Published in final edited form as:

Eur J Intern Med. 2003 February ; 14(1): 32–38.

Serum levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-1 to -6 and their relationship to bone metabolism in osteoporosis patients

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Abstract

Background—Insulin-like growth factor (IGF) system components are important regulators of bone formation. Alterations of individual IGF system components have been described in osteoporosis (OP) patients; however, no study has addressed changes in free IGF-I and in all six IGF binding proteins (IGFBPs).

Methods—A cross-sectional study was performed in 45 OP patients and 100 healthy matched controls. Serum levels of free and total insulin-like growth factor I (IGF-I), IGFBP-1 through -6, intact parathyroid hormone (PTH), 25-OH-vitamin D₃ (25OHD₃), 1,25-(OH)₂-vitamin D₃ (1,25-(OH)₂D₃), osteocalcin (OSC), bone alkaline phosphatase (B-ALP), and carboxyterminal propeptide of type-I procollagen (PICP) were measured with specific assays. Bone mineral density (BMD) of the lumbar spine was determined by dual-energy X-ray absorptiometry (DEXA).

Results—Compared with age- and sex-matched control subjects, OP patients showed a 73% decrease in free IGF-I, a 29% decrease in total IGF-I, a 10% decrease in IGFBP-3, and a 52% decrease in IGFBP-5 levels; they had higher levels of IGFBP-1 (4.1-fold), IGFBP-2 (1.8-fold), IGFBP-4 (1.3-fold), and IGFBP-6 (2.1-fold). Alterations in IGF system components were most evident in 13 OP patients with vertebral fractures in the past 4 years compared to patients without fractures. In OP patients with fractures, the ratio between IGFBP-4 and IGFBP-5 was increased whereas levels of OSC were decreased.

Conclusions—Our data provide strong indirect evidence for a functional connection between circulating IGF system components and bone metabolism and the susceptibility to fractures in OP patients.

Keywords

Bone markers; Bone mineral density; Bone histology; Vertebral fracture

1. Introduction

Bone remodelling is regulated by systemic hormones and locally produced factors acting in concert to maintain bone mass [1,2]. Insulin-like growth factors (IGFs) are among the most important regulators of bone cell function due to their anabolic effects on the skeleton [3,4]. The key role of the IGF system in the local regulation of bone formation is demonstrated by the finding that ~50% of basal bone cell proliferation could be blocked by inhibiting the actions of IGFs endogenously produced by bone cells in serum-free cultures [4]. Approximately 99% of circulating IGFs are bound to six specific high-affinity IGF binding proteins (IGFBPs) that modulate IGF action in a positive or negative manner [5–7]. IGFBP-3 is the quantitatively predominant IGFBP in circulation and associates with an acid-labile subunit (ALS) after IGF binding, forming a 150-kDa complex. This large complex is essentially limited to the intravascular space and prolongs the plasma half-life of IGFs from a few minutes to several hours. On the other hand, 20–30% of the serum IGFs are found in small (45 kDa) protein complexes that contain IGFBPs but not ALS. The small molecular weight IGF–IGFBP complexes can leave the intravascular space and appear to be the primary vehicle that allows circulating IGFs to reach extravascular tissue binding sites [8]. IGFBPs are often co-expressed with IGFs in various tissues, and circulating as well as locally expressed IGFBPs appear to modify IGF action by either inhibiting or promoting IGF bioactivity. For example, IGFBP-4 inhibits while IGFBP-5 stimulates IGF actions in bone cells [7,9,10]. Many of the systemic bone-regulating hormones appear to recruit IGF system components to mediate their effects on bone cells. This is evident from a recent study demonstrating a lack of anabolic effects of parathyroid hormone (PTH) on bone in IGF-I knockout mice deficient in both local and systemic sources of IGF-I [11].

The findings that serum levels of IGF system components are altered during physiological and pathological states when bone metabolism is distorted provide indirect evidence for an important role for IGF system components in bone remodelling. PTH infusion increased circulating levels of insulin-like growth factor binding protein-3 (IGFBP-3) levels in healthy women but not in patients with inflammatory active rheumatoid arthritis [12]. The serum levels of IGFBP-3 were decreased in patients with idiopathic osteoporosis [13] while in elderly women with hip and spine fractures increased IGFBP-4 serum levels have been reported that were positively correlated to elevated PTH concentrations [14]. More recently, we have found that serum levels of IGF-I, IGFBP-3, and IGFBP-5 were reduced by 40–50% in hip fracture patients compared to age-matched control subjects [15]. In osteopenic type-1 diabetes patients, we observed that bone mineral density (BMD) was positively correlated with stimulatory IGF system components (IGF-I, IGFBP-3, and IGFBP-5) and negatively correlated with inhibitory IGF system components (IGFBP-1 and IGFBP-4) [16]. Based on the above findings and a study that demonstrated a significant positive correlation between serum IGF-I levels and the osteoblastic surface measured by histomorphometric techniques [17], it can be speculated that circulating levels of IGF-I and IGFBPs may affect bone formation and may contribute, at least in part, to osteopenia and osteoporosis.

The aim of this study was to investigate the serum levels of all six IGFBPs along with free and total IGF-I in patients with osteoporosis (OP) and in age- and sex-matched healthy subjects. Based on the known actions of IGFBPs in bone cells, we predicted an increase in serum levels of IGFBP-4 and IGFBP-6 (inhibitory) and a decrease in IGFBP-3 and IGFBP-5 (stimulatory) in OP subjects compared to control subjects. Because free IGF-I and not IGFBP-bound IGF is more bioavailable to interact with signalling type I receptor in target tissues, we also predicted a decrease in the circulating level of free IGF-I in OP subjects.

2. Subjects and methods

2.1. Patients

In this cross-sectional study, we investigated 45 Caucasian OP patients. The study was performed according to the rules of the local ethics committee. A total of 14 patients had a history of a bone fracture (mean 2.5 years, range 0.5–4.2 years) before the study. The diagnosis and therapy of OP was based on clinical, radiological, and bone histological findings. The OP group consisted of subjects with idiopathic OP (unknown cause, $n=10$), involutional OP (type I (postmenopausal), $n=6$; type II (age-related), $n=15$), and secondary OP due to hypogonadism ($n=3$), insulin-dependent diabetes mellitus ($n=2$), vitamin D deficiency ($n=7$), or previous therapy with corticosteroids ($n=3$). As for the different causes of OP, the therapy regimen was individually designed (e.g. stimulators of bone formation, such as fluorides, in low-turnover OP, or inhibitors of bone turnover, such as estrogens and bisphosphonates, in high-turnover OP). All OP patients received calcium supplementation (0.5–1.0 g/day). Vitamin D (cholecalciferol 1000 IE/day) was given to patients with vitamin D deficiency to normalize serum levels.

2.2. Radiological and histological assessment of bone mass

X-rays of the lumbar spine were performed in all OP patients. BMD of the lumbar spine was assessed by dual-energy X-ray absorptiometry (DEXA; HOLOGIC QDR 1000, Siemens Medical Systems, Erlangen, Germany) and T-scores were provided. In addition, bone biopsies were performed at the iliac crest in 38 patients and analyzed, as previously described [18].

2.3. Assays

Blood samples were collected under standardized non-fasting conditions. Samples were immediately centrifuged for 10 min at $4000\times g$ and then stored at -20°C until the assays were performed. Serum creatinine, calcium, and phosphate were measured using standard biochemical methods. To assess bone metabolism, we used radioimmunoassay (RIA) to determine: bone-specific alkaline phosphatase (B-ALP; Hybritech, Köln, Germany), osteocalcin (OSC; CIS, Dreieich, Germany), and carboxyterminal propeptide of type-I procollagen (PICP; Pharmacia, Freiburg, Germany). 25-OH-vitamin D_3 (25OHD $_3$) and 1,25-(OH) $_2$ -vitamin D_3 (1,25-(OH) $_2\text{D}_3$) were measured with specific RIAs (Incstar, Stillwater, MN, USA) with a less than 2.5% cross-reactivity to other vitamin D metabolites. Intact parathyroid hormone (PTH) was measured using a chemiluminescence immunometric assay (Nichols, CA, USA) with intra- and interassay coefficients of variation of less than 7%.

The components of the IGF system were measured as previously described [16,18–20]. The normal range of IGF-I and IGFBP levels was obtained from 100 age- and sex-matched healthy blood donors (mean age 57 ± 2.6 years; female/male: 49/51) who were randomly recruited from the University Hospital Ulm. Free IGF-I levels were determined by a specific RIA (DSL, Frankfurt, Germany) with intra- and interassay coefficients of variation of less than 4 and 8%. Total IGF-I was determined by an IGFBP-blocked specific RIA (Mediagnost, Tuebingen, Germany) with a cross-reactivity to IGF-II of less than 0.05% and intra- and interassay coefficients of variation of less than 4 and 8%. IGFBP-1 was assessed by enzyme immunoassay (Mediagnost) with intra- and interassay coefficients of variation of less than 4 and 8%. IGFBP-2 was determined by a specific RIA (DSL) with a cross-reactivity to other IGFBPs of less than 0.05% and intra- and interassay coefficients of variation of less than 5 and 10%. IGFBP-3 was determined by a specific RIA with intra- and interassay coefficients of variation of less than 3.5 and 7.5% and no cross-reaction with IGFBP-1 or IGFBP-2 (Mediagnost). IGFBP-4 and IGFBP-5 were measured by specific RIAs with intra- and interassay coefficients of variation of less than 10%, as previously described [19,20]. IGFBP-6 was determined by a specific RIA

(DSL) with a cross-reactivity to other IGFBPs of less than 0.1% and intra- and interassay coefficients of variation of less than 4 and 10%.

2.4. Statistical analysis

Results are expressed as mean±S.E.M. Data were analyzed via a one-way analysis of variance (ANOVA). The analysis was separated for the individual subgroups. Correlations between variables (Pearson's correlation coefficient) were assessed using univariate linear regression analysis. A *P*-value below 0.05 was considered to be statistically significant.

3. Results

3.1. Clinical data and bone markers

Patient characteristics and laboratory parameters are shown in Table 1. Of the 48 subjects with OP, 14 had had a consolidated bone fracture within the past 4 years (13 vertebral fractures, one hip fracture). OSC levels and BMD were diminished in all OP patients compared to age- and sex-matched healthy controls. The 14 patients who had osteoporotic fractures showed significantly lower OSC levels (6.3 ± 1.1 ng/ml) than OP patients without fractures (9.3 ± 1.4 ng/ml; $P < 0.05$). BMD was similar in both subgroups of OP patients (with fracture -1.9 ± 0.5 S.D., without fracture -2.2 ± 1 S.D.). Seven patients had a T-score below -2.5 . Patients had normal levels for serum creatinine, calcium, phosphate, PTH, 25OHD₃, 1,25-(OH)₂D₃, B-ALP, and PICP. Bone markers and clinical data were not significantly different between male and female patients.

3.2. Bone biopsy findings

Bone biopsies were performed in 38 OP patients using an electric drill. In all cases, typical characteristics of OP, such as reduced trabecular bone volume and diminished trabecular interconnections, were found. Additional pathological findings were high-turnover osteopathy (HTO) in eight patients (age 63 ± 3.5 years), low-turnover osteopathy (LTO) in six patients (age 57 ± 4 years), and osteomalacia (OM) in nine patients (age 50 ± 5.5 years). LTO patients had lower B-ALP values (4.4 ± 1.0 ng/ml) than the HTO patients (11.6 ± 2.7 ng/ml; $P < 0.05$). OM patients had the highest PTH levels (53 ± 20 pg/ml), consistent with mild secondary hyperparathyroidism due to low 25OHD₃ levels (26.1 ± 8 ng/ml). All other patients had PTH levels in the mid-normal range, including the LTO patients.

3.3. IGF system components in OP patients and control subjects

When compared with age- and sex-matched control subjects, OP patients had a 73% decrease in free IGF-I, a 29% decrease in total IGF-I, a 10% decrease in IGFBP-3, and a 52% decrease in IGFBP-5 levels; they had higher levels of IGFBP-1 (4.1-fold), IGFBP-2 (1.8-fold), IGFBP-4 (1.3-fold), and IGFBP-6 (2.1-fold). Mean values±S.E.M. and statistical significances are shown in Table 2. Female patients had lower levels of IGF-I, but higher levels of IGFBP-4 than males. Alterations in IGF system components were most evident in OP patients with bone fractures. These patients had lower levels of free IGF-I, total IGF-I, IGFBP-3, and IGFBP-5 than OP patients without fractures (Fig. 1), but higher levels of IGFBP-1, IGFBP-2, IGFBP-4, and IGFBP-6 (Fig. 2).

When OP patients were divided into subgroups according to the bone histological findings, the HTO subgroup had significantly ($P < 0.05$) higher levels of IGFBP-2 (907 ± 115 ng/ml), IGFBP-4 (604 ± 97 ng/ml) and IGFBP-6 (196 ± 15 ng/ml) than LTO patients (503 ± 51 , 498 ± 83 and 172 ± 21 ng/ml, respectively). The OM subgroup had significantly ($P < 0.05$) lower IGFBP-3 levels (2266 ± 241 ng/ml) than HTO (2726 ± 165 ng/ml) or LTO patients (2619 ± 400 ng/ml).

3.4. Relationship between IGF system components and clinical data

Correlations between IGFs and IGFBPs were assessed by linear regression analysis (Table 3). In all OP patients, free and/or total IGF-I correlated positively with IGFBP-3 and IGFBP-5 and negatively with IGFBP-1, IGFBP-2, and IGFBP-4. Furthermore, a positive correlation was obtained between free IGF-I and total IGF-I ($r=0.55$, $P<0.005$).

Age was positively correlated with free IGF-I ($r=0.32$, $P<0.05$), IGFBP-2 ($r=0.46$, $P<0.01$), IGFBP-4 ($r=0.27$, $P<0.05$), and IGFBP-6 ($r=0.39$, $P<0.05$), but negatively with total IGF-I ($r=-0.65$, $P<0.001$). Interestingly, the ratio of free versus total IGF-I levels showed a significant positive correlation with age ($r=0.45$, $P<0.01$). Thus, higher levels of free IGF-I may compensate for the age-related decrease in total IGF-I levels. Free IGF-I levels correlated positively with OSC levels ($r=0.28$, $P<0.05$). On the other hand, BMI correlated negatively with IGFBP-1 ($r=-0.41$, $P<0.01$) and IGFBP-2 ($r=-0.55$, $P<0.005$).

4. Discussion

This study confirms earlier findings that a deficiency in serum levels of stimulatory IGF system components (IGF-I, IGFBP-3, and IGFBP-5) occurs in OP patients compared to age- and sex-matched control subjects. It also shows for the first time that serum levels of free IGF-I are lower in OP patients. Furthermore, serum levels of inhibitory IGFBPs (IGFBP-4 and IGFBP-6) were higher in OP patients than in age-matched control subjects. Thus, these data are consistent with the hypothesis that an imbalance between stimulatory and inhibitory IGF system components occurs in OP patients and that this is further aggravated in OP patients with bone fractures.

Although the results of our study may be somewhat limited by the cross-sectional study design and the various causes of OP, these results and past findings provide strong indirect evidence that alterations in circulating levels of IGF system components could, in part, contribute to the impairment of bone formation and associated bone loss in OP. The following lines of evidence support this concept: (i) stimulatory IGF system components were decreased in OP patients in this study, matching data from previous reports in patients with osteopenia and OP [13,15,16,21]; (ii) significant positive correlations have been reported between serum levels of stimulatory IGF system components and BMD, on the one hand [13,15,16], and bone histological osteoblastic surface, on the other hand [17]; and (iii) systemic administration of IGF-I, which increases circulating IGF-I levels, stimulates bone formation parameters in animal and human models [22,23].

Because free IGF-I is much more bioavailable to target tissues than IGFBP-bound IGF-I, we expected the free IGF-I level to correlate with bone formation markers in serum. Accordingly, we found that free IGF-I levels were significantly lower in OP subjects than in corresponding age-matched normal subjects and that they correlated positively with serum OSC levels. However, free IGF-I levels did not correlate significantly with other bone formation markers, namely, BAP and PICP. Future studies with larger numbers of subjects are needed to evaluate the relative importance of free IGF-I in the regulation of bone formation.

If changes in systemic circulating levels of stimulatory IGF system components contribute to bone formation changes, one would expect disruption of liver-derived IGF-I, which contributes mainly to circulating IGF-I, to cause an impairment in bone formation. In this regard, our recent studies have shown that although circulating IGF-I was reduced by 80% in liver IGF-I knockout mice, BMD was not compromised in these mice compared to corresponding control mice [24]. In contrast to disruption of liver IGF-I, disruption of IGF-I throughout the body results in severe impairment in the acquisition of BMD in mice and humans [25,26]. There are several potential explanations for this discrepancy. First, serum levels of IGF system components in

OP subjects may reflect bone cell production of IGF system components and bone cell-produced IGF system components may exert a greater role than systemic circulating IGF system components. In this regard, most of the systemically administered IGF-I exists as a free form bioavailable to bind to signalling IGF-I receptor compared to circulating IGF-I in serum, which is mostly bound to IGFBPs and, therefore, not bioavailable [7]. Second, circulating IGF-I in serum may not play a major role in the acquisition of bone mass but may exert a significant effect on age-related bone loss. Third, an imbalance in multiple components of the circulating IGF system in the serum may exert a greater effect on bone formation compared to a reduction in circulating level of IGF-I alone, as found in the liver IGF-I knockout mice. In any case, future studies are needed to evaluate the cause and effect relationship between the deficiency in IGF system and age-related bone loss in OP patients.

The mechanisms that contribute to the changes in circulating levels of IGF system components in OP patients can only be speculated at the present time. In this regard, it is known that growth hormone is one of the major regulators of circulating levels of IGF-I, IGFBP-3, and IGFBP-5 [7] and that a deficiency in growth hormone is associated with a severe reduction in serum levels of these stimulatory IGF system components. It remains to be established whether a reduction in growth hormone levels contributes in part to the deficiency in the circulating level of stimulatory IGF system components in OP patients. In terms of inhibitory IGFBPs, serum IGFBP-4 levels were elevated in patients with secondary hyperparathyroidism [14,18]. The potential mechanisms that contribute to the increase in circulating levels of inhibitory IGFBPs in OP patients remain unknown at this time.

A new finding of our study was that serum IGFBP-6 levels are elevated more than twofold in OP patients. In terms of the significance of this finding, we have recently found that IGFBP-6 is a strong inhibitor of osteoblast differentiation and that the effect of IGFBP-6 may in part be mediated by an IGF-independent mechanism [27]. Further studies are needed to address the significance of higher serum IGFBP-6 levels to impairment in bone formation in OP patients.

In conclusion, our data provide strong indirect evidence for a functional connection between circulating IGF system components and bone metabolism and the susceptibility to fractures in OP patients. The major findings of the present study support the idea that the diminished levels of free and total IGF-I, IGFBP-3, and IGFBP-5, as well as the increased levels of IGFBP-1, -2, -4, and -6, may contribute to diminished bone formation in OP. This is supported by a recent double-blind, placebo-controlled pilot study demonstrating that the administration of recombinant human IGF-I, complexed with its predominant binding protein-3, induce significant positive musculoskeletal effects in OP patients with proximal femoral fractures [28]. Further studies are needed to determine to what extent circulating IGF/IGFBP levels reflect local changes in bone and whether measuring individual components can be useful in the diagnosis and evaluation of treatment of metabolic bone disease such as primary and secondary OP.

Acknowledgments

The authors wish to thank Rosa Herzog and Sabine Schilling for their excellent assistance in performing the RIAs and Joachim Brueckel for his suggestions to the manuscript. This work was supported by the Landesforschungsschwerpunkt Baden-Württemberg: 'Wachstum-sfaktor-Modulation als therapeutisches Prinzip' to P.M. Jehle and an NIH grant (AR31062) to S. Mohan.

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Fig. 1. Stimulatory IGF system components. Serum levels of free IGF-I, total IGF-I, IGFBP-3, and IGFBP-5 in control subjects, in patients with osteoporosis (OP), and in OP patients who have had osteoporotic fractures within the past 4 years (#). Mean values $\pm$ S.E.M. are given. Differences between groups are indicated.

Fig. 2. Inhibitory IGF system components. Serum levels of IGFBP-1, IGFBP-2, IGFBP-4, and IGFBP-6 in control subjects, in patients with osteoporosis (OP), and in OP patients who have had osteoporotic fractures within the past 4 years (#). Mean values $\pm$ S.E.M. are given. Differences between groups are indicated.

Table 1

Clinical data and serum parameters of bone metabolism in osteoporosis (OP) patients

OP patients	All	Females	Males
<i>n</i>	48	26	22
<i>Clinical data</i>			
Age (years)	60.4±2	66.3±2	54±3
BMI (kg/m ²)	25.9±0.7	25.2±0.9	24.4±1
BMD (S.D. T-score)	-2.3±0.13	-2.2±0.15	-2.4±0.2
<i>Serum parameters</i> (reference values)			
Creatinine (37–110 µmol/l)	86±3	83±3	89±5
PTH (10–65 pg/ml)	41±6	35±4	51±11
25OHD ₃ (16–74 ng/ml)	30±7	31±4	29±1
1,25-(OH) ₂ D ₃ (10–60 pg/ml)	34±2	32±4	37±1
OSC (female: 11–39 ng/ml) (male: 13–48 ng/ml)	8.3±1	7.6±1	9.1±1.8
B-ALP (female: 7.5–15.7 ng/ml) (male: 8–16.8 ng/ml)	11.6±1.4	9.8±1.2	13.6±2.7
PICP (female: 50–170 ng/ml) (male: 38–202 ng/ml)	162±15	177±26	143±9

Data are given as mean values±S.E.M. As assessed by ANOVA, there were no significant differences between female and male OP patients. B-ALP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; OSC, osteocalcin; PTH, parathyroid hormone; PICP, carboxyterminal propeptide of type-1 procollagen; 1,25-(OH)₂D₃, 1,25-(OH)₂-vitamin D₃; 25OHD₃, 25-OH-vitamin D₃.

Table 2

Serum levels of IGF system components in osteoporosis (OP) patients compared to healthy control subjects

IGF system components	OP patients, females	OP patients, males	Controls, all	All
<i>n</i>	26	22	48	100
Free IGF-I (ng/ml)	0.89±0.3	0.55±0.1	0.74±0.2 [*]	2.7±1.3
Total IGF-I (ng/ml)	73±7 [#]	121±9	96±7 [*]	135±15
IGFBP-1 (ng/ml)	10.2±1.6	8.3±3.2	9.4±1.6 [*]	2.3±2.1
IGFBP-2 (ng/ml)	931±100	729±101	837±100 [*]	474±21
IGFBP-3 (ng/ml)	2814±186	2600±190	2716±186 [*]	3087±230
IGFBP-4 (ng/ml)	552±47 [#]	425±45	494±47 [*]	396±30
IGFBP-5 (ng/ml)	224±12	212±13	219±12 [*]	454±44
IGFBP-6 (ng/ml)	215±17	201±19	209±17 [*]	99±5

Data are given as mean values±S.E.M. Differences between female and male OP patients and between OP patients and control subjects were assessed by ANOVA

^{*} $P<0.05$, OP patients versus controls;

[#] $P<0.05$, female versus male OP patients.

Table 3

Relationship between free and total IGF-I and IGFBP-1 to -6

	Free IGF-I	Total IGF-I
IGFBP-1	-0.16	-0.03
IGFBP-2	+0.14	-0.50**
IGFBP-3	+0.15	+0.29*
IGFBP-4	-0.08	-0.28*
IGFBP-5	+0.34*	+0.54**
IGFBP-6	+0.25	-0.07

Correlations between variables were assessed by univariate linear regression analysis. Pearson's correlation coefficients (*r*) and statistical significances (**P*<0.05, ***P*<0.01) are given.