Endocrine, vol. 7, no. 1, 87–91, August 1997 0969-711X/97/7:87-91/$9.25

Serum Insulin-Like Growth Factor Binding Protein (IGFBP)-4 and IGFBP-5 Levels in Aging and Age-Associated Diseases

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Because impaired bone formation is a major contributor to the pathogenesis of senile (type II) osteoporosis, and because insulin-like growth factors (IGFs) have been shown to be important stimulators of bone formation in vitro and in vivo, studies have been focused towards clarifying the role of IGF system in the age-related impairment in bone formation. To evaluate if changes in circulating and bone cell production of IGF binding protein (IGFBP)-4 and IGFBP-5 could contribute to age-related impairment in bone formation, the authors measured serum and skeletal levels of IGFBP-5, and serum levels of IGFBP-4 during aging. Circulating levels of IGFBP-4 increased with age, whereas that of IGFBP-5 declined with age. Serum IGFBP-4 and IGFBP-5 levels showed significant positive correlations with serum levels of parathyroid hormone (PTH) and IGFs, respectively. In addition, skeletal content of IGFBP-5 declined with age. The age-related changes in IGFBP-4 and IGFBP-5 production could contribute, in part, to the decrease in osteoblast cell proliferation and deficiency in bone formation.

Key Words: Bone formation; osteoblasts; osteoporosis; parathyroid hormone.

Introduction

Bone formation is essential to all aspects of bone physiology, including embryological development, postnatal growth, bone remodeling, which occurs throughout life, and the repair of injuries. In adults, bone formation occurs exclusively during bone remodeling. During bone remodeling, if bone formation does not keep pace with bone resorption, bone loss and eventually osteoporosis ensues. The amount of new bone formed at a given site is subject to regulation by systemic factors, including hormones, and local factors including growth factors. Of the various local regulators, the insulin-like growth factors (IGFs) are unique regulators of bone formation in that they act both systemically and locally to regulate bone formation. Regarding systemic IGF actions, several studies have shown that systemic administration of IGF-I, which increases serum level of IGF-I, causes a marked increase in bone formation in both animals and humans *(1,2)*. In addition, increases in serum levels of IGFs during growth hormone therapy and during puberty correlate with increases in bone formation markers *(3,4)*, emphasizing that circulating IGFs are potential regulators of bone formation. Regarding local IGF actions, it is noteworthy that IGFs are the most abundant growth factors produced by human osteoblast line cells in culture and, moreover, they are the most abundant growth factors stored in human bone *(5)*. The finding that 50% of the basal bone cell proliferation can be blocked by inhibiting the actions of endogenously produced IGFs provides unambiguous evidence that locally produced IGFs contribute to basal bone cell proliferation in vitro *(6)*. The only true negative feed back system regulating bone formation is mechanical strain, and there is evidence that the effect of mechanical strain to increase bone formation is mediated, at least in part, by the locally produced IGFs *(7)*. If the IGFs (systemic and local) are important regulators of bone formation, the authors should see a deficiency in bone formation in IGF knockout mice, and indeed, this observation has been made in mice lacking functional IGF-I or IGF-II genes *(8,9)*.

As one might expect from a regulatory system with multiple effector inputs involved in diverse actions, the IGF system is composed of many components in addition to the IGFs themselves, including type I and type II IGF receptors, stimulatory insulin-like growth factor binding proteins (IGFBPs), inhibitory IGFBPs, and IGFBP proteases *(10–12)*. Although there appears to be some redundancy in this system, each of the components appears to play a physiological role in mediating IGF action. Of the IGF system components, much attention has been focused

Received March 31, 1997; Accepted May 20, 1997.

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recently on the IGFBP component because of the evidence that IGFBPs modulate IGF actions in a variety of cell types including osteoblasts, and because osteoblast cell production of IGFBPs is regulated by a variety of systemic and local factors *(10,11)*. Although human osteoblasts derived from various skeletal sites have been shown to produce variable levels of IGFBP-1 through -6, the principal focus of this article is on two of the IGFBPs, namely IGFBP-4 and IGFBP-5 for the following reasons:

1) IGFBP-4 is the major IGFBP produced by human osteoblasts in culture and is a potent inhibitor of IGF-induced bone cell proliferation *(6,13)*.

2) IGFBP-5 is the major IGFBP stored in human bone, and in sharp contrast to IGFBP-4, potentiates IGF-induced bone cell proliferation *(13–15)* (Fig. 1).

3) IGFBP-4 and IGFBP-5 show considerable changes in response to clinical disease states and osteoregulatory agents *(16,17)*.

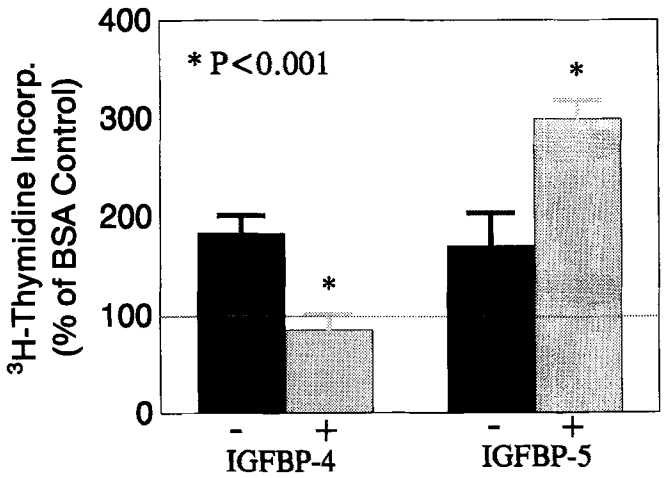

Fig. 1. Effects of IGFBP-4 and IGFBP-5 on IGF-II-induced human bone cell proliferation. IGF-II was added at 10 ng/mL whereas IGFBP-4 or IGFBP-5 was added at 100 ng/mL. Values are mean ± SD of eight replicate wells per treatment (adapted from ref. *13*).

IGFBP-4 and IGFBP-5 Levels in Human Serum

In serum, most of the IGFs (about 75%) circulate as a 150–200 kDa complex, the remaining IGFs circulate as a 50 kDa complex (about 25%). Only a small amount of IGFs (< 1%) circulate in a free form *(17)*. The 150–200 kDa IGF complex consists of 7.5 kDa IGF-I or IGF-II, plus 38–43 kDa IGFBP-3 and 80–90 kDa non-IGF binding acid labile component termed acid labile subunit (ALS). Baxter and coworkers *(18)* have proposed that ALS does not bind to IGFBP-3 unless IGFBP-3 is bound to either IGF-I or IGF-II. However, the question of whether ALS can form a binary complex with IGFBP-3 in the absence of IGF ligand is controversial at this time based on other recent reports. The 50 kDa complex consists of IGF-I or IGF-II bound to one of the remaining five IGFBPs. Comparison of the relative amounts of IGFBP-4 and IGFBP-5 in adult human serum with the relative abundance of other known IGFBPs revealed that the levels of these two IGFBPs are higher than the levels of IGFBP-1, IGFBP-2, and IGFBP-6, but less than 15% of the IGFBP-3 level *(17)*. There is a 50% molar excess of IGFBPs over IGFs in serum, which implies that a small percentage of IGFs remain in the free form.

If IGFBP-4 and IGFBP-5 act in a reciprocal manner to regulate IGF actions, it would be important for these two components to be regulated by different mechanisms to produce a complementary effect in response to changes in physiological and pathological conditions. Otherwise, parallel changes in stimulatory IGFBP-5 and inhibitory IGFBP-4 would cancel each other out. Consistent with the idea that different mechanisms may regulate serum levels of IGFBP-4 and IGFBP-5, we have found that serum levels of stimulatory IGFBP-5 showed significant positive correlations with both IGF-I and IGF-II concentrations while serum levels of IGFBP-4 showed a weak negative correlation or no correlation *(16,19,20)*.

Functions of IGFBP-4 and IGFBP-5 in Serum

IGFs are present in the circulation, and throughout the extracellular space in a high concentration to provide a readily available reserve for their function as endocrine hormones. This function of IGFs as endocrine hormones is feasible because of the presence of IGFBPs. In this regard, IGFBPs may function not only to increase the half-life of the IGFs but may also act to control the transport of IGFs from vascular space into tissue spaces depending on the local needs *(17,21)*. In addition to endocrine effects, IGFs may function locally in a variety of cell types including brain, muscle, and bone *(17)*. Because IGFBP-4 and IGFBP-5 can modulate the biological activity of IGFs both in a positive and negative manner, and because IGFBPs are differentially regulated in various tissues, IGFBPs may also play a central role in the local regulation of IGF actions. More recently, it has been suggested that IGFBP-5 may also regulate cell function independent of the IGFs *(17)*.

In Vivo Studies on Serum Regulation of IGFBP-4 and IGFBP-5 Levels

If IGFBP-4 and IGFBP-5 function to regulate local bone formation by modulating IGF actions, then one might expect agents that stimulate bone formation would increase the effective concentration of IGFBP-5 and/or decrease the effective concentration of IGFBP-4 while agents that inhibit bone formation would have the opposite effects. Herein the authors explore this premise. In addition, they anticipate serum levels of IGFBP-4 and IGFBP-5 to show con-

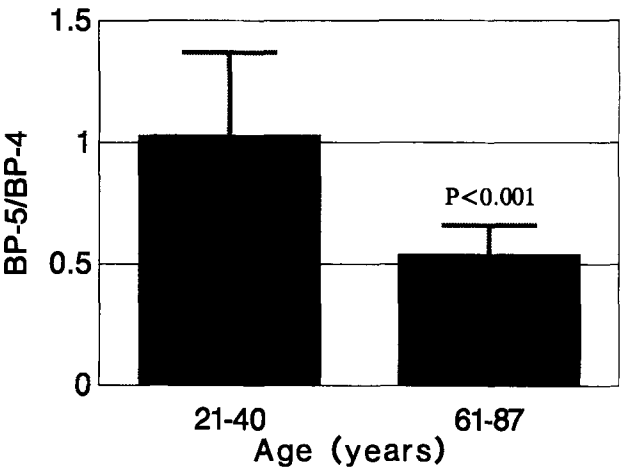

Fig. 2. Relative ratio of stimulatory IGFBP-5 to inhibitory IGFBP-4 in human serum during aging. Serum IGFBP-4 and IGFBP-5 levels in normal healthy men and women were determined by RIA as described previously *(16)*.

siderable changes in response to clinical disease states affecting bone metabolism, a premise that they will also address. These two premises will be evaluated by analyzing the perturbations of these two IGFBPs in 3 different contexts (i.e., changes during aging, osteoporosis, and GH treatment) as discussed below.

Changes During Aging

The authors found that serum IGFBP-4 levels showed an age-related increase in pooled data from 102 men and women between ages 23–87 yr ($r = 0.54$, $p < 0.001$). The mean serum IGFBP-4 level was 35% higher in the 61–87 yr age group compared with the 23–40 yr age group *(16)*. In contrast to IGFBP-4, the serum levels of IGFBP-5 showed an age-related decrease ($r = -0.48$, $p < 0.001$). The mean serum levels of IGFBP-5 was 35% lower in the 61–87 yr age group compared with the 21–40 yr age group. Since serum levels of the stimulatory IGFBP decreased with age, whereas that of the inhibitory IGFBP increased with age, the authors determined the molar ratio of IGFBP-5 to IGFBP-4 in young and old subjects. Changes in this ratio are a measure of the combined growth promoting actions of these two IGFBPs. Figure 2 shows that the molar ratio of IGFBP-5 to IGFBP-4 declined significantly from 1 to 0.54 with advance in age, which suggests that the endocrine IGF actions may decrease with age.

Since multiple tissues contribute to circulating serum levels of IGFBP-4 and IGFBP-5, the authors evaluated if similar age-related changes in IGFBP levels occur in bone. As a means of testing this hypothesis, they measured the skeletal content of IGFBP-5 in human femoral cortical bone of 44 men and 20 men between 20–64 yr of age *(22)*. The authors found that the skeletal levels of IGFBP-5 decreased by 28% between 20–29 yr and 54–64 yr of age *(22)*. Moreover, the authors found that IGFBP-5 levels in both serum and bone substance showed significant positive correlations with IGF-I and IGF-II. Based on these data, they speculate that the decrease in the amount of stimulatory IGFBP-5 in serum and the skeleton could contribute, in part, to the age-related impairment in bone formation (i.e., as skeletal IGFBP-5 is released during bone resorption, it could promote bone formation at the remodeling site).

Changes in Osteoporotic Subjects

To determine if changes in the serum level of IGFBP-4 and IGFBP-5 could be involved in the pathogenesis of age-related osteoporosis, the authors measured circulating concentrations of IGFBP-4 and IGFBP-5 in 50 elderly women and 50 healthy age-matched controls *(23)*. Although the mean serum IGFBP-4 level did not differ significantly between the control and the osteoporotic group, an age-associated increase in serum IGFBP-4 was found in the osteoporotic, but not in the control group. In contrast to serum IGFBP-4 level, the mean serum IGFBP-5 level was reduced by 45% ($p < 0.001$) in the osteoporotic group compared to control group. In addition, serum levels of IGFBP-5 were found to be predictive of serum osteocalcin and femoral neck bone density when pooled data from normal and osteoporotic populations were analyzed by age-adjusted multiple regression models. These data are consistent with the hypothesis that changes in the concentration of IGFBP-5 may cause an impairment in bone formation in osteoporotic subjects.

Changes During Growth Hormone Treatment

A number of in vitro and in vivo studies have demonstrated an important effect of growth hormone on the regulation of bone formation *(24)*. The finding that growth hormone deficiency in childhood and adolescence results in retarded growth and that treatment of children with growth hormone deficiency with growth hormone increases growth supports an important role for growth hormone in bone metabolism. To test the hypothesis that growth hormone mediates its effect on bone not only by upregulating the production of IGF-I but also by increasing the production of IGFBP-5, the authors measured serum levels of IGF system components before and after growth hormone treatment in growth hormone deficient children *(3)*. The authors found that serum levels of IGFBP-5 were significantly increased compared to pretreatment values during the entire 12 mo treatment period. Moreover, the increases in serum IGFBP-5 levels showed significant positive correlation with the increase in serum levels of bone alkaline phosphatase, thus suggesting that the growth hormone-induced increase in serum IGFBP-5 may, in part, mediate the anabolic effects of growth hormone.

In Vitro Studies on Bone Cell Production of IGFBP-4 and IGFBP-5

Studies on IGFBP-4 and IGFBP-5 production using osteoblasts (derived from humans, rats, and mice) demon-

Table 1
Effects of Systemic and Local Agents on Cell Proliferation and IGFBP-4 and IGFBP-5 Levels in Human Osteoblast Cell Conditioned Medium

Effector	Proliferation	IGFBP-4 Level	IGFBP-5 Level
Progesterone	Increase	Decrease	Increase
Glucocorticoid	Decrease	No change	Decrease
1,25 dihydroxyvitamin D3	Decrease	Increase	No effect
Parathryoid hormone	ND[a]	Increase	ND[a]
IGFs	Increase	Decrease	Increase
Transforming growth factor-β	Increase	Decrease	Increase
Bone morphogenetic protein-7	Increase	Decrease	Increase

[a]ND = Not determined.

strate that a number of local and systemic factors influence the production of these two binding proteins. These studies also demonstrate that the regulation of IGFBP-4 and IGFBP-5 production is complex involving both transcriptional and post transcriptional mechanisms. Table 1 shows that bone cell expression of IGFBP-4 and IGFBP-5 are differentially regulated by physiological agents that affect bone cell proliferation. These data reveal that in general, agents that increase osteoblast proliferation increase production of IGFBP-5 and decrease production of IGFBP-4 while agents which inhibit cell proliferation had the opposite effects on the production of these two IGFBPs *(10,11,25)*. Although, these data emphasize a role for IGFBP-4 and IGFBP-5 in mediating the effects of osteogenic agents on cell proliferation, further studies are needed to establish a cause and effect relationship between changes in IGFBP production and cell proliferation.

Potential Role of IGFBP-4 and IGFBP-5 in the Pathogenesis of Senile Osteoporosis

Based on previously published *(10–17)* in vitro and in vivo findings, the authors have proposed a model which implicates the age-related changes in the IGF system components in the pathogenesis of type II senile osteoporosis. According to this model (Fig. 3), serum calcium level decreases in elderly women who have inadequate calcium intakes. This leads to an increase in the production of parathyroid hormone (PTH) to reestablish serum calcium homeostasis. The authors found that the serum levels of PTH correlated with serum levels of IGFBP-4 *(19)*, and they also found PTH treatment increases production of IGFBP-4 in human osteoblasts *(19)*. These findings could explain at least in part the authors' clinical finding of increased serum IGFBP-4 levels with advance in age.

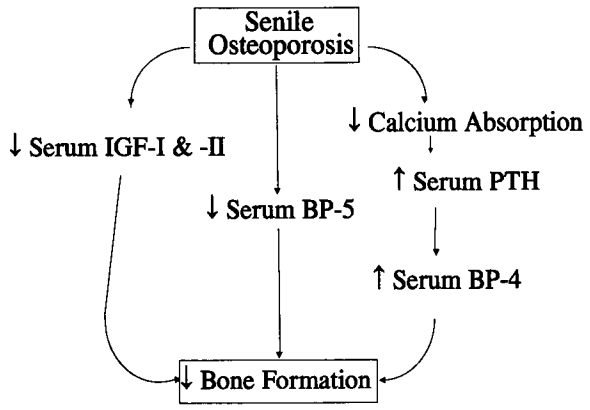

Fig. 3. Model demonstrating how age-related changes in IGF system components could lead to impairment in bone formation and contribute to the pathogenesis of senile osteoporosis (*see* text for details).

In addition to changes in bone cell production of IGFBP-4, the following changes in the production of stimulatory IGF system components could contribute to the impairment in bone formation in senile type II osteoporosis

1. Serum levels of IGFs decline with age and we have indirect evidence that bone cell production of IGFs decrease with age (i.e., decrease in skeletal content of IGFs)
2. Serum IGFBP-5 levels decrease with age and the authors have indirect evidence that there is a reduction in bone cell production of IGFBP-5 with age (i.e., decrease in skeletal content of IGFBP-5)
3. Serum IGFBP-4 levels increase with age

Thus, the underproduction of the stimulatory components and the overproduction of an inhibitory component of the IGF system occurs as a consequence of aging, and these changes lead to age-associated changes in the local as well as hormonal actions of IGFs, all of which could contribute to the pathogenesis of type II osteoporosis.

Conclusion

The following features suggest that IGFBP-4 and IGFBP-5 are important components of the IGF system in bone

1. IGFBP-4 and IGFBP-5 modulate IGF actions in bone cells
2. IGFBP-4 and IGFBP-5 represent the most abundant IGFBPs in osteoblast cell conditioned medium and bone matrix, respectively, and
3. IGFBP-4 and IGFBP-5 production is regulated by key osteoregulatory agents in vitro and in vivo

Although a number of in vitro and in vivo findings support a role for age-associated changes in IGF system components in the pathogenesis of type II osteoporosis, further studies are needed to establish a cause and effect relationship between changes in bone cell production of IGF system components and impairment in bone formation seen in senile osteoporotic patients.

Acknowledgments

This work was supported by funds from National Institutes of Health (AR31062) and the Veterans Administration.

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