MINIREVIEW

From Mouse to Man: Redefining the Role of Insulin-Like Growth Factor-I in the Acquisition of Bone Mass[1]

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The insulin-like growth factor system (IGF) has been linked to the process of bone acquisition through epidemiologic analyses of large cohorts and *in vitro* studies of bone cells. But the exact relationship between expression of IGF-I in bone and skeletal homeostasis or pathologic conditions, such as osteoporosis, remains poorly defined. Recent advances in genomic engineering have resulted in the development of better *in vivo* models to test the role of IGF-I during development and maintenance of the adult skeleton. It is now established that skeletal expression of IGF-I is critical for differentiative bone cell function. It may also be essential for the full anabolic effects of parathyroid hormone on trabecular bone and for some component of biomineralization. Evidence from conditional mutagenesis studies suggests that serum IGF-I may represent more than a storage depot or permissive factor during the final phase of skeletal acquisition. This work re-examines the original tenets of the "somatomedin hypothesis" in light of these newer mouse models and their remarkable skeletal phenotypes. The implications are far reaching and suggest that newer approaches for manipulating the IGF regulatory system may one day be useful as therapeutic adjuncts for the treatment of osteoporosis. Exp Biol Med 228:245–252, 2003

Key words: IGF-I; periosteum; osteoblasts; bone volume; conditional mutagenesis

[1] This manuscript is an update of a previously published minireview entitled "Insulin-Like Growth Factors and Bone: The Osteoporosis Connection Revisited" (Proc Soc Exp Biol Med 219:1-7, 1998).

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1535-3702/03/2283-0245$15.00

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Nearly half a century ago, Salmon and Daughaday made the seminal observation that a circulating factor mediated the biologic activities of growth hormone (1). From those pioneering studies arose the "somatomedin hypothesis," a prelate suggesting that insulin-like growth factor-I (IGF-I) was an endocrine hormone with distant tissue targeting (2). Since those early days, tremendous progress has been made in understanding the role of IGFs, their binding proteins, and their receptors, in various physiologic and pathologic states. Paradoxically, the greatest advances have come in defining the local (paracrine and autocrine) activities of IGF-I in normal and neoplastic states, especially in relation to cell cycle activity and programmed cell death. In part this has been a function of better models, more sensitive read outs, and technological advances including gene chip microarray analyses. With the advent of such tools, the role of IGF-I in cell signaling has become established for virtually all tissues. Still to be defined, however, are the precise physiologic control mechanisms that define its expression and, importantly, how circulating IGF-I is involved in bone remodeling.

In contrast with the spectacular gains in delineating the cellular aspects of IGF actions, progress with *in vivo* modeling has been slow and complex. Recently, however, two developments have forced investigators to re-examine the role of circulating IGF-I in the homeostatic processes of mammals. The first development was the discovery that high normal concentrations of serum IGF-I were a major risk factor for development of breast, prostate, and colon cancer (3). The second was technologic and relates to the use of genetic engineering in mice to define the physiologic activity of the IGF regulatory system. These studies have opened new vistas for exploring functional changes in the IGF pathway of many tissues. The skeleton has been no

exception, and in fact has benefited greatly from the use of mouse models that are genomically altered. Previous *in vivo* models lacked sufficient mechanistic insights, were not comparable to the human skeleton, and paled as developmental models. One need only look at two previous reviews on IGF-I and bone completed by our group in this journal over the last decade to appreciate how far we have progressed in a few short years (4, 5). Currently, investigators have mouse models that over and underexpress IGF-I, the type I IGF receptor, five of the six insulin like growth factor binding proteins (IGFBP), the growth hormone (GH) receptor, and at least one IGFBP-specific protease. In addition, using Cre recombinase and bone-specific promoters, such as osteocalcin and collagen 1A1, targeted deletion or overexpression of IGF components in the skeleton has been accomplished. The creation of a liver-specific IGF-I deletion by Cre technology has unexpectedly reopened the debate about the original somatomedin hypothesis. The resulting phenotypes, as will be discussed in this article, are not only interesting, but also provocative, in part because they challenge many of the current paradigms about skeletal growth and IGF-I.

Yet despite such progress, we still do not understand exactly how IGF-I is partitioned between the circulation and the skeleton, nor how that translates into the acquisition and maintenance of bone mass. Although we have made substantial progress since the pioneering work of Salmon and Daughaday and have moved very rapidly towards the theorem that local IGF-I activity determines tissue fate, investigators remain intrigued by the original somatomedin hypothesis. In this work, we will re-examine the role of both circulating and skeletal IGF-I in skeletal growth and maintenance. We will focus almost exclusively on IGF-I and leave the IGFBPs and the IGFBP proteases to recently published reviews (6). The goal of this article is not to ignore those other components of the IGF regulatory system but rather to relate the current view of the somatomedin hypothesis to our understanding of bone acquisition. As such, much of the focus will center on *in vivo* animal models.

The Physiology of IGF-I in the Skeleton

The skeleton is a highly organized and physiologically active organ, continuously remodeling itself to preserve skeletal integrity while providing a reliable and constant source of calcium for the circulation and thus for all other tissues. The mammalian skeleton is uniquely designed for its protective and structural roles. There is an outer surface of cortical bone that surrounds the inner trabecular elements. Marrow bathes the trabecular skeleton whereas cortical bone is nourished by periosteal vessels and a series of canaliculi connecting osteocytes to lining cells and osteoblasts. Gravitary forces help model the cortical skeleton, although periosteal osteoblasts and the underlying growth plate are principally responsible for longitudinal growth. Both cortical and trabecular bone undergo remodeling, but the frequency of this process is much less in the cortex than in the trabecular components of the spine and distal femur.

Numerous growth factors and cytokines, each of which contributes to coupling bone dissolution (i.e., resorption) to new bone formation, orchestrate bone remodeling within the skeletal compartments. Pre-osteoblasts, derived from mesenchymal stromal cells and under the influence of a key transcription factor (Cbfa1, i.e., core binding factor I or RUNX2), represent target cells for initiation of the remodeling cycle (7). Systemic and local factors enhance preosteoblasts differentiation and this, in turn, leads to the synthesis and release of macrophage colony-stimulating factor and receptor activator of nuclear factor kappa B ligand (RANKL; Ref. 8). These two peptides are necessary and sufficient for the recruitment of bone-resorbing cells, i.e., the osteoclasts. Once bone resorption occurs, calcium, collagen fragments, and growth factors, such as the IGFs and transforming growth factors (TGFs), are released from the bony matrix. The latter factors enhance the recruitment of osteoblasts to the bone surface, thereby setting the stage for collagen synthesis and matrix deposition/mineralization (5). The entire remodeling cycle in humans takes approximately 90 days, with the majority of time consumed by the elaborate process of bone formation and subsequent mineralization.

IGFs are major components of both the organic skeletal matrix and the circulation. Indeed, the serum of most mammals contains significant concentrations of both IGF-I and IGF-II, bound to high and low molecular weight IGFBPs (9). Similarly, the skeletal matrix also is highly enriched with these growth factors and other noncollagenous proteins, including all six IGFBPs and several IGFBP proteases. In addition, the type I IGF receptor is present on both osteoblasts and osteoclasts. It is now reasonably certain that skeletal IGFs originate from two sources: 1) *de novo* synthesis by bone forming cells (i.e., pre-osteoblasts, and fully differentiated osteoblasts) and 2) the circulation. In fact, some skeletal IGFs probably make their way into the matrix by way of specialized canaliculi and sinusoids within the bone microcirculation (5, 10). IGFs, bound to IGFBPs, can also be found within the marrow milieu in close contact with the endosteal surface of bone. But, by most accounts, the vast majority of IGF-I in bone is derived from local osteoblastic synthesis. Yet during active bone resorption, as the matrix is dissolved, significant amounts of IGF-I and II are released from storage (i.e., binding to IGFBP-5 and hydroxyapatite). Subsequently, both IGFs recruit precursor osteoblasts, and possibly early osteoclasts to the bone surface where remodeling is occurring (5, 10, 11).

The IGFs act in diverse patterns via endocrine/autocrine/paracrine pathways to regulate the differentiative functions of both osteoblasts and osteoclasts. Because the IGFs are stored within the skeletal matrix and are released during bone resorption, IGF-I and -II may be the critical coupling proteins that keep bone resorption closely linked to formation. However, several cytokines and differentiation

factors also work in a manner analogous to the IGFs, some with greater potency on osteoblasts than others (5, 10). Likely, the orchestration of bone remodeling requires the activity of both osteoblast-derived and systemic proteins, working through time-, tissue-, and dose- dependent circuits to maintain a balanced bone turnover rate. Notwithstanding the presence of multiple growth factors and cytokines, IGF-I in particular, is an important differentiative factor for osteoblasts.

Several aspects of IGF-I as it relates to bone physiology remain poorly defined. For example, although IGF-I can increase thymidine incorporation in most cells, its role as a mitogen in pre-osteoblast proliferation seems relatively limited. Even less clear is the effect of IGF-I on osteoclast differentiation. Several studies have suggested that under physiologic conditions, IGF-I can enhance osteoclast recruitment, although more recent work in some laboratories has failed to show a major direct effect of IGF-I on recruitment or differentiation of premature osteoclasts (11). However, Rubin *et al.* demonstrated that stromal cell production of RANKL is markedly enhanced by IGF-I in a dose- and time-dependent manner (12). This would be consistent with its role as a coupling factor in bone remodeling, activating bone resorption through the pre-osteoblastic lineage. Despite some uncertainty most lines of evidence point to an important modulatory function in the bone remodeling cycle for IGF-I produced by osteoblasts.

Regulation of IGF-I in Bone Cells

One clue to understanding the effects of circulatory versus skeletal IGF-I on bone remodeling lies in studies of IGF-I gene regulation. Osteoblast-like cells from rodents and human *in vitro* express both IGF-I and IGF-II mRNA transcripts and their expression is altered by the addition of various skeletal growth factors (e.g., interleukin [IL]-6, TGF-B, fibreblast growth factor (FGF); Ref. 5). Similarly, *in vivo* studies have revealed that the major hormones regulating bone turnover also affect IGF-I expression *in vitro*. These include parathyroid hormone (PTH), estrogen, glucocorticoids, and 1,25-dihydroxyvitamin D. There is growing evidence that the anabolic actions of PTH on bone are mediated largely through increased local IGF-I expression (13–15). PTH exerts its effect on IGF-I through increased cyclic AMP (cAMP) production and enhanced gene transcription (16–18). Estradiol also enhances IGF-I synthesis at the transcriptional level in rat bone cells transfected with estrogen receptors (19). However, no consensus estrogen responsive element has been identified within the cloned promoter regions of the *IGF-I* gene. Hence, estrogen likely acts through the cAMP-dependent C/EBP pathway either as an inhibitor in some cell lines and species or as a stimulator of IGF-I transcription in rat and human osteoblast.

There is also unique genetic programming of skeletal IGF expression. Rosen *et al.* demonstrated that for two healthy inbred strains of mice (C3H and C57BL6), of the same body length and size, serum, and skeletal IGF-I content differed by as much as 30% and these interstrain differences in IGF-I expression were also observed in calvarial osteoblasts maintained *in vitro* (20, 21). However, promoter usage differed such that hepatic P2 promoter expression was nearly 5-fold greater in C3H than B6 mice, whereas P1 promoter transcripts were not different by strain (Adamo, personal communication). However, P1 IGF-I expression in the femurs of C3H was significantly greater than B6 without differences in P2 transcripts. Hence, heritable regulators of IGF-I must be strain and tissue specific, although their identities are unknown.

Besides systemic regulators of IGF-I, local factors in combination with systemic hormones modulate the skeletal IGF regulatory circuit. Growth factors, such as FGF-2, and cytokines, such as the interleukins, regulate IGF-I expression in osteoblasts. BMP-2 increases IGF-I and II mRNA expression in rat osteoblasts and may be a critical factor in early osteoblast recruitment within the remodeling unit (13). BMP-7 also has a very potent effect on both IGF-I and II production in bone cells and antisense IGF-I and IGF-II oligonucleotides block BMP-7-induced alkaline phosphatase expression (14). TGF-B increases IGF-I and IGFBP-3 expression in human marrow stromal cells (22). IL-6 upregulates IGF-I expression mRNA in osteoblasts, while its effect on hepatic expression is the opposite (13). Prostaglandins regulate IGF-I and -II expression and are produced locally by bone cells, thereby providing a major paracrine regulatory circuit in the skeleton (15). Mechanical loading is also a stimulus for enhanced IGF-I expression in bone cells, possibly through the induction of PGI2 and PGE2. Strain induced production of PGI2 has been shown to immunolocalize to osteocytes, where IGF-II is released. PGE2, also generated by strain, tends to localize to osteoblasts and can induce the generation of either IGF-I or IGF-II (23). As such, it is clear that there is a complex regulatory circuit for IGF-I. Additionally similar redundancy and complexity exists for the IGFBPs, the type I IGF receptor, and the various proteases that cleave the IGFBPs from their ligand, thereby modulating IGF bioactivity (23).

In Vivo Studies with Genetically Altered Mice

Although *in vitro* studies provide investigators with major clues as to the role of IGF-I in cell signaling and cycling, the effects of IGF-I in the intact animal are also of prime importance for physiologists and bone biologists. *In vivo* studies using recombinant hIGF-I in animal models have demonstrated that this growth factor can enhance longitudinal growth, periosteal circumference, and bone mineral density. Locally synthesized IGF-I is also anabolic to bone. Using *in situ* hybridization, Shinar *et al.* found a close correlation between IGF-I expression and osteogenesis during rat development (24). Also, estrogen treatment of ovariectomized rats resulted in decreased calvarial IGF-I mRNA that preceded reduction in bone formation. Similarly, Watson *et al.* noted that IGF-I expression in osteoblasts by *in situ* hybridization was markedly enhanced by PTH admin-

istration (25). Lean *et al.* undertook a novel study of genes expressed in rat osteocytes after a single, acute episode of dynamic loading to reproduce physiological strains in bone, and found that IGF-I mRNA expression in osteocytes preceded increases in IGF-I expression and matrix formation in overlying surface osteoblasts (26). These types of studies are useful in providing a more global portrait of IGF-I actions in bone.

Genetic modifications in specific components of the IGF system have provided tremendous insights into the role of the IGFs and IGF-IR *in vivo* (Table I and Ref. 27). For example, mice lacking the *IGF-I* gene appear to develop normally but are much smaller, have lower cortical bone density, and frequently die in the postnatal period. The postnatal survivors exhibit an interesting phenomenon. Although cortical bone mass and femur length are reduced, and these animals do not respond to PTH administration in respect to its anabolic skeletal properties, trabecular bone density, and connectivity are actually much greater than wild types (28). Whether this represents compensation (i.e., an increase in GH secretion) for the absence of IGF-I or is caused by the lack of IGF-I in cancellous bone, thereby reducing osteoclast recruitment and bone resorption, still needs to be determined. Interestingly, IGF-I heterozygotes (IGF-I +/–) mice on a CD-1 background with serum IGF-I concentrations half that of wild types, also exhibit reduced cortical bone mineral density (BMD) as well as shorter femur lengths and reduced periosteal circumference (B. Kream, personal communication).

Ubiquitous overexpression of IGF-I in mice using a metallothionein promoter results in increased body weight and disproportionate overgrowth of some organs but normal skeletal size and morphology (29–32). However, overexpression of IGF-II does not cause major changes in skeletal growth and bone turnover in mice (33). Similarly, mice nullizygous for the IGF-I receptor demonstrate extreme organ hypoplasia, delayed skeletal calcification, severe growth retardation, and invariably die postnatally. Crossbreeding of the IGF-I (–/–) and IGF-IR (–/–) mice yields a phenotype indistinguishable to that observed in the IGF-IR null mice, suggesting that IGF-I interacts exclusively with the IGF-IR. By contrast, mice lacking the *IGF-II* gene show no delay in ossification and have normal sized skeletons (34).

Despite the success of these model systems, a daunting challenge has been to sort out the relative contribution of IGF-I and growth hormone to skeletal growth and acquisition. As we alluded to previously, IGF-I knockout mice have markedly enhanced GH secretion. Although this cannot overcome absence of the *IGF-I* gene, for example, in relation to the dynamics of the growth plate, it may be responsible for some unique phenotypic aspects of these mice (e.g., the trabecular skeletal changes). Another mouse model has been helpful in dissecting the effects of GH from IGF-I in bone. *Little* mice are spontaneous mutants with a single amino acid substitution in the growth hormone-releasing hormone receptor (35). This results in a very small animal (i.e., about 10 g) with normal reproductive capacity, normal pituitary function excepting an absence of GH secretion, significant obesity, and very low serum IGF-I concentrations (25 ng/ml compared with 250 ng/ml in the background strain). The skeleton of these animals includes a small growth plate and reduced cortical bone volume, consistent with reduced animal size. But surprisingly, trabecular bone volume is maintained in these mice, as is femoral strength and skeletal expression of IGF-I. Further support for these observations has been reported in humans carrying the same mutation as the *little* mouse. Adults with this disorder are short, but like Laron dwarfs with GH resistance syndrome, do not have reduced volumetric bone mass, or

Table I. Genetically Altered IGF Mouse Models and their Skeletal Phenotypes (Ref. 25 and Others)

Mouse	IGF alteration	Skeletal phenotype	Reference
IGF-I Tg	Global overexpression IGF-I	Normal size; BMD: ND; Inc growth; Inc tail length	25
IGF-II Tg	Global overexpression IGF-II	Normal size; BMD: ND	31
GH Tg	Overexpression of GH	Inc size, Inc BMD; Inc serum IGF-I	25
IGF-I –/–	Global knockout IGF-I	Short bones, low BMD; Virtual absence serum IGF-I	26
IGF-IR –/–	Global knockout of Type I	Lethal growth impairment, poor calcification	25
IRS –/–	Global knockout of IRS Signaling for IGF-I	Severely impaired bone formation small bones, osteopenia	35
IGF-I Tg Targeted	Overexpression IGF-I in bone with OC promoter	Inc BMD and bone formation bone resorption; no size change	40
IGF R –/– Targeted	Conditional IGF Receptor knockout in bone	Dec BMD, Dec bone formation no size change	41
Hepatic IGF-I –/–	Conditional KO of liver derived IGF-I	Dec serum IGF-I; sl decrease bone size; BMD slightly reduced	44
Hepatic IGF-I –/– + ALS –/–	Conditional KO of liver derived IGF-I and ALS	Marked growth retardation, very low serum IGF-I, reduced BMD	43

Note. There is an IGF-I mutant called the Midi-IGF-I mutant. Attempts to ablate *IGF-I* gene function by homologous recombination resulted in a disrupted *IGF-I* gene that retained some function and has some phenotypic alterations in the skeleton including low bone mass.
ND: not done; BMD: bone mineral density; Inc: increased; Dec: decreased; Sl: slight.

increased susceptibility to fractures (36). These findings suggest differential regulation of skeletal components such that the growth plate and the periosteum are influenced more profoundly by circulating IGF-I than is the trabecular skeleton (see Fig. 1). Moreover, these lines of evidence imply that inferences about skeletal integrity based solely on size can be very misleading.

Other components of the IGF system have also been investigated in genetically altered mice and shed some light on the role of this peptide in skeletal maintenance. For example, mice lacking IRS-1, a key downstream effector of the IGF-IR, have severe osteopenia with low bone turnover (37). Experiments using IRS-1 (–/–) osteoblasts demonstrated that IRS-1 deficiency impairs osteoblast proliferation, differentiation, and supports osteoclastogenesis, resulting in low-turnover osteopenia. Global IGFBP-3 overexpression using a phosphoglycerate kinase (PGK) or cytomegalovirus (CMV) promoter results in a reduction in bone volume, trabecular connectivity, and size, changes that are consistent with an antagonistic effect of IGFBP-3 on skeletal IGF-I bioactivity (38). Also, mice globally overexpressing IGFBP-4 have marked growth retardation and disproportionally small bones (39). Additionally, injection of a protease-resistant IGFBP-4 directly into the parietal bones of mice inhibits the anabolic actions of IGF-I (40). Paradoxically, however, IGFBP-4-null mice have reduced weight at birth (10–15%; Ref. 41). One potential explanation is that absence of IGFBP-4 diminishes tissue IGF storage capacity. This would suggest that physiological levels of IGFBP-4 are required for normal growth and that IGFs would be released through the action of IGFBP-4 proteases. These observations further confirm the thesis that IGFBPs can serve to sequester IGF-I and either inhibit or enhance its actions.

Although studies of these mouse models have provided additional insights into our understanding of the actions of the IGF system, proper interpretation of these findings can be challenging. First, the high lethality and severe organ defects in the IGF-I and IGF-IR knockout mice make it difficult to distinguish direct and indirect actions of IGF-I in bone and prohibit study of adult mice. Also, as alluded to above, systemic overexpression of IGF-I or II cannot unequivocally differentiate endocrine versus autocrine/paracrine actions in bone, nor does it necessarily give valid information on the changes in the availability of IGFs in bone local microenvironment. Third, compensatory pathways in knockouts can overcome the absent gene effect and may obscure phenotypic presentations. However, tissue-specific modifications of the IGF system components in mice should theoretically be more useful in that regard. Indeed, targeted overexpression of IGF-I to osteoblasts of mice using the osteocalcin (OC) promoter increases cancellous bone formation rate and volume without changes in osteoblast number, suggesting locally delivered IGF-I exerts its anabolic actions primarily by increasing the activity of resident osteoblasts (42). Surprisingly, cortical bone mass is barely affected by IGF-I overexpression with either the OC or Col1A1 promoters.

However, even models with targeted expression have confounding variables. For example, the skeletal phenotype might result from the overexpression of a transgene and not necessarily reflect the action of IGF-I at its physiological levels. Also, with both the Col1A1 and the osteocalcin promoter, targeted overexpression of IGF-I in mature osteo-

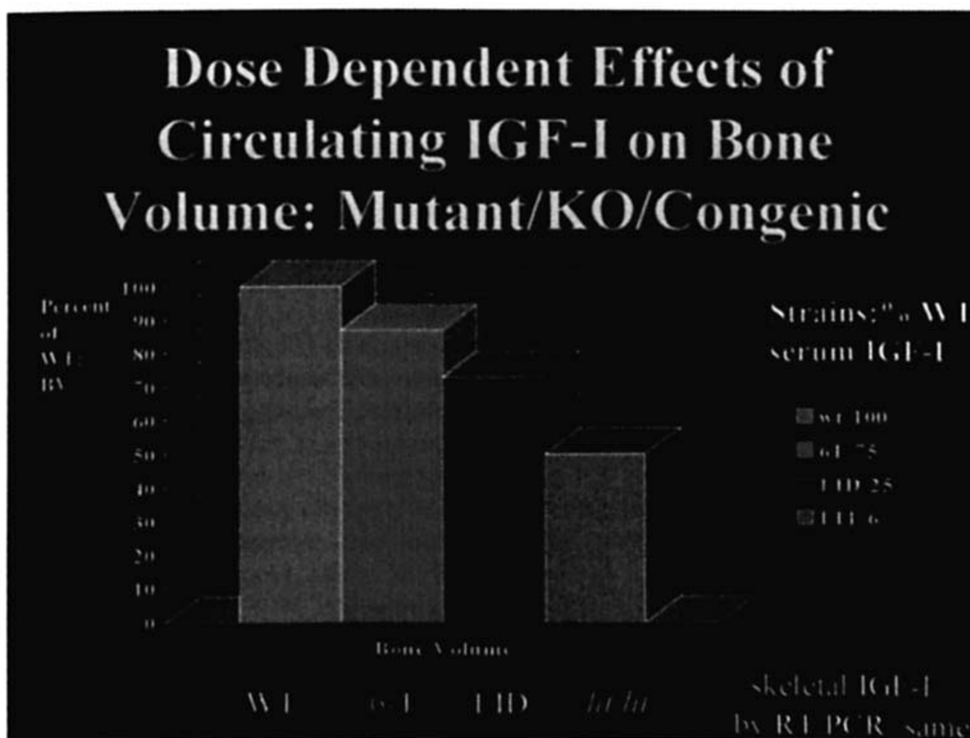

Figure 1. Bone volume by uCT in four different mutant/knockout/congenic strains of mice with variations in serum IGF-1, 6T represents the congenic mouse (6T) constructed for a QTL, which negatively affects circulating IGF-1 (49); LID represents mice with targeted deletion of liver IGF-1 (43); *lit* represents a natural mutation of the growth-releasing hormone receptor, resulting in the absence of GH secretion (33). There is a clear relationship between circulating IGF-1 concentrations (Right, y axis) and bone volume as percent of WT B6 mice.

blasts results in significant osteoclastogenesis and, eventually, enhanced bone resorption. Whether this represents stromal cell enhancement of RANKL as a result of high IGF-I concentrations within the skeleton or changes in IGFBPs as a result of compensation remain to be determined. Finally, the onset and duration of the promoter necessary to drive overexpression in bone may differ considerably, thereby resulting in variable phenotypes. In this regard, the feasibility of conditional mutagenesis of the *Igf1r* gene in osteoblasts using Cre-mediated recombination recently has been demonstrated and theoretically should provide an even more powerful approach to further define the actions of IGF-I in bone (43). Using the osteocalcin promoter and Cre recombinase to selectively delete the IGF type I receptor from osteoblasts, the resulting mice were found to have a significant defect in bone formation with reduced osteoblastogenesis. Even more revealing, however, was the dramatic reduction in bone mineralization that occurred by 3 weeks of age in these animals. As such, these mice provide the first *in vivo* evidence that IGF-I and its receptor are important in the process of biomineralization. More importantly, these mice demonstrate proof of concept in respect to conditional mutagenesis. Newer studies are just now beginning to examine temporal expression patterns using specific inducible systems.

Notwithstanding the remarkable progress in studying skeletal IGF-I actions the relative importance of circulating versus skeletally produced IGF-I in the process of bone acquisition remains an enigma. Cross-sectional and cohort studies in various human populations have implied there is a strong correlation between serum levels of IGF-I and femoral or lumbar BMD (44). Within inbred strains of mice, a similar correlation between cortical BMD and serum IGF-I exists, and is by no means trivial (20, 21). However, these studies provide only indirect evidence for a relationship between circulatory IGF-I and bone acquisition. Fortunately, recent work by several groups using genetically modified mice, have provided new and valuable insights. Yakar *et al.* were the first to examine the effects of knocking out hepatic IGF-I expression and/ or the acid-labile subunit (ALS) protein that binds IGF-I in the circulation to IGFBP-3 (45, 46). When hepatic IGF-I expression alone is deleted (i.e., liver IGF-I- deficient LID mice), serum IGF-I declines by 75%, femur length and weight are reduced by about 6%, whereas overall growth velocity is maintained (46). Despite modest changes in femoral length, cortical bone volume in LID mice is reduced 26%. This is also associated with a marked reduction in periosteal circumference and cross-sectional area (Fig. 1). Similarly, mice with selective knockout of the ALS and reduction in serum IGF-I to the same degree as LIDs exhibit a nearly identical diminution in cortical bone volume as the LIDs. However, the double knockout mice (hepatic IGF-I and ALS) had even lower concentrations of serum IGF-I (i.e., 90% reduction), significant growth retardation, and low cortical bone mineral density (45). Unlike the LIDs and ALS knockout mice, trabecular bone density was also significantly compromised in the double knockouts. Moreover, the growth plates of these mice were significantly disordered. Interestingly, the level of free IGF-I was dramatically increased in the knockouts, although the circulating half-life of IGF-I was significantly shortened and growth hormone secretion increased several fold. Still, these data suggest there may be a major role for serum IGF-I in determining bone size and mass. This conclusion is supported by a comparison of cortical bone volume across various mouse models in relation to changes in circulating IGF-I (Fig. 1).

A similar conclusion about the somatomedin hypothesis was derived through a different approach but with genomic engineering of several mouse strains. Lupu *et al.* performed a very detailed analysis of skeletal phenotypes in three artificially derived dwarf strains, the IGF-I knockout, the GH receptor knockout, and combined GHR/IGF-I-null mice (47). The authors established that chondrocyte growth and development were related to GH- and IGF-I-independent actions. When both GH and IGF-I are deleted, the profound skeletal phenotype for the double knockout mouse is one of extremely reduced size (i.e., 5 g) and virtually no detectable serum IGF-I (47). Moreover, Lupu *et al.* showed that virtually all of the circulating IGF-I in mice was related to hepatic transcripts from GH induction. By contrast many other tissues exhibited GH independent expression of IGF-I transcripts in the double knockouts. The major conclusion from this comprehensive analysis was that the IGF system is the major determinant of both embryonic and postnatal growth and that the postnatal expression of IGF-I is a function of GH secretion. In other words there is a convergence of growth signaling pathways by GH and IGF-I effectors, but each has some degree of independence from the other. More importantly, based on these two studies, it is likely that the endocrine role of IGF-I in skeletal development may have been significantly underestimated.

More experimentation is required, and many questions remain, particularly in relation to skeletal IGF-I expression and regulation. An alternative strategy for defining the interaction between IGF-I and bone acquisition involves generation of new mouse strains that are not knockouts or transgenics, but rather have allelic differences at key genomic points. The generation of congenic mice carrying small regions of specific chromosomes (also called quantitative trait loci) has advanced our understanding of the genetic regulation of many phenotypes. The congenic strategy involves the donation of a quantitative trait locus from one inbred strain to another using repetitive backcrossing to one parental strain (48). After 10 generations, congenic mice carry the chromosomal region of interest on the homozygous background of the recipient strain. From there, investigators can define the full genetic effect of a particular quantitative trait locus, and determine whether there are gene × gene interactions.

Two inbred strains, C3H/HeJ and C57/B6, are particularly useful models for creating congenic mice to study the

interaction of bone mass and serum IGF-I (49, 50). These two strains have differences in peak bone acquisition that correlate very closely with differences in serum IGF-I (49, 50). Bouxsein *et al.* produced a congenic mouse by transferring a section of chromosome 6 from C3 H mice (an inbred strain with high BMD and high serum IGF-I) to C57B6 (a strain with low BMD and low IGF-I) over 10 generations (51). This 20-centimorgan segment contained a major QTL from C3H that regulated serum IGF-I in a negative direction (i.e., lowering IGF-I circulating concentrations in the high IGF-I strain), and accounted for nearly 15% of the variability in that phenotype. After 10 generations of mice, both male and females carrying the Chr 6 QTL (c3/c3;named 6-T) but homozygous throughout the rest of the genome for B6 (b6/b6) exhibited 25% lower circulating IGF-I levels, 13% less total bone volume, and a nearly 50% reduction in trabecular bone density (see Fig. 1; Ref. 51). More intriguing, however, is the finding that IGF-I expression in the femur and neonatal calvariae of the congenic did not differ from the background strain. These congenics are proof that a QTL found through whole genome scanning of F2 mice, when transferred to a different background, strongly influences circulating IGF-I concentrations, volumetric bone mass, and bone strength. These data would imply that the genetic determinants of serum IGF-I could have a major impact on bone mass, even when changes in skeletal IGF-I expression are not present.

Conclusions

In the beginning (i.e., in the late 1950s), IGF-I was considered an endocrine effector, mediating growth hormone actions at sites remote from the liver. Later, we learned that circulating IGF-I concentrations differed considerably from cellular expression and secretion. Moreover, we found tissue-specific regulation of IGF-I that determined cellular pathways of proliferation, differentiation and death. Along the way, it became apparent that the somatomedin hypothesis was inadequate to explain the role of IGF-I in both normal and pathologic states. However, in the last half decade that tenet has shifted once again. Seminal epidemiologic studies by Pollack and others have noted a strong although not causal relationship between high normal serum levels of IGF-I and the development of breast, prostate and colon carcinoma (3, 52, 53). Genetically engineered mouse models have helped partition the role of circulating versus local IGF-I in tumorigenesis and bone acquisition, allowing us to redefine the somatomedin hypothesis once again. This ubiquitously expressed peptide continues to challenge our intellectual resources. The future of somatomedin research promises to be both exciting and groundbreaking.

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