Insulin-like growth factor I and bone mineral density: experience from animal models and human observational studies

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The insulin-like growth factor (IGF) system has been linked to the process of bone acquisition through epidemiological analyses of large cohorts and in vitro studies of bone cells. However, the precise relationship between the expression of IGF-I in bone and skeletal homeostasis or pathological conditions such as osteoporosis, remains poorly defined. Recent advances in genomic engineering have resulted in the development of better in vivo models for testing the role of IGF-I during development and the maintenance of the adult skeleton. Evidence from conditional mutagenesis studies of IGF-I has opened up a new area of in vivo analysis. These studies suggest that serum IGF-I levels may represent more than a storage depot or permissive factor during skeletal acquisition and that tissue IGF-I is essential for normal bone formation. The implications from these animal models 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.

Key words: IGF-I; bone mineral density; bone remodelling; genomic models.

Nearly half a century ago Salmon and Daughaday1 made the seminal observation that a circulating factor mediated the biological activities of growth hormone (GH). Subsequently, tremendous progress has been made in understanding the role of insulin-like growth factors (IGF-I), their binding proteins and their receptors, in various physiological and pathological states.2 Paradoxically, the greatest advances have come in defining the paracrine and autocrine activities of IGF-I in normal and neoplastic states, especially in relation to programmed cell death. In part, such progress has been a function of better models, more sensitive read outs and greater technological advances including gene chip micro arrays. With the advent of such tools, the role of IGF-I in cell signalling has become established for virtually all tissues. Still to be defined, however, are the precise physiological control mechanisms that define its expression and, importantly, how circulating and skeletal IGF-I is involved in bone remodelling.
In contrast to in vitro work, progress from in vivo modelling has been frustratingly slow. 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 might be protective against osteoporosis yet was a major risk factor for the development of breast, prostate and colon cancer. The second development was technological and related to the use of genetic engineering in mice to define IGF-I activity. These studies have opened new vistas for exploring functional changes in the IGF pathways for many tissues. The skeleton has been no exception and, in fact, has benefitted greatly from the use of mouse models that are genomically altered. The mechanisms surrounding the relationship between circulating levels of IGF-I and bone mineral density (BMD) and/or fracture could not previously be probed in humans and early in vivo models lacked sufficient mechanistic insights, were not comparable to the human skeleton and did not consider developmental aspects. Currently, investigators have mouse models that over- and under-express IGF-I, the Type I IGF receptor, five out of the six IGF binding proteins (IGFBPs), the GH receptor and at least one IGFBP specific protease. In addition, by utilising Cre recombinase and bone specific promoters such as osteocalcin and collagen I A1, targeted deletion or over-expression of IGF components in the skeleton and liver have been accomplished. Temporal studies are currently underway to pinpoint when and where skeletal IGF-I has its greatest impact. The resulting phenotypes from these models are not only interesting, but provocative, in part because they challenge many of the current paradigms about skeletal growth and IGF-I.

Yet despite such progress, we 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. In this chapter, experience from cohort studies and animal models will be used to explore the role of both circulating and skeletal IGF-I in skeletal growth and maintenance.

THE PHYSIOLOGY OF IGF-I IN THE SKELETON

The skeleton is a highly organised and physiologically active organ, continuously remodelling 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 while cortical bone is nourished by periosteal vessels and a series of canaliculi connecting osteocytes to lining cells and osteoblasts (OB). Gravitary forces help model the cortical skeleton although periosteal OBs and the underlying growth plate are principally responsible for longitudinal growth. Both cortical and trabecular bone undergo remodelling, 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 the coupling of bone dissolution (i.e. resorption) to new bone formation, orchestrate bone remodelling within the skeletal compartments. Pre-osteoblasts (pre-OBs), derived from mesenchymal stromal cells and under the influence of a key transcription factor (i.e. core binding factor I (Cbfα1) or RUNX2) represent target cells for the initiation of the remodelling cycle. Systemic and local factors enhance pre-OB differentiation, and this, in turn, leads to the synthesis and release of macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL). These two
peptides are necessary and sufficient for the recruitment of bone resorbing cells, i.e. the osteoclasts (OC). 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 OBs to the bone surface, thereby setting the stage for collagen synthesis and matrix deposition/mineralisation. The entire remodelling cycle in humans takes approximately 90 days, with the majority of time being consumed by the elaborate process of bone formation and subsequent mineralisation.

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. Similarly, the skeletal matrix is also highly enriched with these growth factors and other non-collagenous proteins including all six IGFBPs and several IGFBP proteases. In addition, the Type I IGF receptor is present on both OBs and OCs. It is now reasonably certain that skeletal IGFs originate from two sources: (i) de novo synthesis by bone forming cells (i.e. pre-OBs and fully differentiated OBs) and (ii) the circulation and/or marrow. In fact some skeletal IGFs probably make their way into the matrix by way of specialised canaliculi and sinusoids within the bone microcirculation. 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-2 and IGFBP-5). Subsequently, both IGFs recruit precursor OBs and, possibly, early OCs to the bone surface where remodelling is occurring.

The IGFs act in diverse patterns via endocrine/autocrine/paracrine pathways to regulate proliferative and differentiative functions of both OBs and OCs. Since 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 OBs than others. The orchestration of bone remodelling probably requires the activity of both OB-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 growth factor for OBs.

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-OB proliferation is still not totally clear. Recent evidence from studies of human marrow cells suggest that IGF-I can significantly enhance OB precursor proliferation. Similarly, some studies have suggested IGF-I is critical for OB differentiation while others have demonstrated, through chip array studies, down-regulation of IGF-I expression during the final terminal differentiation of OBs (Rowe, pers. comm.). Even less clear is the effect of IGF-I on OC differentiation. Several studies have suggested that under physiological conditions, IGF-I can enhance OC 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 OCs. Rubin et al recently demonstrated that stromal cell production of RANKL is markedly enhanced by IGF-I in a dose- and time-dependent manner. This would be consistent with its role as a coupling factor in bone remodelling, activating bone resorption through the pre-osteoblastic lineage. Despite some uncertainty, most lines of evidence point to an important modulatory function in the bone remodelling cycle for IGF-I produced by OBs.
REGULATION OF IGF-I IN BONE CELLS

One clue to understanding the effects of circulatory versus skeletal IGF-I on bone remodelling can be found in IGF-I gene regulation. OB-like cells from rodents and humans, 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-6 (IL-6), TGF-B, fibroblast growth factor (FGF)). 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), oestrogen, 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. PTH exerts its effect on IGF-I through increased cyclic AMP (cAMP) production and enhanced gene transcription, although the transcriptional effects may vary according to species, or within mouse strains. Oestradiol also enhances IGF-I synthesis at the transcriptional level in rat bone cells transfected with oestrogen receptors. However, no consensus oestrogen responsive element has been identified within the cloned promoter regions of the IGF-I gene. Hence, oestrogen probably acts through the c-AMP 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 OB.

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 (B6)), of the same body length and size, serum and skeletal IGF-I content differed by as much as 30% and these inter-strain differences in IGF-I expression were also observed in calvarial OBs maintained in vitro. However, promoter usage differed, such that hepatic P2 promoter expression was nearly five-fold greater in C3H than B6 mice, while P1 promoter transcripts were not different by strain. Furthermore, P1 IGF-I expression in the femurs of C3H was significantly greater than that for B6 without any differences in P2 transcripts. Hence, heritable regulators of IGF-I must be strain- and tissue-specific, although their identities are unknown.

As well as 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 OBs. BMP-2 increases IGF-I and II mRNA expression in rat OBs and may be a critical factor in early OB recruitment within the remodelling unit. BMP-7 also has a very potent effect on both IGF-I and -II production in bone cells and anti-sense IGF-I and IGF-II oligonucleotides block BMP-7 induced alkaline phosphatase expression. TGF-B increases IGF-I and IGFBP-3 expression in human marrow stromal cells. IL-6 up-regulates IGF-I expression mRNA in OBs, while its effect on hepatic expression is the opposite. Prostaglandins regulate IGF-I and -II expression and are produced locally by bone cells, thereby providing a major paracrine regulatory circuit in the skeleton. 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 immunolocalise to osteocytes, where IGF-II is released. PGE2, also generated by strain, tends to localise to OBs and can induce the generation of either IGF-I or IGF-II. Finally, in prostate cancer cells, IGF-I up-regulates its own expression. As such, it is clear that there is a complex regulatory circuit for IGF-I. In addition, 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.
IGF-I peaks during puberty at or about the same time as acquisition of peak bone mass. In addition, serum IGF-I levels decline with ageing along a slope that is similar to age-related bone loss.\textsuperscript{25,26} Hence, it is not surprising that the role of circulating IGF-I in bone cell metabolism and bone turnover has been the subject of significant scrutiny. Intuitively, alterations in circulating IGF-I could play a role in modulating bone remodelling and thereby affect bone mass and fracture risk. For example, growth hormone deficient (GHD) individuals with low serum IGF-I and low serum IGFBP-3 have reduced BMD and a significantly greater risk of osteoporotic fractures.\textsuperscript{27} In adults with acquired GHD, serum IGF-I levels (as well as IGFBP-3) correlate closely with femoral and spine BMD.\textsuperscript{28} However, attempts to correlate BMD with serum IGF-I in older individuals have produced conflicting results, thereby making a true ‘cause and effect’ relationship more difficult to prove. In part this may relate to tissue-specific expression of IGF-I and its regulation in the liver (which contributes the majority of circulating IGF-I) and other tissues. But, it also speaks to the issue of the multi-layered regulation of serum IGF-I, from GH to nutritional status to physical activity and insulin production.

Two studies in larger cohorts of men and women have suggested a more powerful relationship between IGF-I and bone. Langlois and colleagues measured serum IGF-I and bone density in 425 women and 257 men (aged 72–94 years) from the Framingham Heart Study.\textsuperscript{29} These investigators corrected for several confounding variables including weight, height, protein intake, smoking, mobility, weight change and body mass index (BMI). Serum IGF-I was positively associated with BMD at all sites of the hip, radius and lumbar spine in women after adjustment for all those factors. A threshold effect of higher BMD was evident at each of three femoral sites and the spine for women in the highest quintile of serum IGF-I (i.e. serum levels > 180 ng/ml) versus those in the lower four quintiles. These data are somewhat more powerful and suggest that IGF-I levels are associated with greater BMD in older women.

Recently, Bauer et al\textsuperscript{30} reported on the relationship of IGF-I and IGFBP-3 to hip fractures in 9704 women from the Study of Osteoporotic Fractures (SOF). In this prospective study, sera were measured for IGF components in 148 women who subsequently sustained hip fractures after 4.0 years of follow up and in 349 women randomly selected from the cohort. Women in the lowest quartile of IGF-I (< 80 ng/ml) had a 60% greater risk of hip fracture and incident vertebral fracture than did other women. Moreover, adjustment for calcaneal BMD did not change those associations and IGFBP-3 was not associated with a greater risk of fractures. This was the first prospective study identifying IGF-I as a potential risk factor for fracture in older individuals. Subsequently, Garnero et al\textsuperscript{31} reported a nearly identical finding of higher risk among older postmenopausal women in the lowest quartile of serum IGF-I from a French cohort. But these findings are not totally surprising since IGF-I levels fall with protein calorie under-nutrition and catabolic states and older women with recent or past weight loss are at higher risk for hip fractures.\textsuperscript{32} Hence, it is unclear if this risk factor has pathogenic implications. Indeed, low IGF-I levels can be induced in mice or humans simply by reducing protein intake. Whether this is sufficient to cause suppression in bone turnover and bone loss remains to be defined.

Studies in another series of individuals who have osteoporosis at a young age also point toward a pathogenic role for IGF-I in the development of low bone mass. Idiopathic osteoporosis in men (IOM) is a condition that is characterised by low serum
IGF-I levels, a family history of osteoporosis, low bone turnover with decreased bone formation, hypercalcemia in some patients and very low bone mass with fractures before the age of 60. In a cohort of subjects with IOM, Kurland et al noted that serum IGF-I levels correlate with lumbar BMD and that men with this condition have levels of circulating IGF-I almost one standard deviation below age-matched controls despite normal GH dynamics. Moreover, it was noted that those same individuals with IOM have a higher frequency of a recessive polymorphism in the IGF-I gene which, independent of GH, accounts for a nearly 20% reduction in serum IGF-I levels compared with men or women without that specific genotype. More recently, Utterlinden et al noted, in a much larger cohort, that low serum IGF-I level was associated with the absence of a 19 base-pair repeat in the IGF-I gene and these women and men had lower femoral BMD and reduced bone geometric properties. These data suggest that serum IGF-I levels may reflect skeletal activity, especially in respect to skeletal geometry and density. Moreover, this regulation may be completely independent of GH.

IN VIVO STUDIES WITH GENETICALLY ALTERED MICE

Although cohort studies provide investigators with major clues as to the role of IGF-I in bone, while in vitro studies provide a mechanistic pathway, the effects of IGF-I in the intact animal are also of prime importance for endocrinologists and clinicians. In vivo studies using recombinant human IGF-I (hIGF-I) in animal models have demonstrated conflicting results. However, genetic modifications in specific components of the IGF system have provided investigators with the sharpest insights into the role of the IGFs and IGF-I receptor (IGF-IR) in vivo (Table 1). 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 with respect to its anabolic skeletal properties, trabecular bone density and connectivity are actually much greater than in the wild-types. Whether this represents compensation (i.e. an increase in GH secretion) for the absence of IGF-I, or is due to the lack of IGF-I in cancellous bone, thereby reducing OC 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 that are half that of the wild-types, also exhibit reduced cortical BMD as well as shorter femur lengths and reduced periosteal circumference (B. Kream, pers. comm.).

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. However, overexpression of IGF-II does not cause major changes in skeletal growth and bone turnover in mice. Similarly, mice nullizygous for the IGF-IR demonstrate extreme organ hypoplasia, delayed skeletal calcification, severe growth retardation and invariably die postnatally. Cross-breeding of the IGF-I (−/−) and IGF-IR (−/−) mice yields a phenotype that is indistinguishable from 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.

Despite the success of these model systems, a daunting challenge has been to sort out the relative contribution of IGF-I and GH to skeletal growth and acquisition.
As alluded to previously, IGF-I knockout mice have markedly enhanced GH secretion. Although this cannot overcome the 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 GH releasing hormone receptor.49 This results in a very small animal (i.e. about 10 g) with normal reproductive capacity, normal pituitary function except for an absence of GH secretion, significant obesity and very low serum IGF-I concentrations (25 ng/ml compared to 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, nor increased susceptibility to fractures.50 These findings suggest a 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. Moreover, these lines of evidence imply that inferences about skeletal integrity based solely on size can be very misleading.

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Source: reference 25 and others. There is an IGF-I mutant called the Midi-IGF-I mutant. Attempts to ablate the 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. IGF, insulin-like growth factor; Tg, IGF-IR, IGF-I receptor; IRS, insulin receptor; substrate; GH, growth hormone; KO, knockout; ND, not done; BMD, bone mineral density; Inc, increased.
Other components of the IGF system have also been investigated in genetically altered mice and they shed some light on the role of this peptide in skeletal maintenance. For example, mice lacking insulin receptor substrate-1 (IRS-1), a key downstream effector of the IGF-IR, had severe osteopaenia with low bone turnover.\(^{51}\) Experiments using IRS-1 (−/−) OBs demonstrated that IRS-1 deficiency impairs OB proliferation, differentiation and supports osteoclastogenesis, resulting in low-turnover osteopaenia. Global IGFBP-3 over-expression utilising a PGK or 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.\(^{52}\) Also, mice globally over-expressing IGFBP-4 have marked growth retardation and disproportionately small bones.\(^{53}\) Interestingly, overexpression of IGFBP-4 in bone alone using an osteocalcin promoter results in reduced BMD, along with blunted growth. Furthermore, injection of a protease resistant IGFBP-4 directly into the parietal bones of mice inhibited the anabolic actions of IGF-I.\(^{54}\) Paradoxically, however, IGFBP-4 null mice have reduced weight at birth (10–15%).\(^{55}\) One potential explanation is that the 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 hypothesis that IGFBPs can serve to sequester IGF-I and either inhibit or enhance its actions.

While 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. Firstly, 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 the study of adult mice. Secondly, 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 the bone local microenvironment. Thirdly, compensatory pathways in knockout models 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 OBs of mice using the OC promoter increases cancellous bone formation rate and volume without changes in OB number, suggesting that locally delivered IGF-I exerts its anabolic actions primarily by increasing the activity of resident OBs.\(^{56}\) Surprisingly, cortical bone mass is barely affected by IGF-I overexpression with either the OC or Col1A1 promoters.

But, 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 OBs 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 action 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 OBs using Cre-mediated recombination has recently been demonstrated and theoretically should provide an even more powerful approach for further defining the actions of IGF-I in bone.\(^{57}\) Using the osteocalcin promoter and Cre-recombinase to selectively delete the IGF Type I receptor from OBs, 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 mineralisation 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 bio-mineralisation. More importantly, these mice demonstrate proof of the concept with 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 that there is a strong correlation between serum levels of IGF-I and femoral or lumbar BMD. Within inbred strains of mice, a similar correlation between cortical BMD and serum IGF-I exists and it is by no means trivial. 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 protein (ALS) that binds IGF-I in the circulation to IGFBP-3. 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%, while overall growth velocity is maintained. Despite modest changes in femoral length, cortical bone volume in LID mice is reduced by 26%. This is also associated with a marked reduction in periosteal circumference and cross-sectional area. Similarly, mice with selective knockout of the ALS and a reduction in serum IGF-I to the same degree as LID mice, 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 BMD. Unlike the LID 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 GH secretion increased several fold. Still, these data suggest that 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.

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 (GHR) knockout and the combined GHR/IGF-I null mice. These authors established that chondrocyte growth and development was related to the independent actions of GH and IGF-I. 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. 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 signalling pathways by GH and IGF-I effectors, but each has some degree of
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 the 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 (QTL) from one inbred strain to another using repetitive backcrossing to one parental strain. 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 QTL 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 level. These two strains have differences in peak bone acquisition that correlate very closely with differences in serum IGF-I levels. Bouxsein et al produced a congenic mouse by transferring a section of chromosome 6 (Chr 6) from C3H 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. 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 males 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. 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.

CONCLUSION

IGF-I is an important local and systemic factor for the growth and development of the skeleton. Epidemiological studies have suggested a causal relationship between serum levels of IGF-I and fracture risk and/or bone density. In vivo animal models have demonstrated that circulating IGF-I is necessary for bone modelling, that skeletal expression of IGF-I is required for optimal trabecular bone mass and that both circulating and skeletal IGF-I play a role in mineralisation. Defining the developmental aspects of IGF-I physiology in bone will require more temporal studies using tissue specific genomic manipulation. As such, the future holds great promise for better defining the importance of IGF-I in the growing and adult skeleton.
References


