasone inhibits IGF-I expression in rat tibia treated with GH (88). Glucocorticoids can also have significant transcriptional effects on bone-specific IGFBPs produced by osteoblasts (see IGFBPs and Bone). For example, in human bone cells, dexamethasone suppresses IGFBP-5 expression while it stimulates IGFBP-4 production (89). Unfortunately, there are no studies to date which have prospectively examined the relationship between circulating IGF-I (or IGFBPs) and bone mass in steroid-treated individuals.

The other major calciotropic hormone which regulates skeletal IGFs is $1,25(OH)_2D_3$. Linkhart and Keffer (90) noted in mouse calvaria that physiologic concentrations of $1,25(OH)_2D_3$ (10^{-10}–10^{-7} *M*) inhibited both basal and PTH-stimulated IGF-I (but not IGF-II) production. In humans administered large doses of $1,25(OH)_2D_3$ (2–3 μg/day), serum IGF-I concentrations fell by 50% while IGFBP-4 increased by 70% (91). In contrast, $1,25(OH)_2D_3$ stimulates IGF-I production and secretion in UMR-106 cells and in short-term cultures of HBCs (92, 93). Again, no prospective studies have studied the relationship between the positive effects of $1,25(OH)_2D_3$ on bone mass and systemic or skeletal IGFs.

Numerous local factors also regulate skeletal IGF synthesis and action. TGF-β stimulates IGF-I release from rabbit osteoblasts (94). This effect is magnified when PTH and TGF-β are added simultaneously. Likewise, in neonatal mouse calvaria, TGF-β stimulates release of both IGF-I and IGF-II (90). In UMR-106 cells, TGF-β increases the accumulation of radiolabeled IGF-I (95). In chondrocytes however, TGF-β suppresses basal and stimulated IGF-I production (94). Similarly, basic fibroblast growth factor (bFGF) suppresses IGF-I and IGF-II synthesis in 22-day-old osteoblast-enriched cells from fetal rat calvaria (96).

PGE_2 an important intermediary in the BMU, may also be a skeletal regulator of IGF production. In rat osteoblasts, PGE_2 stimulates IGF-I production while IL-1 has a similar effect in neonatal mouse calvaria (97). The osteoblastic response to IL-1 may be mediated through PGE_2, since indomethacin blocks the rise in mRNA transcripts for IGF-I (98). Besides prostaglandins, platelet derived growth factor (PDGF) suppresses IGF-I production in osteoblasts (96). Finally, it should be remembered that the IGFs are potent autocrine factors which control osteoblastic proliferation, differentiation, and IGF production. This autoregulation is mediated partly through specific changes in other components of the IGF regulatory system (i.e., IGFBPs and IGFBP-specific proteases).

IGFBPs and Bone

Both systemic and local effects of IGF-I are modulated by a group of proteins, the insulin-like growth factor binding proteins (IGFBPs), which have high affinity and specificity for the IGFs. To date, six IGFBPs of different molecular weights—IGFBP-1, -2, -3, -4, -5, and -6—have been identified in various tissues, including bone (5, 99–102). These IGFBPs act to enhance or to inhibit the proliferative and mitogenic effects of IGF-I and IGF-II, depending on the particular IGFBP, the cell type, and the physiological milieu (103, 104). The action of the IGFBPs is partially dependent on protease activity which is specific for individual IGFBPs and active in defined physiological states. Theoretically, a fall in physiological pH would initiate the release of a protease, causing the disassociation of IGFs from the binding complex, allowing free IGFs to assume biological activity (105).

The IGFBPs modulate the proliferative and mitogenic effects of IGF-I and IGF-II in response to the physiological environment and cell type. Most cells produce not only IGFs, but also IGFBPs that are, in turn, capable of having endocrine, paracrine, and au-

tocrine roles in regulating the effects of IGFs. Studies utilizing numerous osteoblast-like cell types have shown both stimulatory and inhibitory IGF effects when exogenous IGFBPs were supplied (100, 106, 107). The nature of the cellular response depends on the IGFBP preparation and the test conditions, particularly the concentration of individual IGFBPs and the time of exposure. Although several different osteoblast-derived cell lines are used interchangeably to study IGFBP production and effect, Hassager *et al.* (108) have shown that each cell line has not only a unique pattern of basal IGFBP secretion, but a specific response to hormonal stimuli. Normal HOB cells cultured from explants of human trabecular bone and SV40-transformed HOB (HOBIT) cells produced similar patterns of IGFBP secretion, but responded differently to hormonal stimulation (108). Rat osteosarcoma cell lines (ROS 17/2.8, UMR-106-01) and human osteosarcoma cell lines (U-2, MG-63, TE-85) produced different IGFBPs and responded differently to various stimuli (108). In addition, rat osteosarcoma lines differed from each other both in the IGFBPs produced and in response to hormonal stimuli (108). Since basal and stimulated secretion of IGFBPs is cell-line specific, it is important to identify the experimental model and to resist extrapolation of results directly to other cell lines or to human bone. Table I illustrates the myriad of calciotropic factors that modulate the skeletal IGFBPs.

The defined roles for the IGFBPs include sequestering IGFs to inhibit insulin-like activity, prolonging the half-life of IGFs and enhancing the biological response of target tissues to IGFs by directing the peptides to particular cell types (105, 109–111). The mechanism by which the IGFBPs present IGFs remains unclear, since it is not known if all targeted cells have receptors for the IGFBPs. To date, all six identified IGFBPs have been shown to be produced by osteoblast-like cells, depending on the particular cell type and the experimental conditions.

Table I. The Bone IGF System: Regulation by Local and Systemic Factors[a]

IGF component	Regulation
IGFs	
IGF-I	PTH, PGE_2, GH, $1,25(OH)_2D$, E_2, DEX, IGF, TGF-β
IGF-II	PGE_2, IGFs, IL-I, TGF-β, bFGF
IGFBPs	
IGFBP-2	1,25(OH)D, T_3, PTH, insulin, progest
IGFBP-3	DEX, GH, E_2, IGFs
IGFBP-4	DEX, PTH, 1,25(OH)D, GH, IGFs
IGFBP-5	DEX, PTH, IGFs, PGE_2
IGF receptors	
Type 1	DEX, IGFs
Type 2	IGFs
IGFBP-specific proteases	IGFs, androgens, ? estrogens

[a] DEX = dexamethasone; $1,25(OH)_2D$ = dihydroxyvitamin D; E_2 = 17β-estradiol; progest = progesterone; GH = growth hormone. See text for references and Table II.

Human IGFBP-1 is a 25-kD protein which is produced by UMR-106 osteoblast-derived rat osteosarcoma cells (112). Although IGFBP-1 is primarily an acute metabolic modulator (i.e., IGFBP-1 is regulated principally by insulin and glucagon), it has been shown to inhibit both the basal and IGF-I stimulated growth of chick cartilage *in vitro* (107). On the other hand, IGFBP-1 had no effect on IGF-I-induced mitogenesis in chick osteoblasts (100). It is thought that of the six IGFBPs, IGFBP-1 is least likely to play a significant role in regulation of the bone remodeling process in humans.

Human IGFBP-2 is a 31-kD protein (113) which preferentially binds IGF-II (114). This IGFBP is produced in calvarial cells from newborn rats and in rat osteosarcoma cells (5). IGFBP-2 levels increase in rat osteoblasts in response to $1,25(OH)_2D_3$, triiodothyronine (T_3), PTH, and progesterone, but do not respond to GH or retinoic acid (8, 115, 116). Insulin and dexamethasone have been shown to decrease production of IGFBP-2 in rat osteoblasts (8, 115). One report regarding the biological effect of IGFBP-2 has shown that recombinant human (Cys^{281})IGFBP-2 inhibits IGF-I stimulated bone cell proliferation and bone collagen synthesis in 21-day-old fetal rat calvariae and could therefore interfere with bone remodeling (116).

The GH dependent human IGFBP-2 is a 53-kDa acid-stable binding subunit that complexes with an acid-labile 85-kD subunit plus IGF-I or IGF-II; the three components form the 150-kDa IGF binding protein, the transport molecule for the majority of IGFs in circulation (117). This N-glycosylated IGFBP is produced by various cell types including rat calvarial osteoblasts, HBCs cultured from rib and PyMS, an osteoblast-like cell line (7, 118). IGFBP-3 secretion is stimulated in these cells by GH and by IGF-I (118, 119). Other hormones that increase IGFBP-3 production in rat osteoblasts include estradiol, T_3, and PTH (116, 118). On the other hand, cortisol inhibits IGFBP-3 release (118). In addition, prostaglandin E_2 stimulates the production of cAMP, IGF-I, and IGFBP-3 in rat calvarial osteoblasts (120).

Locally produced IGFBP-3 may enhance the anabolic effects of IGF-I on bone. Comparison of the effect of native IGF-I and (QAYL)-IGF-I, an IGF-I mutant with reduced affinity to IGFBPs, has shown that the effects of native IGF-I were enhanced in the presence of IGFBP-3 (106, 121). Similarly, in PyMS cells that constitutively express IGFBP-3, IGF-I had a greater growth stimulatory effect than RCT-3 cells

which do not produce IGFBP-3 (106). Schmid and Ernst (32) have suggested that IGFBP-3 provides an enhancer loop whereby systemic hormones regulate the availability of IGFs to bone cells. In addition, IGFBP-3 may increase the half-life of IGFs by serving as a local reservoir from which IGFs can be continuously released into the local environment for autocrine or paracrine action (4, 118).

IGFBP-4 is a 24-kD binding protein purified from conditioned medium of HBCs that inhibits IGF bioactivity (100). Addition of IGF-I or IGF-II to TE-89 human osteosarcoma cells or to rib HBCs, decreases IGFBP-4 and increases bone cell proliferation (7). Conversely, addition of exogenous IGFBP-4 to MC3T3-E1 cells, a clonal osteoblast-like mouse calvarial cell line, inhibits cell proliferation (101). IGFBP-4 represents more than 90% of all IGFBPs secreted from clonal mouse osteoblasts and exhibits inhibition of IGF-induced mitogenesis (122). In clonal mouse osteoblasts and HBCs, the addition of $1,25(OH)_2D_3$ increases IGFBP-4 and decreases [^{3}H]thymidine incorporation (91, 122). These data suggest that increased IGFBP-4 inhibits the proliferative effect of IGF-I and could initiate a switch in osteoblast behavior from proliferation to differentiation (122).

Regulation of IGFBP-4 in HBCs is multifaceted. PTH and cyclic AMP analogues increase synthesis and secretion of IGFBP-4 (123). Steroid hormones also modulate IGFBP-4 production. Dexamethasone stimulates and progesterone inhibits IGFBP-4 production (124). In addition, both IGFs suppress IGFBP-4 mRNA transcripts (124). Preliminary evidence suggests that circulating levels of IGFBP-4 may reflect local bone cell regulation. In one report, elderly women with hip fractures had high levels of a 24-kD IGFBP (likely to be IGFBP-4), which was closely correlated with circulating levels of PTH (125). In another small study, serum IGFBP-4 nearly doubled after chronic exposure to high-dose oral $1,25(OH)_2D_3$ (91). This increase was very similar to the $1,25(OH)_2D_3$-induced increases in steady state mRNA transcripts for IGFBP-4 in HBCs (91).

IGFBP-5 has a molecular weight of 29 kD and is the most abundant binding protein in cerebrospinal fluid with preferential binding for IGF-II (126). IGFBP-5 is produced in cultures of normal mouse osteoblasts and is able to associate directly with the osteoblast surface and to stimulate mitogenesis in the absence of IGF-II (99, 124). In UMR-106-01 rat osteosarcoma cells, IGFBP-5 mRNA expression is increased by PTH and other cyclic AMP analogs (10). In contrast, IGF-I increases accumulation of IGFBP-5 in conditioned medium from UMR-106-01 cells without changing IGFBP-5 mRNA expression (10). Similarly, in neonatal mouse calvaria, PTH and PGE_2 both stimulate release of IGFBP-5 from bone matrix but do not alter mRNA levels (127). In HOB cells treated with dexamethasone (10^{-8} *M*) IGFBP-5 mRNA expression is reduced 70% (128).

In skeletal models, IGFBP-5 promotes the biological action of IGF-I and IGF-II. This is in sharp contrast to IGFBP-4 which inhibits IGF-induced mitogenesis. This functional difference may be related to the mechanistic aspects of binding protein action on bone cells. For example, IGFBP-5 binds to HBCs and produces a dose-dependent enhancement of IGF binding to the Type 1 or 2 receptor (129). IGFBP-4 does not bind to HBCs but rather prevents direct growth factor binding to the IGF receptor (129). It appears from preliminary data that IGFBP-5 can capture the IGFs (due to IGFBP-5 binding sites) and shuttle growth factors to their respective receptors (124, 129).

In addition, IGFBP-5 may be critical in bone remodeling for two other reasons. First, IGFBP-5 has a very strong affinity for hydroxyapatite, while IGF-II does not (5). IGF-II exists in bone as a large molecular weight complex, rather than in its native form. This would suggest that IGFBP-5 fixes IGFs to the skeletal matrix. During remodeling, as mineralization proceeds, osteoblasts synthesize growth factors and binding proteins. At a defined time, synthesis and release of IGFBP-5 by bone cells permits attachment of these recently synthesized growth factors to the newly mineralized matrix (124). Second, although IGFBP-5 specific proteases are present in bone (IGFBP-5 fragments can be measured in conditioned medium by immunoblot analysis), IGFBP-5 is relatively resistant to IGF-II-induced proteolysis (130). In contrast, IGFBP-4 proteolysis is stimulated by IGF-II. From these data it appears that skeletal IGFs are capable of autoregulating their own biologic activity by modulating IGFBP specific proteases (see IGFBP proteases).

IGFBP-6, a 32-kD IGFBP has been detected in MC3T3-E1 cells and in osteosarcoma cells (101, 126). However, the regulation and action of IGFBP-6 in skeletal tissue remains to be defined.

IGFBP-Specific Proteases

One important function of the IGFBPs is to maintain pericellular reservoirs of IGFs by combining to form IGF/IGFBP complexes that are incapable of binding to IGF receptors, In turn, the high-affinity binding between IGFs and IGFBPs can be significantly reduced or totally negated by proteases specific for individual IGFBPs and activated by particular physiological states. The biological significance of these enzymatic molecules is an area of intense investigation, but the consensus hypothesis is that by altering the binding capacity of the IGFBPs for their ligands at the appropriate time, the IGFBP proteases

free IGFs to stimulate anabolic activity in target cells (10, 131–133). The first IGFBP-specific protease to be reported was a pregnancy associated IGFBP-3 protease that was active from Day 12 onward in rat and mouse, and from the second trimester in human pregnancy sera (123, 134, 135). Further investigation of this IGFBP-3 protease revealed tissue specificity whereby proteolytic activity was prominent in reproductive tissues, but did not affect IGFBP levels in nonreproductive tissues (136). Following these original observations, IGFBP protease activity has been identified in a number of cell types including dermal fibroblasts that secrete an IGFBP-4 specific protease that is preferentially dependent on IGF-II activation (10, 133). Serum IGFBP-3 specific protease activity has been noted in severely ill patients and has been shown to respond to parenteral nutrition, suggesting that protease activity was a mechanism to increase the availability of IGFs in an attempt to improve the nitrogen balance and to counter the catabolic state induced by severe illness (137). The induction of specific IGFBP-3 protease has also been reported in patients following major heart surgery and after cholecystectomy (136, 138).

Numerous proteases have been identified in bone, and recently an IGFBP-3 specific protease was described in PyMS cells and in human osteoblastic osteosarcoma (Saos-2/B-10) cells, both of which constitutively produce IGFBP-3 (131). In these two models, intact IGFBP-3 blocked IGF-I-induced DNA and glycogen synthesis, but truncated IGFBP-3 resulting from proteolytic cleavage did not. Local regulation of IGF-I activity by IGFBP-3 appears to be dependent upon the proteolysis of IGFBP-3, suggesting that this IGFBP-3-specific protease is essential for IGF-I-induced growth of osteoblasts. The plasmin system is also involved in the release of IGFs from IGFBPs in bone (139). In MG-63 human osteosarcoma cells, activation of plasminogen to plasmin by either urokinase plasminogen activator or tissue-type plasminogen activator secreted by these osteosarcoma cells resulted in dissociation of the IGF/IGFBP complex and potentiated IGF biological activity (139).

Clinical interest has been piqued by the possibility that IGFBP-specific proteolysis modulates the paracrine interactions between prostate adenocarcinoma cells and osteoblastic cells (112). A 35-kD IGFBP protease that has activity for IGFBP-1 and IGFBP-2 has been partially purified from PA-III rat prostate adenocarcinoma cells (112). The activity of this protease increases the bioavailability of osteoblast-derived IGFs and thereby could support the growth of PA-III cells (112). Even more intriguing is the finding that prostate-specific antigen (PSA), a 34-kD androgen-dependent glycoprotein produced exclusively by prostate epithelium, is a serine protease that can dissociate IGFBP-3 *in vitro* (140).

Disseminated prostate adenocarcinoma is associated with marked osteoblastic metastases. Although normal prostatic epithelial cells do not produce IGFs (prostatic stromal cells are the source of local IGFs), these cells express high concentrations of the Type 1 IGF receptor. PSA promotes IGFBP-3 proteolysis and IGFBP-3 fragments are detected by ligand blot analysis following neoplastic transformation of prostatic epithelial cells. These transformed cells also show a significant proliferative response to IGF-I (Plymate, Ware, and Birnbaum, personal communication). Enhancement of PSA activity, which is observed in widespread metastatic prostate cancer, may indirectly provide a ready source of growth factors necessary for autocrine and paracrine proliferation. At the same time, increased IGF bioavailability could cause marked enhancement of osteoblastic proliferation, a characteristic of this malignancy. The bone-forming properties of prostatic tumor cells could have considerable relevance for the development of pharmacological agents which promote bone formation without directly stimulating resorption. In conclusion, it seems reasonable to predict that more IGFBP-specific proteases will be identified and these enzymes will have a major regulatory role in determining IGF bioactivity.

Diagnostic and Therapeutic Aspects of the Skeletal IGFs

Diagnostic Utility. In this review, evidence has been presented that the skeletal IGF regulatory system is critical for bone remodeling. It has been well established that bone mineral loss is a predictable accompaniment of aging. Recently, it has been reported that serum IGF-I and IGFBP-3 decline with age (65, 141, 142). If changes in skeletal IGFs are reflected in circulating concentrations of growth factors, then serum measurements of IGF-I or the IGFBPs could be used to assess patients for their potential risk of osteoporosis.

Bennett *et al.* (141) reported age-associated changes in serum IGF-I and IGF-II. However, these investigators did not find a statistically significant correlation between bone density and serum IGF-I or IGF-II when age was held constant. More recent cross-sectional studies in elderly men and women have also failed to demonstrate a relationship between bone density (or osteoporotic fracture) and IGF-I or IGF-II (124, 142). In a recently completed longitudinal study of 18 elderly women (mean age—77 years) followed for two years, neither IGF-I nor IGFBP-3 correlated with femoral or lumbar bone mass (143).

However, cross-sectional studies in slightly

younger men and women suggest a possible relationship between serum growth factors and bone mass. In a preliminary communication, Wuster *et al.* (144) noted that serum IGFBP-3 and IGF-I were consistently lower in osteoporotic patients than controls or subjects with degenerative arthritis. Johansson *et al.* (145) and Ljunghall *et al.* (146) reported low levels of IGF-I and IGFBP-3 in young males (30–57 years) with idiopathic osteoporosis. These investigators also noted a consistently strong correlation between IGF-I and bone mineral densities of the spine and forearm. Similarly, two independent cross-sectional studies have recently confirmed reduced bone density (compared with age-matched controls) in adult patients with acquired GH deficiency, independent of gonadal steroid replacement (29, 147). Furthermore, there were very strong correlations between biochemical determinants of growth hormone (IGF-I, GH response to GHRH, IGFBP-3) and femoral bone mineral density although a significant relationship between IGF-I and spinal bone mass did not exist. These findings suggest that the long-term presence of GH (and probably IGF-I) is important for skeletal integrity.

Systemic factors other than GH also regulate skeletal IGFs and IGFBPs. Sustained perturbations in PTH affect bone mineral density. However, as alluded to previously, there are few longitudinal studies which have examined the relationship between IGFBPs and bone turnover. In a cross-sectional study, IGFBP-4 levels (by ligand blot analysis) were considerably higher in elderly women with hip and spine fractures than in age-matched controls (125). Additionally, a very strong correlation existed between serum IGFBP-4 binding and immunoreactive PTH. In a related pilot study, changes in serum IGFBP-4 from summer to winter, predicted seasonal bone loss in elders. The IGFBP-4 increase during winter was parallel to a significant seasonal rise in PTH (148). However, these studies are only preliminary, since data were derived from ligand blot analysis, a semiquantitative method for measuring IGFBPs. Also, it is uncertain if changes in serum IGFBP-4 originate from skeletal tissue or are produced by endothelial cells, fibroblasts, or other cell types which contribute to the circulating pool. Confirmation of these data await a specific radioimmunoassay for IGFBP-4 currently in development.

In sum, there are no convincing data that serum IGFs (or IGFBPs) can predict bone mass or bone loss in GH sufficient subjects. In acquired GH deficiency, serum IGF-I or IGFBP-3 predict femoral bone density, regardless of gonadal steroid status. However, serum IGF-I is not a useful clinical marker of bone formation. As our understanding of the IGFBPs increases, and radioimmunoassays are developed for IGFBP-4, -5, and -6, it is possible that the clinical usefulness of serum IGF measurements will expand considerably.

Potential Utility of IGFs in the Management of Osteoporosis. Therapeutic goals in osteoporosis include restoration of bone mass and prevention of bone loss. A third aim, which cannot be attained with current therapies, is augmentation of bone mineral density. Almost all drugs in the present therapeutic armamentarium for osteoporosis affect the skeletal IGF system either indirectly or directly (see Table II).

Gonadal steroids slow activation of the remodeling unit by blocking bone resorption. It is beyond the scope of this review to examine in depth the mechanisms responsible for this effect. However, oral estrogens and tamoxifen lower serum IGF-I and IGFBP-3 probably via direct hepatic suppression (65, 149, 150). Interestingly, transdermal estradiol has no effect on circulating IGF-I, yet this mode of estrogen delivery preserves bone mass (151). To date, it is uncertain how circulating IGF-I affects bone remodeling in estrogen-treated women, because the primary effect of estrogen on bone remodeling is to slow bone resorption. However, it is conceivable that gonadal steroids may regulate skeletal IGF and IGFBP synthesis. As noted previously, 17-β estradiol increases IGF-I and IGFBP-3 mRNA expression in rat osteoblasts (118). In addition, androgens enhance IGF-II binding to mouse calvarial cells and progesterone inhibits IGFBP-4 production in HBCs (152, 153). Despite the fact that human osteoblasts have estrogen and androgen receptors, gonadal steroid effects on bone formation have been difficult to demonstrate *in vivo*. More studies are required to establish if the skeletal IGF system is directly responsive to hormonal replacement therapy.

Calcitonin (CT), a peptide hormone produced by parafollicular cells in the thyroid, directly inhibits bone resorption by acting on its osteoclast receptor. CT is used for the treatment of osteoporosis primarily because it blocks bone resorption (not unlike gonadal steroids). In humans, CT does not appear to affect osteoblasts or bone formation directly. However, CT stimulates in a time- and dose-dependent manner the release of ACTH and β endorphins from the pituitary (154). These stimulatory properties may explain the analgesic effects of CT. Although there is no evidence that CT directly stimulates GH release, in one pilot study, nasal CT increased β-endorphin concentrations and this increase correlated closely with a modest rise in serum IGF-I (155). More detailed studies will be needed to define whether there are indirect changes in skeletal IGFs as a result of CT.

Two hormones, GH and PTH, are currently under intense investigation in multicentered clinical trials. Each has a major impact on the IGF regulatory system. More than 35 years ago, Raben treated a pituitary

Table II. Therapeutic Options in Osteoporosis: Relationship of Current and Future Treatment Regimens to the IGF System

Drug[a]	Bone IGF	Serum IGF	BP protease	Res/form	FDA phase (Ref.)
		Indirect effects			
Est	± ↑ IGF-I	↓ IGF-I	?	−/±	approved[b] (63, 146)
Tamox	?	↓ IGF-I	−/±	−/±	III[c] (146)
Test	? ↑ IGF-I	↑ ↑ IGF-I	? ↑	−/±	±use[b] (128)
CT	—	? ↑ IGF-I	?	−/0	approved (150)
PTH	↑ ↑ IGF-I	? ↓ IGF-I	?	+ +/+ + +	II, animal (19)
GH	↑ IGF-I	↑ ↑ IGF-I	?	+ +/+ +	animal[b] (123)
1,25D	↕ IGF-I	↓ IGF-I	?	− ?/+	±use (91)
		Direct effects			
IGF-I	↑ ↑ IGF-I	↑ ↑ IGF-I	↑	+/+ +	animals[b] (177, 178, 183)
IGF-II	↑ ↑ IGF-II	↑ ↑ IGF-II	↑	+/+ +	animals (127)
BP-3	±IGF-I	↑ ↓ IGF-I	−	0/+ ?	animals (179)
BP-3 + IGF-I	↑ ↑ IGF-I	↑ ↑ IGF-I	−	+/+ +	animals (180, 181)

[a] Drug: a list of curretly used agents for treatment of osteoporosis, either approved by the FDA or used clinically (±use) without defined approval for osteoporosis indication. Tamox = tamoxifen; Est = estrogen; Test = testosterone; CT = calcitonin; 1,25D = 1,25-Dihydroxyvitamin D_3.

[b] Multicenter trial in progress: NIA Trophic Factors.

[c] Tamoxifen—National Breast Cancer Prevention Trial.

Res/form = drug effect on resorption and formation; FDA phase refers to the state of testing for drug development. Animals-treatment limited to animal studies at present in the United States except for IGF-I which is being studied in the NIA Trophic Factors Study.

dwarf with human GH for ten months and reported a significant increase in linear height (156). Since then, rhGH has made therapy more accessible and less expensive, although controlled longitudinal studies concerning efficacy in regards to linear growth are scarce. Clearly, rhGH has an important therapeutic role in replacement therapy for GH-deficient children with short stature. However, recent attention has focused on GH administration to nonelderly, GH-deficient adults and older men and women with or without osteoporosis. Data on bone mineral density and biochemical markers of bone turnover are few from the first group, although, in one study, lumbar bone density and IGF-I both increased after replacement therapy with rhGH (157). In addition, significant changes in muscle mass also result from GH replacement therapy. Therefore small changes in bone density could be a result of enhanced muscle performance rather than direct skeletal changes.

The effects of GH treatment on bone turnover and density in the elderly are currently being examined in great detail, in part because of publication and media reports from a relatively small study in 1990 by Rudman *et al.* (158). In a group of 21 elderly men with low plasma IGF-I concentrations (<350 U/L defined by an older IGF-I standard), GH administration three times/week for six months caused a decline in adipose tissue mass, an increase in lean body mass, a rise in plasma IGF-I, and a surprising 1.6% increase in lumbar BMD (158). Bone density in eight other sites, including the radius and proximal femur, did not change. Marcus *et al.* (159) studied 18 healthy men and women over age 60 with varying doses of rhGH (0.03–0.12 mg/kg daily) for seven days. Serum PTH, $1,25(OH)_2D_3$, osteocalcin, IGF-I, and urinary hydroxyproline all increased after the 7-day trial. These findings suggest that bone resorption, as indicated by hydroxyproline, and bone formation, as indicated by osteocalcin and $1,25(OH)_2D_3$, were enhanced. In a related study, Marcus (160) examined spine and hip BMD in 22 healthy elderly women. Eleven women received rhGH for 6 months and eight continued therapy for one year. No changes in BMD at the hip or spine were noted at 6 or 12 months, although serum osteocalcin and bone alkaline phosphatase both remained elevated over the course of the study (160).

Older GH deficient people given GH replacement therapy have significant increases in osteocalcin with concomitant rises in urinary deoxypyridinoline, a sensitive marker of bone resorption (161). In a slightly different study but with similar results, Ljunghall *et al.* (146) noted that male patients with idiopathic osteoporosis and low serum IGF-I responded to a 5-day course of rhGH with increased bone specific alkaline phosphatase, indicating formation, and increased urinary calcium/creatinine, indicating resorption. Similarly, Brixen *et al.* (162) administered rhGH to normal men for 7 days and found acute increases in osteocalcin, urinary calcium, and urinary hydroxyproline. Thus, GH treatment (to elders) or replacement (to some elders with low IGF-I, GH-deficient patients, or younger subjects with low IGF-I) is associated with a rise in serum IGF-I and activation of the complete bone remodeling sequence. Preliminary evidence

would suggest little or no bone augmentation occurs, since the entire BMU is activated.

Published series examining the effects of GH on bone mass and fracture rates in osteoporotic subjects are limited. Aloia *et al.* (163–165) published three studies using hGH for 2–24 months. In two studies, radial bone density declined after GH treatment, even though one group received salmon CT to block bone resorption in combination with GH for two years. If CT was given sequentially with GH (2 months GH, 3 months CT), radial BMD did not change, although total body calcium increased. Bone histomorphometry in the latter study failed to reveal evidence of altered bone remodeling. Correlations with IGF-I were not reported in these studies.

In sum, substantial evidence indicates that GH initiates a persistent increase in bone remodeling activity. Due to physiologic coupling in the BMU, there is little, if any, net change in bone mass. Whether the coupling signal operative in the remodeling unit after GH administration is IGF-I cannot be determined with certainty at the present time. However, based on recent *in vitro* studies noted above, it is conceivable that skeletal IGF induction leads both to sustained bone formation through autocrine and endocrine effects on the osteoblast, and to increased bone resorption, through paracrine actions on osteoclast precursors. It is currently concluded that GH therapy for idiopathic osteoporosis is probably not indicated. Furthermore, any enthusiasm for GH therapy must be tempered by potentially significant side effects (e.g., carpal tunnel syndrome, fluid retention, and insulin resistance).

GH directly induces IGF-I production in the liver which subsequently leads to measurable increases in plasma IGF-I (65). The same cannot be said for PTH which appears to have minimal effects on systemic IGF-I yet stimulates bone formation probably through modification of the skeletal IGF system. PTH, when administered intermittently, has greater anabolic properties on bone than GH (166–168). Therefore, despite some stimulation of bone resorption, the anabolic properties of PTH may provide greater therapeutic benefits for patients with osteoporosis than GH.

PTH is a remodeling activator. Patients with primary or secondary hyperparathyroidism have increased bone resorption but enhanced bone formation (78). Histomorphometric studies of these patients have shown that there are increased numbers of osteoblasts, more trabecular surfaces covered with osteoid, and new remodeling units (77). These changes can be duplicated in experimental animals by intermittent (<once/day) administration human PTH at low doses. With subcutaneous delivery, trabecular and, to a lesser extent, cortical bone mass are enhanced (166). Conversely, high doses or continuous treatment with PTH leads to increased bone resorption, hypercalciuria, and inhibition of bone formation (166). A series of studies using PTH for the treatment of postmenopausal osteoporosis was initiated nearly a decade ago. In a large multicenter European trial, 400 units of human PTH(1–34) daily for 6 months produced an increase in trabecular bone formation and trabecular bone volume, as well as enhanced skeletal accretion of Ca^{47} (167). Although these results were encouraging, concern soon arose about cortical bone loss. Subsequent studies combined PTH with $1,25(OH)_2D_3$ in men with osteoporosis, PTH with calcitonin in postmenopausal women, PTH with estrogens in relatively younger postmenopausal women and PTH after one year of estrogen replacement in older postmenopausal women (76, 77, 168, 169). In each trial, PTH produced a marked increase in trabecular bone mass and no change in cortical bone density. Combined cyclical therapies such as these may offer the best potential for using PTH in the treatment of osteoporosis. However, the mechanisms responsible for differential enhancement of trabecular bone mass after PTH therapy remain uncertain. The effects of PTH on the skeletal IGF regulatory system may provide some important clues to its action in bone.

Several lines of evidence, primarily from rodent studies, support the hypothesis that the anabolic effects of PTH on the skeleton are mediated through the IGFs. First, as noted previously (see Regulation of IGFs in Bone), exposure of rat organ cultures to PTH increases the synthesis and release of IGF-I (79). The anabolic effect of PTH is blocked by addition of anti-IGF-I antibodies. Second, continuous PTH administration in rats, dogs, and humans does not stimulate bone formation (57, 166, 170). Rather, collagen synthesis and osteoblast replication are suppressed. Third, and possibly most important, low-dose intermittent PTH administration increases bone mass in normal and estrogen-deficient rats (171). However, hypophysectomy drastically reduces this effect. In this model, growth hormone replacement, but not estrogen, androgen, or thyroid hormone, partially restores the anabolic effects of PTH on bone. Fourth, human PTH administration in oophorectomized rats leads to increased bone mass and enhanced IGF-I mRNA transcripts in bone (83).

The response of skeletal IGFs to PTH may depend on the age of the animal and the mode of PTH administration. In the aged oophorectomized rat model (two-year-old rats), intermittent treatment with human PTH enhanced bone mineral content (BMC), while IGF-I reduced BMC and increased bone resorption (172). When PTH was combined with IGF-I, the anabolic effects of PTH on periosteal and endocortical surfaces were blunted. In contrast, 24-month-old rats given in-

termittent PTH treatment and continuous intravenous IGF-I had accelerated bone formation rates and more osteoid surfaces covered with alkaline phosphatase positive cells than animals treated with PTH alone (173).

There are no histomorphometric and/or biochemical data in humans to support the previously cited rodent studies. Therefore, it is premature to conclude that IGF-I is responsible for the anabolic action of PTH on human bone. Nor have the IGFBPs been examined in detail. However, in one pilot study, chronically elevated PTH levels in patients with primary or secondary hyperparathyroidism were associated with increased serum IGFBP-4 (125, 174). In contrast, osteoporotic patients administered intermittent subcutaneous PTH showed a statistically significant decline in serum IGFBP-4 (174). It is apparent that further studies will be needed to clarify the relationship between the IGF regulatory system and PTH. However, it is certain that intermittent low-dose PTH, given with or without anti-resorptive agents, can augment bone mass (170). Its clinical usefulness will be determined in the next few years.

With the advent of recombinant DNA technology, it is now possible to generate factors which act directly at the cellular level in the remodeling unit. rhIGF-I is the first of what promises to be a large series of peptides developed to stimulate bone formation directly through the osteoblast. More than a decade ago, Froesch and colleagues demonstrated convincingly that IGF-I stimulated growth of hypophysectomized rats in a dose-dependent manner (175). Since then, other studies have documented the growth-promoting properties of IGF-I in transgenic mice that are GH deficient and mutant dwarf rats (26, 176). However, much of the increase in body weight occurs because of growth in previously atrophied kidneys and spleen, not in bone.

In contrast to rhIGF-I administration in rodents, Tixier-Boichard *et al.* (177) failed to demonstrate changes in statural growth (although body weight increased) in sex-linked dwarf-mutant (SLD) chickens administered rhIGF-I. IGF-I had no effect on growth and little effect on systemic hormone levels in normal chicks in that same study. rhIGF-I administered to spontaneously diabetic BB rats also did not result in changes in epiphyseal width, osteoblast surfaces or osteocalcin concentrations (53). In ovariectomized rats, addition of rhIGF-I only partially restored femoral calcium and trabecular bone volume with no effect on osteoblasts or osteoclasts. In contrast, human PTH almost completely prevented femoral bone loss (178). In a different study of six-month oophorectomized rats, rhIGF-I increased midshaft tibial BMD by enhancing periosteal bone apposition (179). However, IGF-I did not increase bone strength or stiffness which was observed when the anti-resorptive agent, pamidronate, was used (179). Spencer *et al.* (180) used an entirely different model system and infused IGF-I into the arterial supply of the right hindlimb in ambulatory rats for up to 14 days. Trabecular bone formation increased 22% compared with the contralateral limb, while cortical bone formation was only slightly enhanced (180). There was no evidence of osteoclastic activation and the effects were age-independent.

Extensive studies involving rhIGF-I administration to humans are currently underway or just beginning. The primary focus for these trials has been Type I and Type II diabetes mellitus, since rhIGF-I can lower plasma glucose, suppress endogenous insulin production, and increase glomerular filtration rate (181). However, the results of rhIGF-I administration on skeletal mass and bone turnover in metabolically normal humans have recently been described. Johansson *et al.* (182) administered 160 μg/kg/day of rhIGF-I for seven days to one man with idiopathic osteoporosis and a borderline low serum IGF-I level (182). Bone-specific alkaline phosphatase, osteocalcin, and the carboxyterminal peptide of procollagen Type I all increased by more than 40% over seven days (182). However, two markers of bone resorption (urinary calcium/creatinine ratio and hydroxyproline) also increased (182). Four weeks after treatment, markers of bone formation remained elevated but resorptive indices returned to normal (182). Eberling *et al.* (183) administered varying doses (30–180 μg/kg/day) of subcutaneous rhIGF-I to 18 healthy postmenopausal women over 8 days. Except at the lowest dose, rhIGF-I significantly increased markers of both formation (procollagen peptide) and resorption (deoxypyridinoline cross-links). There were only minor side effects, except with the highest dose (180 μg/kg/day), which was associated with development of edema and tachycardia. Interestingly, none of the women developed hypoglycemia.

The theoretical benefits of using IGF-I over GH include (i) more direct activation of bone formation (it is conceivable that GH cannot induce IGF-I in senescent osteoblasts); (ii) less frequent occurrence of side effects (fluid retention, carpal tunnel syndrome); and (iii) improved glucose tolerance. However, IGF-I has a relatively short half-life and can produce hypoglycemia. Therefore, very recent efforts have focused on combining rhIGF-I with rhIGFBP-3. Not only does IGFBP-3 prolong the half-life of IGF-I in the circulation, but, by virtue of its own stimulatory properties (see IGFBPs and Bone), may enhance the mitogenic action of IGF-I.

Three preliminary studies in rats have recently been presented and the results are encouraging. Brommage *et al.* (184) administered either IGF-I or IGF-I coupled to IGFBP-3 to ovariectomized rats (5.5

months of age) daily for 8 weeks. Both treatments increased femoral and tibial bone density more than saline controls (184). Greater than 50% of the rats treated with rhIGF-I developed hypoglycemia, while less than 10% did in the IGF-I/IGFBP-3 group. Narusawa *et al.* (185) gave IGF-I or IGF-I/IGFBP-3 to older (8 months of age) rats months after oophorectomy and sciatic nerve surgery. Both IGF-I and IGF-I/IGFBP-3-treated rats had restoration of bone mass which was lost after estrogen withdrawal. However, the rats who received IGF-I/IGFBP-3 had greater increments in bone formation, increased osteoblast recruitment, and enhanced mineralization. Similarly, Bagi *et al.* (186) treated 6-month-old ovariectomized and sham operated rats with IGF-I or IGF-I/IGFBP-3 for eight weeks. Again, the greatest increase in cancellous bone mass, mineral apposition rate, and bone formation occurred in the group treated with the IGFBP complex. Most promising was the finding that thickness of existing trabeculae was increased by 20%.

In determining efficacy of a particular treatment for osteoporosis, prevention of fractures is the most important criteria. Fractures result from low bone mass and reduced bone strength. Increased trabecular thickness often translates into greater bone strength. Therefore, changes noted in rodents with IGF-I/IGFBP-3 may have particularly important ramifications. However, it is obvious that many more studies will be needed to confirm these findings.

Despite some preliminary data concerning the efficacy of rhIGF-I on bone, it is premature to consider growth factors as a revolutionary new treatment for osteoporosis. In studies with IGF-I alone, bone resorption is accelerated, thereby limiting bone mass accretion. Furthermore, although IGF-I may produce fewer side effects than GH, Eberling *et al.* (183) still observed tachycardia, weight gain, and orthostatic hypotension in normal subjects treated for 8 days. The studies with IGFBP-3 and IGF-I are promising, but it is much too early to assess their future role in treating metabolic bone diseases. Hence, considerable therapeutic problems remain before clinicians can use growth factors as anabolic agents for age-related or postmenopausal osteoporosis.

Conclusion

The skeletal IGF regulatory system is intimately involved in normal and aberrant bone remodeling. There is little doubt that perturbations in this complex growth factor network modify the physiology of the BMU. However, the precise role of circulating IGFs, local and systemic IGFBP-specific proteases and IGF receptors still require further definition, especially in relation to altered states of bone turnover. Nevertheless, newer therapeutic approaches utilizing bone specific IGFBPs, with and without IGFs, are on the horizon. So much progress has been made in the last half a decade that the future looks very promising. Indeed, there appears to be a very strong connection between insulin-like growth factors and osteoporosis.

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