Insulin-like growth factor-binding proteins and bone metabolism

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Conover CA. Insulin-like growth factor-binding proteins and bone metabolism. Am J Physiol Endocrinol Metab 294: E10–E14, 2008. First published November 14, 2007; doi:10.1152/ajpendo.00648.2007—Insulin-like growth factor-binding proteins (IGFBPs) are important regulators of bone metabolism. However, their precise roles are not fully understood, since IGFBPs can have both enhancing and inhibiting effects on IGF action, depending on context and posttranslational modifications, as well as IGF-independent effects. This review focuses on recent findings from cell culture, rodent models, and clinical studies concerning local IGFBP-2, IGFBP-4, and IGFBP-5 action in bone.

Insulin-like growth factors

It is well established that the insulin-like growth factors (IGF-I and IGF-II) are critical regulators of bone metabolism and that the family of six structurally similar IGF-binding proteins (IGFBPs) have tremendous influence over the biological effectiveness of the IGFs. Since systemic administration of IGF has therapeutic limitations, regulation of local IGF action in bone using IGFBPs has been considered. However, IGF/IGFBP interactions are complex, and the big unanswered question in the field is “Why do you need six IGFBPs?” In their native or recombinant state in solution, all six IGFBPs bind IGFs with high affinity, thereby preventing interaction with receptors and effectively inhibiting IGF action. There is some preferential binding of IGF-II over IGF-I for a few of the IGFBPs, and except for IGFBP-6 this preference is modest, so the need for six IGFBPs cannot be explained solely on that basis. Now there is increasing awareness that there is more to the IGFBP story than simple sequestering of IGF. What we are discovering is that the IGFBPs can both enhance and inhibiting effects on IGFs, depending on cell type and context. Furthermore, certain IGFBPs have been reported to have IGF-independent effects. IGFBP expression is known to be regulated by skeletal factors, but “What function do IGFBPs serve in bone?” It has been difficult to assign a specific physiological role to any individual IGFBP, since ultimate cell responses depend on cell phenotype, relative concentration of IGFBP and ligand, and posttranslational alterations of the IGFBP, including specific proteolysis and extracellular matrix interactions that can alter structure and function. A better understanding of IGFBP biological effects in bone will be necessary to further the goal of therapeutic efficacy in various bone disorders such as osteoporosis.

This short review focuses on recent findings concerning local IGFBP action in bone from cell culture, rodent models, and, where available, clinical studies. Although bone cells are capable of expressing all six IGFBPs, the emphasis here will be on IGFBP-2, IGFBP-4, and IGFBP-5. The interested investigator is encouraged to read the many excellent book chapters and reviews that delve into more detail on other aspects of the IGF system (15, 23, 36, 42).

IGFBP-2

IGFBP-2 generally inhibits IGF action when added to osteoblastic cells in culture (14, 19, and Fig. 1A). Also, transgenic mice overexpressing IGFBP-2 exhibit skeletal deficiencies (18). In a recent clinical study, the increasing levels of circulating IGFBP-2 observed with age were associated with deleterious effects on bone density in men and women (2). Common to all of the studies is that IGFBP-2 was in substantial molar excess to IGFs. On the other hand, Palermo et al. (37) reported that IGFBP-2, when added at equimolar concentrations, played a potentiating role in IGF-II-mediated rat osteoblastic cell differentiation in vitro. Studies in cultured human bone cells suggested a mechanism for this enhancing effect. It was shown that IGFs, and especially IGF-II, enhanced IGFBP-2 binding to extracellular matrix produced by human osteoblasts (26). IGF-II or IGFBP-2 alone had little or no affinity for osteoblast extracellular matrix. IGFBP-2 was the only one of the six IGFBPs that showed this IGF-II-induced enhancement of matrix binding. IGFBP-2, upon binding IGF-II, likely undergoes a unique conformational change that unmask glycosaminoglycan binding sites. Once IGF-II-IGFBP-2 is associated with matrix, IGF-II would need to be available to receptors on osteoblasts and preosteoblasts in order to be effective. It was shown that in a bone matrix-rich environment the IGF-II-IGFBP-2 complex was as effective as IGF-II alone in stimulating osteoblast proliferation. IGFBP-2 alone had no effect on osteoblast function in these studies. There was no evidence of proteolysis and no apparent change in IGFBP-2 affinity for IGF with matrix association that would favor kinetic equilibrium toward receptor binding (12). An alternative model would have the IGF-II in complex with both IGFBP-2 and glycosaminoglycans as bioavailable to receptor. In this way, matrix-associated IGFBP-2 would act as a delivery and a linkage molecule promoting the concentration of IGF-II in the bone microenvironment and enhanced access to IGF receptors (Fig. 1B). Although an attractive model with similarities to basic fibroblast growth factor interaction with cells (44, 46), a direct test of this tripartite model has not yet been reported.

Animal studies support the hypothesis that IGFBP-2 in complex with IGF-II can preferentially target bone and increase bone mineral density (BMD) following short-term infusion. In one such study, osteopenia of the femur was induced by...
unilateral sciatic neurectomy in rats (11). At the time of surgery, miniosmotic pumps containing vehicle or equimolar IGF-II/H18528IGFBP-2 were implanted under the neck skin. After 14 days, sciatic neurectomy resulted in a 9% loss in BMD in the femur of the surgical limb that was prevented by treatment with IGF-II/H18528IGFBP-2. As expected, the control limb experienced no change in femur BMD over the 14 days with vehicle treatment, but IGF-II/H18528IGFBP-2 treatment resulted in a significant increase in BMD.

In vivo data obtained in patients with hepatitis C-associated osteosclerosis, who show marked increases in skeletal mass as adults, and in prepubertal children with constitutively tall stature further suggest that elevation in circulating IGFBP-2 and IGF may facilitate targeting of IGFs, in particular IGF-II, to skeletal tissues with a subsequent stimulation of osteoblast proliferation and activity (20, 26). Interestingly, parathyroid hormone, which is the first truly anabolic agent available for the treatment of osteoporosis, appears to stimulate production of IGF and IGFBP-2 by osteoblastic cells (21, 29), and in this case IGFBP-2 may serve to sequester locally produced IGFs in bone leading to a stimulation of bone formation.

Thus, relative concentrations of IGFBP-2 and IGFs and posttranslational modification of IGFBP-2, such as occurs with extracellular matrix association, can profoundly alter IGFBP-2 function in bone.

**IGFBP-4**

IGFBP-4 is a negative regulator of IGF action in a variety of cell types, including bone (49). Indeed, IGFBP-4 was origi-
nally isolated from human bone cell culture media by Mohan et al. (33) as “inhibitory IGFBP”. Miyakoshi et al. (30) showed an inhibitory effect of locally administered IGFBP-4 on bone growth in vivo. Also, transgenic mice overexpressing IGFBP-4 in osteoblasts show a decrease in bone volume and cortical bone density (48). These effects were attributed to the sequestration of IGF-I by IGFBP-4 and consequent impairment of IGF-I action in skeletal tissue of these transgenic mice (Fig. 1A). However, the concept of IGFBP-4 as an inhibitor of IGF action was reassessed when knockout of the IGFBP-4 gene in mice resulted in a significant reduction in body size rather than the expected increase that would be associated with loss of an inhibitor (38). The current interpretation of these findings is that, in vivo, IGFBP-4 binds IGF, serving as a pericellular reservoir for this important growth factor. Thus, loss of the reservoir function in the IGFBP-4 knockout mouse resulted in diminished local IGF-stimulated growth. The other part of the story is that bioactive IGF could be released from this reservoir by posttranslational modification of IGFBP-4; i.e., the affinity of IGFBP-4 for IGF is reduced through proteolysis, thus releasing the IGF for acute receptor activation (Fig. 1B). An IGFBP-4 protease produced by cultured cells, including osteoblasts, was shown to cleave IGFBP-4 mid-molecule and potentiate the effectiveness of exogenous IGF-stimulated growth (17, 25, 10). Studies using wild-type and protease-resistant IGFBP-4 provided evidence that IGFBP-4 proteolysis may be important for bone formation in vitro and in vivo (31, 39). The IGFBP-4 protease expressed by human fibroblasts and osteoblasts was identified as pregnancy-associated plasma protein-A (PAPP-A) (28). PAPP-A is the major, if not the only, IGFBP-4 protease produced by bone and enhances IGF bioactivity in bone cells in vitro (8, 22). Moreover, PAPP-A has been shown to be important for bone growth in vivo. Deletion of the PAPP-A gene in mice led to diminished growth and delayed bone development (5, 9), whereas overexpression of PAPP-A in mice using a rat type I collagen promoter enhanced bone growth (40).

Thus, as with IGFBP-2, not only the presence and amount of IGFBP-4 but also posttranslational modification, in this case proteolysis, determines inhibitory and enhancing effects of IGFBP-4 on IGF action in bone.

**IGFBP-5**

IGFBP-5 is the most highly conserved of the IGFBPs across species, the most abundant IGFBP stored in bone, and the most controversial of the IGFBPs in terms of its function (45). There are inconsistent and seemingly contradictory findings regarding its role in regulating bone growth. IGFBP-5 has been reported to both inhibit and enhance IGF actions in bone in vitro and in vivo (4, 13, 16, 31, 34, 41, 43). The inhibitory effect is presumed to be due to the ability of IGFBP-5 to bind and sequester IGFs (13, and Fig. 1A). Interestingly, in transgenic mice with global overexpression of IGFBP-5, there is a sex-related decrease in BMD, with transgenic adult males affected more severely than transgenic females (43). Transgenic mice overexpressing IGFBP-5 in bone show transient impairment of osteoblast function (16). Binding of IGFBP-5 to extracellular matrix protects it from proteolysis (see below) and reduces its binding affinity for IGFs (34). Thus, for the potentiating effect, it has been proposed that binding of the IGF-I-IGFBP-5 complex to extracellular matrix reduces the affinity of IGFBP-5 for IGF-I, resulting in release of IGF-I to receptors (Fig. 1B). Along this line, administration of IGF-I in complex with equimolar IGFBP-5 stimulated periosteal bone formation in rats; IGFBP-5 alone had no effect (6). Furthermore, with the addition of another layer of complexity, IGFBP-5 has been shown to stimulate bone formation in vitro and in vivo via IGF-independent mechanisms. The most convincing piece of evidence was a direct effect of a local injection of IGFBP-5 on osteoblast function in IGF-I knockout mice (31). IGFBP-5 possesses a nuclear localization sequence (NLS) in its carboxy-terminal domain. Amaar et al. (1) discovered that IGFBP-5 interacts with four-and-a-half LIM protein-2 and hypothesized that this interaction facilitates transport into the nucleus where the complex then modulates transcription of genes involved in osteoblast proliferation and/or differentiation. However, Andress et al. (4) showed that daily subcutaneous injections of carboxy-truncated IGFBP-5 given to ovariectomized mice for 8 wk resulted in enhanced bone formation. Therefore, the COOH-terminal portion (containing NLS) is not required for the bone anabolic effect of IGFBP-5, and other IGF-independent mechanisms may be involved. These could include IGFBP-5 binding sites that are not IGF receptors, which have been identified on osteoblast surfaces (3, 34, and Fig. 1C).

The above-mentioned cell culture and in vivo models used “gain-of-function” approaches, exogenous protein, or overexpression, which might not necessarily provide insight into physiological function in bone where there is already abundant expression of IGFBP-5. Therefore, Yin et al. (47) took a “loss-of-function” approach using RNA interference to knock down endogenous IGFBP-5 expression in U2 osteosarcoma cells. Their data indicated that endogenous IGFBP-5 was important for maintaining bone cell survival and differentiation but had little effect on cell proliferation. On the other hand, exogenous IGFBP-5 was proapoptotic in these cells, which may be due to inhibition of IGF-I-mediated cell survival. This important study points out the possible opposing actions of endogenous and exogenous IGFBP as well as the possible differential effects of IGFBPs on osteoblast proliferation, differentiation, and survival. In addition, most of the focus has been on osteoblasts, but data from Kanatani et al. (24) suggested that IGFBP-5 could stimulate bone resorption both by promoting osteoclast formation in an IGF-independent fashion as well as by IGF-dependent activation of mature osteoclasts, possibly via osteoblasts.

Thus, IGFBP-5 has considerable potential as a bone anabolic factor, but much more needs to be learned to move toward this goal. Furthermore, IGFBP-5 is a substrate for several proteases expressed by bone cells, including PAPP-A (27), complement component C1s (7), and ADAM-9 (35). The physiological role for IGFBP-5 degradation and whether it is intended for elimination of the actions of the intact protein and/or generation of unique bioactive molecules is unknown.

**Summary**

Although there has been tremendous progress in recent years addressing the question “Why do we need six IGFBPs?”, we are still a long way from definitive answers to “What function do IGFBPs serve in bone?” The challenge now is to advance our knowledge of mechanisms, cell interactions, and posttrans-
lational modifications in order to better understand the role of the IGFBPs in bone metabolism in vivo. This knowledge will also have practical importance when one is considering IGFBPs as therapeutic agents.

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