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# The role of IGF system in the rise and fall in bone density with age

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To evaluate the potential role of the IGF system in the development of peak bone density during growth and in the fall of bone density during aging, we have conducted during the past decade clinical and basic studies, particularly mouse studies involving targeted disruption of genes relevant to growth hormone (GH)/IGF axis. Although the IGF system is composed of two ligands, IGF-I and -II, two receptors, type I and type II IGF receptor, 6 binding proteins and several activators and inhibitors, our discussion will largely focus on the IGF-I ligand which acts predominantly via the type I IGF receptor and the binding protein, IGFBP-5.

In a clinical study of the skeletal changes during puberty, we found that there was a strong correlation between serum IGF-I and DEXA bone density in 65 females ranging in age from 9.4 to 14.4 years ($r$=0.55, $p$<0.001). Serum IGF-I was also correlated with metacarpal thickness in Tanner stage II girls ($r$=0.81, $p$<0 .001). Additionally, serum IGF-I showed a significant positive correlation with serum osteocalcin during puberty ($r$=0.76, $p$<0.001). To establish the cause and effect relationship between the observed changes in BMD and serum IGF-I in humans, we evaluated femur BMD in IGF-I knockout mice compared to controls. Between 23 and 31 days of age, which represents the period of puberty in the mouse, there was a 40% increase in BMD in the control mice but no significant increase was seen in the IGF-I knockout mice, establishing the importance of IGF-I in bone mass accretion during puberty. pQCT values were also decreased in the IGF-1 knockout mice. Consistent with these data, clinical studies in humans have established that loss of IGF-I gene function results in severe osteopenia (a BMD 5 SDs below age-matched controls). These data establish that IGF-I plays a key role in the regulation of peak BMD in both humans and experimental animals.

In order to evaluate the role of GH in regulating IGF-I actions in bone, we compared the skeletal phenotypes of mice lacking GH (lit/lit mouse with a mutation in GH releasing hormone receptor) and IGF-I (targeted IGF-I gene disruption). We studied femur BMD as a function of age from puberty up to 60 days of age in lit/lit and IGF-I knockout mice. Interestingly, the bone density was only slightly less than controls at the beginning of puberty in the GH-deficient mice, suggesting that the role of IGF-I in pre-pubertal rise in bone density is only modestly dependent upon GH. In contrast, during and after puberty both lit/lit and IGF-I knockout mice exhibit much lower BMD than the controls, emphasizing the importance of GH-mediated IGF-I actions during and after puberty.

GH regulates serum IGFBP-5 as well as serum IGF-I and therefore it was not surprising to find a strong positive correlation between metacarpal width and serum IGFBP-5 as well as with serum IGF-I. *In vitro* studies indicate that IGFBP-5 facilitates the proliferative actions of IGF-I in bone cells. In addition to modulating IGF actions, IGFBP-5 also exerts its effects in an IGF-I independent manner as evident from our studies that local administration of IGFBP-5 caused a 100% increase in calvarial bone formation in IGF-I knockout mice. Thus, IGFBP-5 itself is a growth factor that can exert anabolic effects on bone independent of its ligand.

The foregoing observations support an important role for the IGF system components in the development of increased bone density occurring prior to and beyond puberty. We next sought to evaluate the potential role of the IGF system components in age related bone loss. In adults, bone loss is thought to involve poor coupling of bone formation to resorption. In our model of the coupling of formation to resorption

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the sources of growth factor for the formation phase include: 1) Bone from which stored growth factors are released during bone resorption; 2) Osteoclasts, which produces growth factors; 3) Osteoblasts, which are known to secrete a variety of autocrine growth regulators; 4) Osteocytes, which are known to produce IGF-1; and 5) Serum, which contains bone growth factors. With respect to the bone as a source of IGF-I for coupling, we found in a clinical study that the concentration of IGF-I in normal hip bone between 20 and 64 years showed a large fall from 100 to 50 ng/mg protein ($p<0.001$). This was not surprising because GH secretion also declines with age and it is known that GH not only regulates the liver production of IGF-I (which in turn is the major contributor of circulating serum IGF-I) but also osteoblast production of IGF-I. Thus, the decline in local source of IGF-I could contribute to uncoupling of bone formation to resorption.

Another potential growth factor source for bone and other organs participating in mineral metabolism is serum IGF system components. Our study in elderly female hip fracture patients and controls (50 per group) revealed highly significant decreases (31 to 44%) in serum IGF-I, IGFBP-3, and IGFBP-5 (all $p<0.001$). This was attended by a 52% increase in urine pyridinoline and a 21% decrease in serum N-procollagen peptide (both $p<0.001$). This apparent uncoupling of bone formation to resorption is consistent with the observed 30% decrease in femoral neck BMD in the hip fracture patients ($p<0.001$). The possibility that the decrease in serum IGF system components contributed to the low bone formation is consistent with past *in vitro* and *in vivo* findings. The potential mechanism by which IGF system deficiency could cause the increase in bone resorption is explored below.

With respect to the cause of the uncoupling of bone formation to resorption in our fracture patients, work by others has shown that senile osteoporosis is characterized by secondary hyperparathyroidism. This was the case in our study. The serum PTH was increased by 232% ($p<0.001$). Clinical studies by other workers have attributed the bone loss in senile osteoporosis largely to secondary hyperparathyroidism. Consequently, from a mechanistic standpoint, the cause of the secondary hyperparathyroidism is a pivotal issue. Calcium intake was probably low in both groups but detailed diet studies were not done. Serum 25OHD was decreased 57% ($p<0.001$) in the hip fracture group and therefore could have contributed to the PTH differential. However, according to our analyses serum 25OHD accounted for only about 15% of the variation in serum PTH. We, therefore, predicted that the observed IGF-I deficiency could contribute to the high serum PTH based on the findings that GH-deficient adults have a high serum PTH which is corrected by GH treatment.

To further evaluate this hypothesis, we examined serum $1,25(OH)_2D_3$ levels in IGF-I knockout mice and found a highly significant decrease in serum $1,25(OH)_2D_3$. Additionally, vitamin D receptor (VDR) expression in the kidney was reproducibly decreased by approximately 70% in the IGF-I knockout mice. When the IGF-I knockout mice were placed on a low calcium diet, there was a decrease in bone formation and an increase in bone resorption compared to the wild-type mice; these changes are similar to the changes seen in our hip fracture patients who also had a relatively low calcium intake. In confirmation of our postulated role of GH/IGF-I in serum PTH regulation, we found that GH-deficient mice exhibited a high serum PTH which was decreased with GH therapy. The foregoing suggests that IGF-1 deficiency causes a metabolic vitamin D deficient status, one consequence of which is secondary hyperparathyroidism.

The serum and bone changes relevant to bone and calcium metabolism in IGF-I knockout mice and those reported by us and others in senile osteoporosis were strikingly similar. In both, there was a deficiency of IGF-I. In both, there was a decrease in serum $1,25(OH)_2D_3$ (relative to serum PTH) and some parameters of $1,25(OH)_2D_3$ action. In both, the serum PTH was elevated. Hip fracture patients and calcium deficient IGF-1 KO mice both had an increase in bone resorption and a decrease in bone formation, which contributed to a decrease in BMD.

It seems probable that senile osteoporosis is a multi-factorial disease and that a deficiency of IGF system components is only one of several contributing factors. Nonetheless, our work suggests that it is an important contributing factor. As such, the conundrum raised by the foregoing is the cause of the deficiency of serum IGF components. In this regard, even though the IGF levels were clearly different between the two groups, the height was not decreased in the hip fracture group suggesting that GH secretion was not markedly different during bone growth. Other workers have implicated protein deficiency as a cause of the decrease in serum IGF-I that attends aging. This is an important observation, but it seems likely that there will be other important regulatory factors as well.

One reservation in the hip fracture patients about ascribing any bone changes to a deficiency in serum IGF-I alone is that in mice with a conditional knockout of liver IGF-I production which results in 75% reduction in circulating IGF-I did not have a significant bone phenotype. Moreover, we recently found that conditional disruption of the IGF-I gene specifically in osteoblasts using a Cre/LoxP approach resulted in a significantly decreased femur BMD. Our working hypothesis is that, because both paracrine and endocrine IGF-1 production are regulated by GH, a serum IGF-1 deficiency reflects a paracrine as well as an endocrine deficiency.

Our knockout studies in mice and clinical studies strongly support a role for the IGF system components in the development of peak bone density and in the bone loss that occurs in senile osteoporosis. Because our hypotheses involved the extrapolation of data from knockout mice to humans, it is obviously necessary to test our hypotheses directly in clinical studies, which should be feasible. The regulation of the IGF components system is a fundamentally important health issue in that basic studies suggest that a reduced IGF action increases longevity and decreases cancer; whereas, clinical bone studies suggest that such a deficiency could seriously impair the quality of life and perhaps even enhance mortality.