MINIREVIEW

Insulin-Like Growth Factors and Bone: The Osteoporosis Connection Revisited (44310)

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Abstract. **Tremendous advances have been made in knowledge about the pathogenesis and treatment of osteoporosis, a disease that affects more than 25 million Americans. In particular, it has been determined that two major processes are responsible for osteoporotic fractures. These are: 1) bone mass acquisition during adolescence; and 2) bone loss beyond the sixth decade. The former, and possibly the latter, are regulated by genetic and environmental factors. Insulin-like growth factor-I(IGF-I), a ubiquitous polypeptide, assumes a critical role in both of these processes. Very recent studies have elucidated a complex multifaceted IGF regulatory system in bone and have allowed investigators to consider site-directed approaches to therapy. Even more exciting is the prospect that the genetic regulation of peak bone mass may be controlled by components of the IGF regulatory system. Within the last half decade, tremendous strides have been made in defining the regulatory circuits that determine the expression of skeletal and serum IGF-I. These heritable modulators may be similar or identical to regulators of bone mineral density, thereby joining two distinct phenotypes. This minireview highlights some of the new investigations into the role IGF-I plays in the pathogenesis of osteoporosis. Although recent clinical trials with growth hormone and IGF-I in this disease have been relatively disappointing, advances on other fronts have generated considerable excitement, and these promise new and innovative approaches to this crippling disease.** [P.S.E.B.M. 1998, Vol 219]

In the past decade, tremendous strides have been made in defining the role of IGF-I in cell growth and destiny. Investigators have now characterized tissue-specific IGF regulatory systems that consist of ligands (IGF-I,-II), IGF-specific binding proteins (IGFBPs: 1–6), IGFBP proteases, and two IGF receptors (1). In concert, these components are essential for maintaining the functional activity of many, if not all, organ systems. Progress has also been steady in delineating the subcellular signaling cascades activated by IGF-I and the function of both IGF receptors (2). The regulation of IGF-I at a molecular level has also been investigated revealing novel information about the IGF-I gene. In the last 5 years, human and animal studies with IGF-I have occupied center stage and, although the results have been somewhat disappointing, these efforts have opened up newer therapeutic avenues for this peptide in other disease states. Notwithstanding these advances, there remains a tremendous gap in our understanding of IGF-I in several chronic diseases. Nowhere is this more apparent than in osteoporosis. Although several lines of evidence suggest that IGF-I plays a major role in skeletal physiology, precisely how that knowledge can be applied to patients with osteoporosis remains to be determined.

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Several new lines of investigation support the importance of IGF-I in the skeleton. IGF-I enhances osteoblastic differentiation, helps to maintain the osteoblast phenotype, and inhibits collagenase activity (3). These IGF-dependent events result in an increase in matrix apposition and guarantee preservation of the skeleton (4). Studies have also confirmed that bone is a major depot for IGF-I and that aging produces a rather marked decline in skeletal production and storage of this peptide (5). There is mounting evidence that IGF-I can also recruit premature osteoclasts and serve as a coupling agent in the bone remodeling cycle (6). In addition, all six IGFBPs are produced by bone cells and have been shown to modulate IGF-mediated osteoblast proliferation and differentiation (7). Still, it is uncertain how the IGFs support bone mineral density and what effect deficiency states might have on remodeling status and bone structure.

The purpose of this minireview is to revisit the relationship between IGF-I and osteoporosis, highlighting some very recent findings concerning the basic biology of IGF-I in the skeleton. It is likely that data from several of these newer studies will be used to design novel diagnostic and therapeutic approaches to this chronic disease. Despite the tremendous potential of IGF-I, it is becoming more apparent that further investigations will be needed, both at the basic and clinical level, before the full promise of this peptide in osteoporosis medicine will be realized.

Genotypic Interactions Between Two Phenotypes: IGF-I and BMD

A new and exciting development in osteoporosis research is the search for genes that control bone mineral density. Bone mineral density (BMD) is an excellent surrogate for osteoporosis since there is a very strong inverse relationship between low bone mass and fracture risk (8). In fact, low bone density is a better predictor of fracture risk than cholesterol is for coronary heart disease, or blood pressure is for stroke. Furthermore, up to 60% of the variance in BMD can be attributed to genetic factors (9). This has led to widespread screening studies using densitometry to map "bone density genes" that could forecast osteoporosis. Although expectations were high that association studies between BMD and specific candidate loci would quickly lead to discovery of one or more osteoporosis genes, it has become clear that BMD is a complex polygenic trait under the control of multiple genetic factors (9).

One candidate protein that has undergone scrutiny is IGF-I. As noted previously, IGF-I is extremely abundant in bone, is synthesized by osteoblasts, and plays a major role in coupling bone resorption to bone formation. Recent evidence suggests that serum IGF-I is also under genetic control. Comuzzie *et al.* noted in a Mexican American cohort of 582 individuals in 26 pedigrees that there was strong heritability for serum IGF-I (10). We recently reported a similar degree of heritability in serum IGF-I between mother-daughter Askenazi Russian Jewish immigrants (11). Not surprisingly, in our study, significant heritability for calcaneal ultrasound attenuation (i.e., bone mass) was also noted.

There are several other lines of evidence suggesting that serum IGF-I is closely related to bone mineral density. First, several cross-sectional studies have shown a correlation between serum IGF-I levels and BMD at various skeletal sites (12, Kiel, personal communication). Second, acquisition of peak bone mass occurs during rapid adolescent growth, a time when serum IGF-I is also reaching its highest level. Since peak bone density accounts for more than half of the variance in bone mineral density at any time in an individual's life, and more than 70% of peak bone mass is heritable, IGF-I may be very critical in the final determination of osteoporosis risk. In a similar vein, other components of the IGF system, the IGFBPs and IGFBP proteases, are reasonable regulatory gene candidates, recognizing that BMD is a complex polygenic trait, and that genetic interrelationships are certain to be multifactorial. Finally there have been some very recent studies in mice and men that have reinforced the importance of IGF-I in regulating bone mineral density.

Genetic studies in humans are expensive, time consuming, and complicated by environmental variables. Hence investigators have turned to animal models to study the heritability of bone density. Healthy inbred strains of mice are currently being used to map "osteoporosis" genes for several reasons. First, several strains exhibit large differences in femoral bone densities (F-BMD) (13). Second, acquisition of peak bone mass in mice parallels the same process in humans and occurs at 12–16 weeks of age (13). Third, environmental interactions can be kept to a minimum allowing for a clearer evaluation of heritable determinants of the bone density phenotype. Fourth, the finite life span and the ease of measuring genotype and phenotype make mice logical models for genetic analysis. In the last 3 years, investigators have used intercrosses and sibling matings to identify several quantitative trait loci (QTLs) that contribute to the F-BMD phenotype (13). During these studies, it was also noted that for the two inbred strains with the highest and lowest F-BMD, serum IGF-I levels were also found to be at the same two extremes (14). Rank orders of peak BMD in several strains of healthy mice revealed a similar order for serum IGF-I with a 30% difference in serum and skeletal IGF-I between the two extreme bone density strains (14). In fact, after progenitor crosses of the BMD extreme strains (high-low) followed by intercrossing their F_1 offspring, serum IGF-I co-segregated with F-BMD and accounted for 35% of the phenotypic variance in F-BMD for all F_2 mice (14). Moreover, *in vitro* studies of bone cells from the two extreme progenitor strains also revealed the same magnitude of difference in production of IGF-I (14). These findings have reinforced the hypothesis that IGF-I is related to BMD. However, these data do not prove a direct cause and effect between IGF-I and bone density.

Further support for a putative association between these two phenotypes is also emerging in humans. For example,

in men with severe osteoporotic fractures and low BMD, serum IGF-I concentrations are correspondingly low and correlate directly with histomorphometric evidence of reduced bone formation (15). Moreover, in several cohorts of men and women, a homozygous polymorphism within a microsatellite CA(n) repeat within the IGF-I gene (1 kb upstream of the transcription start site) is strongly associated with low serum IGF-I levels (16). In a very recent association study, Rosen *et al.* found a two-fold greater frequency of this homozygous polymorphism in men with asymptomatic low bone mass as well as those with the syndrome of idiopathic osteoporosis (16). Thus, there appears to be a heritable component to the IGF-I phenotype, which could be related to bone density. Potentially, these heritable determinants could strongly impact acquisition of peak bone mass. Future family and pedigree studies will certainly focus on IGF-I and other components of the IGF axis as potentially important genetic determinants of BMD.

IGF-I and Age-Related Osteoporosis

Age-related bone loss is a major cause of osteoporotic fractures in the elderly, yet the pathogenesis of this syndrome is not clear. It is now firmly established that bone resorption increases with advancing age (possibly as a function of secondary hyperparathyroidism and calcium deficiency) (17). Bone loss associated with a rise in bone resorption is reflected by increased serum and urinary markers of bone turnover (17). Also, in elderly women who are rapid bone-losers, there is uncoupling in the remodeling sequence, as bone formation rates cannot match the rapidity of bone resorption (18, 19). Recent evidence suggests that osteoblastic resistance to IGF-I occurs in bone cells harvested from the elderly (19). This resistance, coupled with reduced recruitment of osteoblast progenitors, suggests that age-related bone loss is magnified by defective osteoblastogenesis.

Since the skeletal and circulating IGF regulatory system are important in maintaining differentiated osteoblastic function, much attention has focused on disturbances in IGF-I as one cause of age-related bone loss. Several studies have shown an age-associated reduction in circulating IGF-I, in part due to impaired GH secretion (20). Boonen *et al.* recently noted that cortical and trabecular IGF-I concentrations in the human femoral neck decline by more than one third from the age of 23 to 92 (21). These data, along with an earlier study by Nicholas *et al.* confirm that skeletal IGF-I is markedly reduced in the elderly, and that this decline is similar to the age-associated drop in serum IGF-I (5, 21).

In addition to the well-described reduction in serum and skeletal IGF-I often observed in the elderly, there may be other perturbations in the IGF regulatory system that could contribute to impaired bone formation. In particular, there are likely to be alterations in the production and breakdown of inhibitory and stimulatory IGFBPs (7). Several roles for the IGFBPs and their related proteases have emerged from *in vitro* studies using various osteoblast-derived cell lines. These include sequestering IGFs to inhibit their biological activity, prolonging the half-life of IGFs, and enhancing the biological response of target tissues to IGFs by targeting the peptides to particular cell types or by fixing them in adjacent compartments (22–26). Of particular interest is IGFBP-5, one of the IGFBPs that potentiates IGF action (27). IGFBP-5 is specific for IGF-I and -II but is able to associate directly with the osteoblast surface to stimulate mitogenesis in the absence of both peptides (28, 29). IGFBP-5 is present in the extracellular matrix and may facilitate storage of IGFs by complexing with IGFs and then binding to hydroxyapatite (Fig. 1) (30); this allows the IGFs, once liberated by acid-induced proteolysis, to become available for the bone remodeling process (29, 31).

Regulation of the balance between inhibitory IGFBPs and stimulatory IGFBPs could be an important mechanism in bone remodeling (32). In contrast to the agonistic properties of IGFBP-5, IGFBP-6 is a binding protein that acts to inhibit IGF actions by sequestering IGFs and preventing association with IGF receptors (1). IGFBP-5 declines with age whereas IGFBP-6 increases, and these changes may contribute to reduced osteoblastic activity (1). Similarly, IGFBP-4, an inhibitory IGFBP, is markedly increased in the serum of elderly individuals and is highest among those who sustain a hip fracture (33). Furthermore, IGFBP-4 concentrations correlate closely with PTH, and both rise with age (33, 34). Finally, IGFBP-3, a major circulating binding protein, is also produced by osteoblasts and is regulated by growth hormone (4, 5). Serum IGFBP-3 declines with age and may contribute to changes in bone turnover in the elderly.

Thus, a scenario could be constructed whereby elderly individuals with reduced serum and skeletal IGF-I, IGFBP-3 and IGFBP-5 concentrations develop secondary hyperparathyroidism. This leads to increased bone resorption, but also enhanced production of IGFBP-4, which prevents IGF stimulation of bone formation. Uncoupling in the remodeling unit could markedly increase the risk of osteoporotic fractures as bone mass declines rather dramatically. Furthermore, if IGF-I and the IGFBPs play such a critical role in the process of age-related bone loss, then therapeutic options aimed at the IGF regulatory system could have great potential.

IGF-I as a Therapeutic Option for Osteoporosis

Low bone mineral density as a result of chronic growth hormone deficiency (GHD) in adulthood can lead to osteoporotic fractures (35). Recently, the U.S. FDA approved the use of recombinant human GH (rhGH) for growth hormone deficiency (GHD) in adults. In part, this indication was based on compelling data from the United States and Europe that rhGH treatment for 2 years to growth-hormone-deficient individuals increases bone mass at several skeletal sites (36). However, no studies have shown that rhGH can increase bone mass in the elderly, even though both skeletal and serum IGF-I levels are markedly reduced in the elderly (37). The reasons for this disparate response to rhGH are not

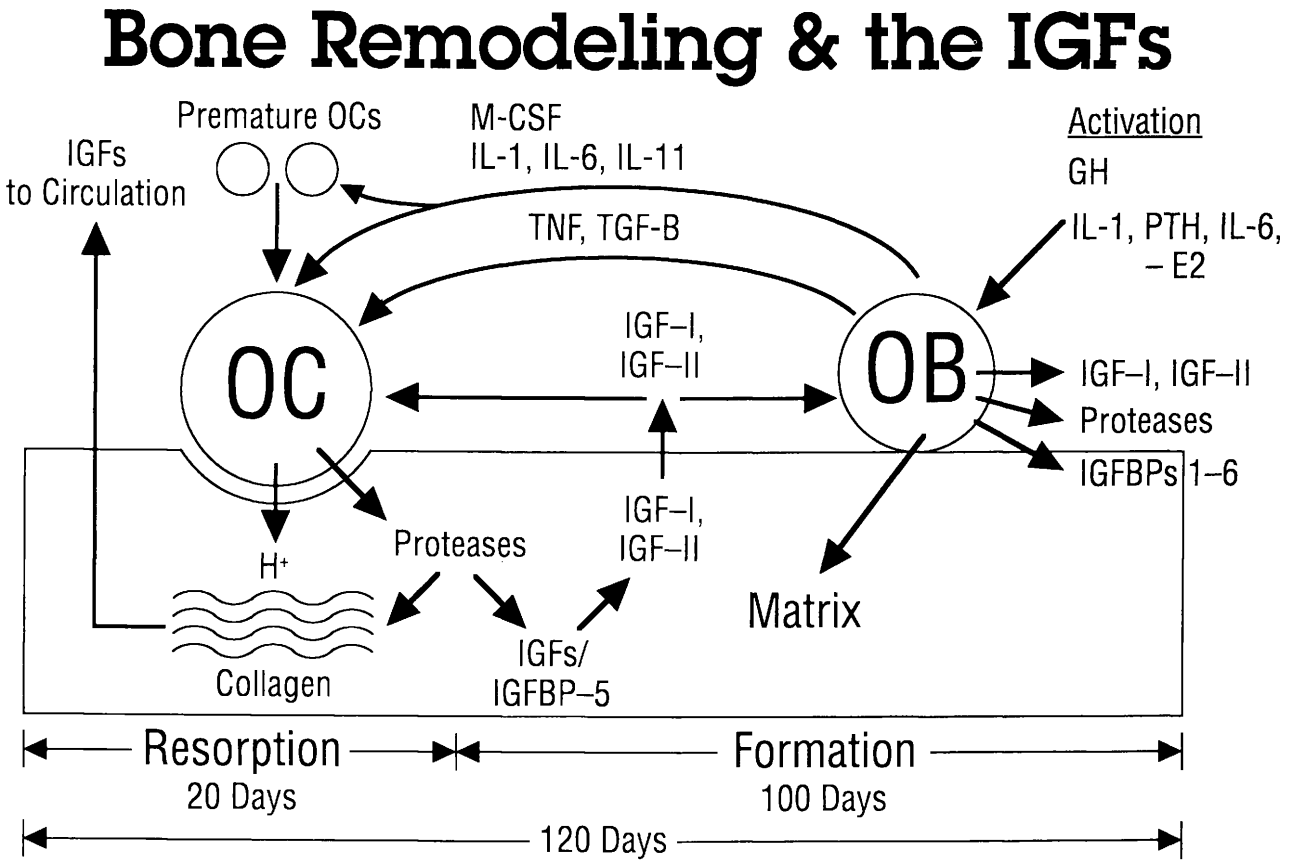

Figure 1. The bone remodeling unit involves the coupling of bone resorption to bone formation. Osteoblast activation results in the release of several cytokines including IL-1, -6 and -11 and M-CSF as well as TGF-b and TNF. These peptides recruit osteoclasts to the skeletal surface where bone resorption occurs. This leads to release of matrix-bound proteins including the IGFs and their binding proteins (IGFBPs). These molecules help to recruit osteoblasts to the resorption lacunae and thereby promote coupling. Differentiated osteoblasts synthesize IGFs and IGFBPs to be deposited within the skeletal matrix and orchestrate collagen synthesis. Both osteoclasts and osteoblasts exhibit IGF Type I receptors. IGFBP proteases are produced by bone cells, and some of these enzymes require activation after protons are generated by osteoclasts.

entirely clear. Originally, Rudman *et al.* reported a 1.6% increase in lumbar BMD following 6 months of rhGH treatment to elderly males with low serum IGF-I (38). Subsequent follow-up of that cohort failed to show a consistent effect from rhGH on spine, hip, or total body BMD (39). Other short-term studies have been unable to show a positive effect from rhGH on bone mineral density, even though markers of bone turnover increase (40–42). Similarly, Holloway *et al.* could not establish a benefit from rhGH treatment alone that was greater than treatment with calcitonin alone (43). Furthermore, MacLean *et al.* recently reported that total body BMD decreased after 1 year of low-dose rhGH in elderly men and women classified as frail by indices of physical performance (44). It is even more notable that the results of these studies were negative despite consistent and significant increases of serum IGF-I into the young normal range. Taken together, these data suggest that IGF-I deficiency was not the pathogenetic factor in age-related osteoporosis, or that other factors, including the IGFBPs, limit the bioactivity of IGF-I in the skeleton of older individuals.

The absence of skeletal anabolic effect from GH in the elderly has not deterred investigations with rhIGF-I and IGF-I/IGFBP-3 as antiosteoporotic treatments. Ebeling *et al.* investigated several doses of rhIGF-I in postmenopausal women and found that bone turnover was stimulated and that low-dose IGF-I could increase bone formation more than resorption (45). Few side effects were noted with rhIGF-I at doses of 30 and 60 μg/kg/day. More recently, Ghiron *et al.* administered low-dose IGF-I (15 μg/kg/day b.i.d.) to elderly women and found a selective increase in bone formation without changes in bone resorption (42). These data suggest that IGF-I in low doses may have an effect on bone turnover and potentially BMD.

Because serum levels of IGFBP-3 have been shown to be reduced in osteoporotic patients, and because of the concern about the long-term safety of IGF-I in elders, an alternative approach for using IGF-I in age-related osteoporosis has emerged (46). IGF-I complexed to IGFBP-3 and administered daily as a soluble complex subcutaneously has been shown to increase serum IGF-I concentrations markedly in the young and elderly without serious adverse effects. Based on earlier animal studies, IGF-I/IGFBP-3 complex can strongly enhance bone formation and bone mass (47). Dose-ranging studies using IGF-I/IGFBP-3 complex (0.3–6.0 mg/kg) in young volunteers and healthy elderly adults has

shown that this agent is safe and well tolerated (D. Rosen, personal communication). Similarly, in a phase I trial, 7 consecutive days of rhIGF-I/IGFBP-3 at doses of 0.5–2.0 mg/kg/day by continuous subcutaneous infusion *via* minipump, produced no serious side effects. Furthermore, procollagen peptide (a marker of bone formation) increased 50% over the 7-day period and remained elevated for an addition 7 days after discontinuation of treatment (D. Rosen, personal communication). Despite a concomitant rise in deoxypyridinoline with complex administration, this rise did not persist post-treatment as was seen with procollagen peptide. Thus, this form of IGF-I could have utility in future studies of patients with osteoporosis.

Other future osteoporosis therapies could center on the IGFBP proteases, some of which are specific for particular IGFBPs and operate only in certain environments (e.g., one IGFBP-4 protease works at a very low pH that is only found at the site of bone resorption). Bone cells in culture and human bone cells *in vivo* produce enzymes that proteolyze the IGFBPs. These include nonspecific matrix metalloproteases, plasmin, and IGFBP specific proteases (48–50). Because IGFBP-5 fragments have been shown to enhance the action of IGFs in bone cells, IGFBP-5 proteases may be important in IGF/IGFBP-mediated bone differentiation (51). In some physiological conditions IGFBP-5 protease will also degrade IGFBP-3 and -4 and proteolysis of IGFBP-5 has been shown to be responsive to parathyroid hormone (PTH) and prostaglandin E_2 (52). IGFBP-4 also has a specific protease that is induced by PTH and estrogen, effectively limiting the inhibitory capacity of IGFBP-4 in medium from osteoblastic cell culture (53). Consequently, induction of specific proteases to release or sequester IGFs *via* IGFBPs could be used therapeutically to manipulate the bioavailability of the IGFs.

GH/IGF-I as Short-Term Treatment of Catabolic States Associated with Osteoporosis

Hip fractures are the most feared complication of the osteoporosis syndrome (54). This is due to the high mortality (upwards of 20%) and the tremendous morbidity associated with this event (54). Although hip fractures do not kill people directly, the baseline nutritional status of those who fracture, the trauma itself, the surgery required for repair, and the predisposing frailty associated with hip fracture, lead to poor outcomes. Very recently, two independent groups have noted a dramatic drop in serum IGF-I in elders after a hip fracture (55, 56). These changes in IGF-I were accompanied by significant declines in femoral BMD and lean body mass 8 weeks after the fracture (56). Even though some of the hip-fracture subjects were malnourished or chronically sick prior to their fracture, it is clear that the injury and the resultant surgery strongly limits IGF-I production. Furthermore, this decline in IGF-I could be linked directly to increases in total body catabolism. Recently, Bonjour *et al.* established that baseline IGF-I after hip surgery was a surrogate marker for prolonged hospitalization postsurgical fixation (56). Since much of the annual cost for osteoporosis (13 billion dollars in the United States) centers around hospitalization and rehabilitation after hip fractures, therapies using GH/IGF-I axis or its components, make sound medical and economic sense.

Several therapeutic strategies have recently been initiated to reduce hospital stays and morbidity after a hip fracture. Bonjour *et al.* demonstrated convincingly that 6 months of protein supplementation to elderly patients after hip fracture increases serum IGF-I by 75%, reduces bone loss by half, improves muscle strength, and shortens rehabilitation times (56). Growth hormone treatment has also been attempted in some elders after hip fracture, but at the present time, those trials have been somewhat inconclusive, and safety has yet to be established.

Another approach is the use of growth hormone releasing hormone (GHRH) or GH releasing peptide analog to induce modest increases in serum IGF-I and reduce protein breakdown. There are several advantages to use of these agents. First, there are few side effects reported with their use in elderly individuals. Second, the growth hormone/IGF-I axis remains intact. Third, the ease of administration (oral or subcutaneous) is appealing. Fourth, the rise in IGF-I with use of these agents is far lower than after treatment with GH or rhIGF-I. Preliminary studies in elders suggest that both oral and subcutaneous forms of these secretagogues raise serum IGF-I only 40%–60% above baseline (57). Functional responses to these short-term therapies have yet to be reported. Finally, there is an ongoing 3-month, placebo-controlled, randomized, phase II clinical trial of IGF-I/IGFBP-3 complex administered for 2 months to 24 elderly men and women who have sustained a hip fracture. Results of that study will be awaited with considerable interest.

Conclusions

The last half decade has produced some exciting and innovative approaches to understanding the relationship of IGF-I to the skeleton. The IGF regulatory system is complex and multifaceted. It is regulated by numerous hormonal and paracrine factors that ultimately control matrix deposition and bone mass. New evidence has emerged that there are also genetic determinants that affect total skeletal and serum IGF-I concentrations. Aging results in a dramatic decline in IGF-I that may lead to accelerated bone loss and osteoporosis. The vast repertoire of IGFBP actions in bone make these peptides particularly important in defining skeletal responses to the IGFs. The potential role of IGF-I as a candidate gene affecting peak bone mass may ultimately determine how clinical investigators will manipulate this system to improve bone density and reduce fracture risk in the 21st century.

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