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Serum Levels of Insulin-Like Growth Factor (IGF); IGF-Binding Proteins-3, -4, and -5; and Their Relationships to Bone Mineral Density and the Risk of Vertebral Fractures in Postmenopausal Women

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Abstract

We previously found that serum levels of insulin-like growth factor I (IGF-I) and IGF-binding protein (IGFBP)-3, but not IFGBP-2, were associated with bone mineral density (BMD) and the risk of vertebral fractures. The aim of the present study was to investigate the roles of IGFBP-4 and -5 in age-dependent bone loss and vertebral fracture risk in postmenopausal Japanese women and to compare them with those of IGF-I and IGFBP-3. One hundred and ninety-three Japanese women aged 46–88 years (mean 62.5) were enrolled in the cross-sectional study. BMD was measured at the lumbar spine, femoral neck, ultradistal radius (UDR), and total body by dual-energy X-ray absorptiometry. Serum levels of IGFBP-4 and -5 as well as IGF-I and IGFBP-3 were measured by radioimmunoassay. Serum levels of IGF-I, IGFBP-3, and IGFBP-5 declined with age, while serum IGFBP-4 increased with age. Multiple regression analysis was performed between BMD at each skeletal site and serum levels of IGF-I and IGFBPs adjusted for age, body weight, height, and serum creatinine. BMD at the UDR was significantly and positively correlated with all serum levels of IGF-I and IGFBPs measured ($P < 0.01$), while BMD at the femoral neck was correlated with none of them. Serum IGF-I level was significantly and positively correlated with BMD at all sites except the femoral neck ($P < 0.01$), while serum IGFBP-3 and -4 levels were significantly and positively correlated with only radial BMD ($P < 0.01$). Serum IGFBP-5 level was positively correlated with UDR BMD ($P < 0.001$) and negatively correlated with total BMD ($P < 0.05$). Serum IGF-I, IGFBP-3, and IFGBP-5 levels were significantly lower in women with vertebral fractures than in those without fractures (mean $\pm$ SD: 97.1 ± 32.1 vs. 143.9 ± 40.9 ng/dl, $P < 0.0001$; 2.18 ± 1.02 vs. 3.23 ± 1.07 μ g/ml, $P < 0.0001$; 223.6 ± 63.3 vs. 246.5 ± 71.5 ng/ml, $P = 0.0330$, respectively). When multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and serum levels of IGF-I and IGFBPs adjusted for age, body weight, height, serum creatinine, and serum albumin as independent variables, IGF-I and IGFBP-3 were selected as indices affecting the presence of vertebral fractures [odds ratio (OR) = 0.29, 95% confidential interval (CI) 0.15–0.57 per SD increase, $P = 0.0003$ and OR = 0.31, 95% CI 0.16–0.61 per SD increase, $P = 0.0007$, respectively]. To compare the significance values, IGF-I, IGFBP-3, and age were

simultaneously added as independent variables in the analysis. IGFBP-3 was more strongly associated with the presence of vertebral fractures than IGF-I and age ($P = 0.0006$, $P = 0.0148$, and $P = 0.0013$, respectively). Thus, after comprehensive measurements of serum levels of IGF-I and IGFBPs, it seems that serum IGF-I level is most efficiently associated with bone mass and that serum IGFBP-3 level is most strongly associated with the presence of vertebral fractures in postmenopausal women among the IGF system components examined.

Keywords

Insulin-like growth factor-binding protein; Insulin-like growth factor; Bone mineral density; Vertebral fracture; Postmenopausal women

Bone remodeling is regulated by systemic hormones and locally produced factors acting in concert to maintain bone mass [1,2]. Insulin-like growth factors (IGFs) are synthesized in osteoblasts and 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 approximately 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]. However, circulating IGF-I, mainly produced in the liver via regulation by growth hormone (GH) and diet, acts in an endocrine manner as well, which activates bone remodeling and exerts anabolic effects on bone tissues [5–7]. Indeed, there is recent evidence that the GH/IGF-I axis plays an important role in maintaining bone mass in adults as well as longitudinal growth of bone in childhood [8–10].

Approximately 99% of circulating IGFs are bound to six specific high-affinity IGF-binding proteins (IGFBPs) that are produced in osteoblasts and other cell types and modulate IGF action in a positive or negative manner [11–13]. In addition, IGFBPs *per se* may directly affect bone and cartilage metabolism [14–16]. A major portion of IGF-I is bound to IGFBP-3, which is a quantitatively predominant IGFBP in the circulation [17]. Serum IGFBP-3 level is considered to be positively regulated by GH and/or IGF-I [18–20]. In a previous report, we demonstrated an age- and body mass index-independent positive correlation between serum IGF-I level and bone mineral density (BMD) of the lumbar spine, femoral neck, and mid-radius in postmenopausal Japanese women [21]. We also found that the serum IGFBP-3 level was significantly correlated with BMD at the mid-radius once age was taken into account [21]. Furthermore, our study suggested that serum levels of IGF-I and IGFBP-3 would be clinically important predictors of vertebral fracture risk because they were significantly lower in subjects with vertebral fractures than those without fractures at any decade [21].

IGFBPs are often coexpressed with IGFs in various tissues, and both circulating and locally expressed IGFBPs appear to modify IGF action by either inhibiting or promoting IGF bioactivity. For example, IGFBP-4 inhibits IGF actions in bone cells, while IGFBP-5 stimulates it [12,22,23]. Thus, it can be speculated that circulating levels of IGFBP-4 and IGFBP-5, as well as IGF-I and IGFBP-3, may affect bone formation and may contribute, at least in part, to osteoporosis.

The present study was performed to investigate whether or not serum levels of IGFBP-4 and IGFBP-5 would be useful for predicting BMD and the presence of vertebral fractures in a fashion similar to IGF-I and IGFBP-3 in the previous study [21]. In addition, we reevaluated the efficiency of serum levels of IGF-I and IGFBP-3 for predicting the presence of vertebral fractures through estimating odds ratios (ORs) with logistic regression analysis in a population slightly larger than that in the previous study ($n = 193$ vs. 165).

Materials and Methods

Subjects

The subjects in this study were 193 Japanese postmenopausal women aged 46–88 years (mean 62.5). Subjects visited an outpatient clinic at Kobe University Hospital to see whether or not they had osteoporosis. Baseline characteristics of subjects are shown in Table 1. The study was approved by the institutional review board of the hospital. Subjects agreed to participate in the study and gave informed consent. All women had been without spontaneous menses for more than 1 year. All serum estradiol levels were <20 pg/ml. Nobody had hepatic or renal dysfunction or nutritional derangements which might cause changes in bone metabolism. All subjects were tested for drugs known to influence bone and calcium metabolism until the time of the present study.

Radiography

Lateral X-rays of the thoracic and lumbar spine were taken. The anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4 to L4 were measured using an electric caliper. A vertebral fracture was diagnosed if at least one of three height measurements along the length of the same vertebrae had decreased by >20% compared to the height of the nearest uncompressed vertebral body. None of the subjects had a history of serious trauma.

BMD and Biochemical Measurements

BMD values of the lumbar spine, femoral neck, radius, and total body were measured by dual-energy X-ray absorptiometry (QDR-2000; Hologic, Waltham, MA). BMD of the lumbar spine (L2–L4) was measured separately and expressed as the mean. Because vertebral compression fractures may cause aberrations in BMD, the data from fractured spines were excluded. Bone mineral content (BMC), bone width (BW), and BMD (defined as BMC/BW) were measured at the distal one-third and ultradistal of the radius using single-photon absorptiometry (Bone Mineral Analyzer Type 2780; Norland, Fort Atkinson, WI). The same operator tested all of the subjects during the study to eliminate operator discrepancies. The coefficients of variation (precision) of measurements of the lumbar spine and mid-radius by our methods were 0.9 and 1.9%, respectively. Z score indicates deviation from the normal age- and sex-matched mean in standard deviation (SD).

After overnight fasting, serum was collected. Serum was promptly separated and stored at –20°C until assay. Serum IGF-I level was measured by radioimmunoassay (RIA) after acid-ethanol extraction [24,25]. Serum IGFBP-3 level was also measured by RIA [18]. The intact form of IGFBP-3 was mostly measured by the assay. Intra- and interassay variations of the measurement were 3–4% and 7–8%, respectively. Serum levels of IGFBP-4 and IGFBP-5 were measured by specific RIAs, with intra- and interassay coefficients of variation of <10%, as previously described [26,27].

Statistical Analysis

Data were expressed as means $\pm$ SD. Statistical significance between two groups was determined using Student's *t*-test. Simple, multiple, and logistic regression analyses were performed using the statistical computer program Statview (Abacus Concepts, Berkely, CA). $P < 0.05$ was considered to be significant.

Results

Serum Levels of IGF-I and IGFBPs with Age

Serum levels of IGF-I, IGFBP-3, and IGFBP-5 declined with age ($r = -0.414$, $P < 0.0001$; $r = -0.219$, $P = 0.0044$; $r = -0.177$, $P = 0.0138$ by single regression analysis, respectively). In contrast, serum IGFBP-4 level tended to increase with age ($r = 0.136$, $P = 0.0627$ by single regression analysis) (Fig. 1). Serum IGFBP-4 level was weakly but not significantly correlated with serum creatinine ($r = 0.138$, $P = 0.0598$ by single regression analysis).

Correlation between IGF System Components

A simple regression analysis showed that IGF-I and IGFBP-3, -4, and -5 were strongly and significantly correlated with each other except between IGF-I and IGFBP-4 ($P < 0.0001$) (Table 2).

Relationship between BMD and Serum Levels of IGF-I or IGFBPs

Since serum levels of IGF-I and IGFBPs are known to be affected by age, body stature, and renal function, a multiple regression analysis was performed between BMD at each skeletal site and the levels of IGF-I and IGFBPs adjusted for age, body weight, height, and serum creatinine (Table 3). BMD at the ultradistal radius (UDR) was significantly and positively correlated with all serum levels of IGF-I and IGFBPs measured ($P < 0.01$), while BMD at the femoral neck was correlated with none of the levels. Serum IGF-I level was significantly and positively correlated with BMD at all sites except the femoral neck ($P < 0.01$), while serum IGFBP-3 and -4 levels were significantly and positively correlated with only radial BMD ($P < 0.01$). Serum IGFBP-5 level was positively correlated with UDR BMD ($P < 0.001$) and negatively correlated with total BMD ($P < 0.05$).

Relationship between the Presence of Vertebral Fractures and Serum Levels of IGF-I and IGFBPs

Next, we compared demographic and biochemical parameters including serum levels of IGF-I and IGFBPs as well as BMD values at each site between women with and without vertebral fractures (Table 4). Women with vertebral fractures were significantly older ($P < 0.0001$), smaller in body weight ($P < 0.0001$), and shorter ($P < 0.0001$) and had significantly lower values of serum albumin and absolute BMD at each of the skeletal sites than women without vertebral fractures ($P < 0.0001$). Among IGF system components, serum levels of IGF-I, IGFBP-3, and IGFBP-5 were significantly lower in women with vertebral fractures than in those without fractures ($P < 0.0001$, $P < 0.0001$, and $P = 0.0330$, respectively).

When multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and serum levels of IGF-I and IGFBPs adjusted for age, body weight, height, serum creatinine, and serum albumin as independent variables (Table 5), IGF-I and IGFBP-3 were selected as indices affecting the presence of vertebral fractures ($P = 0.0003$ and $P = 0.0007$, respectively). To compare the significance values, IGF-I, IGFBP-3, and age were simultaneously added as independent variables in the analysis. IGFBP-3 was more strongly associated with the presence of vertebral fractures than IGF-I and age ($P = 0.0006$, $P = 0.0148$, and $P = 0.0013$, respectively).

Discussion

It is known that the GH secretion rate as well as serum levels of IGF-I and IGFBP-3 decrease with age [9]. It is also known that GH is one of the major regulators of circulating levels of IGF-I, IGFBP-3, and IGFBP-5 [12] and that a deficiency in GH is associated with a severe reduction in serum levels of these stimulatory IGF system components. Although serum GH

levels of the subjects were not assayed, the present data suggest that an age-dependent attenuation of GH is associated with a negative correlation between age and serum levels of these stimulatory IGF system components. In terms of inhibitory IGFBPs, the present study revealed that serum IGFBP-4 level tended to increase with age. Since simple regression analysis showed that serum IGFBP-4 level was weakly correlated with serum creatinine, this increase may be partly explained by an age-dependent decline in the renal clearance of IGFBP-4.

In the present study, multiple regression analysis adjusted for age, body weight, height, and serum creatinine revealed that serum levels of IGFBP-3, -4, and -5 were positively and strongly correlated with radial BMD. In contrast, these IGFBPs were not significantly correlated with either lumbar or femoral neck BMD. These results agree with our previous finding that serum IGFBP-3 level was significantly correlated with radial BMD but not with lumbar BMD after adjusting for age [21]. Thus, IGFBP-3, -4, and -5 might be preferentially involved in the appendicular bone mass.

The positive correlation between IGFBP-4 and radial BMD seems to contradict the well-known inhibitory effect of this protein on IGF action in almost all *in vitro* models [12,28]. However, systemic administration of IGFBP-4 at pharmacological doses has been shown to increase bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism [29]. Two additional *in vitro* studies suggest that IGFBP-4 enhances IGF-I growth stimulation of melanocytes [30] and augments the survival of rat neuronal cells [31]. Thus, IGFBP-4 might function as a stimulator of IGF actions and might increase bone mass under certain conditions.

The end point of treatment for osteoporosis is the prevention of bone fractures attributed to it. Therefore, it is of great importance to predict the risk of osteoporotic fractures. From this point of view, we and others have tried to establish the threshold of osteoporotic fractures of the hip and spine [32–35]. However, it became evident that there is no absolute threshold of BMD for predicting bone fractures that shows sufficiently high sensitivity and specificity. Measurements of biochemical bone markers such as osteocalcin, pyridinoline, and deoxypyridinoline are helpful to predict the rate of bone loss; but it is still difficult to predict the risk of osteoporotic fractures from these measurements [36,37]. Accordingly, the most meaningful evidence from the present study is that serum levels of IGF-I and IGFBP-3 were apparently lower in subjects with vertebral fractures than those without fractures and that their serum levels were strongly associated with the presence of vertebral fractures in postmenopausal women by logistic regression analysis. Although no method for evaluating bone quality or bone geometry is clinically available at present, the presence of vertebral fracture, albeit only in an obtuse way, could be used for the assessment of bone quality in individual patients. A large study on the incidence of vertebral fractures in postmenopausal osteoporosis showed that patients with previous vertebral fractures were more likely to suffer new vertebral fractures [38,39] and hip fractures [38] than those without vertebral fractures during several-year study periods. In this sense, our present findings suggest that IGF-I and IGFBP-3 in the circulation might play some important role in maintaining bone mass not only quantitatively but also qualitatively. Given the strong significance of *P* values in logistic regression analysis, their serum levels could be surrogate markers for evaluating the severity of osteoporosis by efficiently predicting the risk of vertebral fractures.

When IGF-I and IGFBP-3 were simultaneously added as independent variables in multiple regression analysis, IGFBP-3 became more significantly associated with the presence of vertebral fractures than IGF-I ($P = 0.0006$ vs. $P = 0.0148$). This finding might suggest that the significance of IGFBP-3 was independent of any other IGF system components, while that of IGF-I was partly dependent on IGFBP-3. Thus, IGFBP-3 might be the most powerful predictor

of vertebral fractures among IGF system components examined in this study. The predictive value of IGFBP-3 for vertebral fractures was even stronger than that of age ($P = 0.0013$).

This study has some limitations. First, the sample size was not large enough to make definite conclusions. Second, we analyzed only subjects who attended Kobe University Hospital, a tertiary-care center, for an evaluation or treatment of osteoporosis. Therefore, the patients enrolled in this study might have a relatively severe state of the disorder and might not be representative of normal Japanese postmenopausal women. Consequently, assessment of larger numbers of patients from the general population are necessary to determine the usefulness of serum levels of IGF-I and IGFBP-3 for predicting the risk of vertebral fractures. Further studies are also needed to investigate the mechanisms by which circulating IGF/IGFBP levels reflect local changes in BMD and the presence of vertebral fractures.

Several other studies also showed the positive correlations between IGF system components and BMD. Szulc et al. [40] found that IGF-I correlated with total hip BMD in men aged 19–60 years. Krassas et al. [41] reported the positive correlation of IGF-I and IGFBP-3 levels with lumbar and femoral neck BMD in healthy Caucasian men. Gillberg et al. [42] showed that femoral neck BMD is positively correlated with circulating IGF-I and IGFBP-3 in Swedish men. Seck et al. [43] found an association between serum IGF-I and change in femoral neck BMD in women more than 10 years past menopause. Kim et al. [44] reported that serum IGF-I and IGFBP-3 correlated positively with BMD of the lumbar spine and Ward's triangle in Korean postmenopausal women aged 47–60, even when age, BMI, and menopause duration were taken into account in a multiple regression analysis. In contrast, other studies on the Caucasian population found no association between IGF-I and BMD in men or women [45–48]. The reason for the discrepancies between these clinical studies is unclear, but it might be partly explained by ethnic or racial differences. Since the positive correlations of BMD with IGF-I and IGFBP-3 were found in both Japanese and Korean postmenopausal women [21, 44], IGF system components might be related to bone mass more prominently in Asian postmenopausal women than in their Caucasian counterparts.

In a previous paper [21], we found that serum IGFBP-2 level was not related to either BMD or the presence of vertebral fractures. In the present study, we additionally showed that serum IGFBP-4 and -5 levels were related to only radial or total BMD and not associated with the presence of vertebral fractures. Taken together, after comprehensive measurements of IGF system components in the two studies, it seems that serum IGF-I level is most strongly associated with BMD and that serum IGFBP-3 level is most strongly associated with the presence of vertebral fractures in postmenopausal women.

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Fig. 1.
Serum levels of IGF-I and IGFBPs with age.

Table 1**Baseline characteristics of subjects**

Number of subjects	193
Age (years)	62.5 ± 8.0
Age at menopause (years)	48.8 ± 4.0
Body weight (kg)	50.2 ± 7.8
Height (cm)	151.7 ± 5.8
BMI (kg/m ²)	21.8 ± 2.9
Alb (g/dl)	4.22 ± 0.29
Ca (mg/dl)	9.16 ± 0.35
P (mg/dl)	3.70 ± 0.44
Alp (IU/l)	242 ± 71
BUN (mg/dl)	16.6 ± 4.0
Cr (mg/dl)	0.63 ± 0.10
U-Ca/U-Cr	0.249 ± 0.182
IGF-I (ng/ml)	129.4 ± 44.0
IGFBP-3 (μg/ml)	2.97 ± 1.15
IGFBP-4 (ng/ml)	318.2 ± 109.0
IGFBP-5 (ng/ml)	239.3 ± 69.7
L BMD (g/cm ²)	0.740 ± 0.136
L BMD (Z score)	0.08 ± 1.20
F BMD (g/cm ²)	0.604 ± 0.113
F BMD (Z score)	0.26 ± 1.46
1/3R BMD (g/cm ²)	0.531 ± 0.096
1/3R BMD (Z score)	-1.08 ± 1.19
UDR BMD (g/cm ²)	0.314 ± 0.074
UDR BMD (Z score)	-1.29 ± 0.96
Total BMD (g/cm ²)	0.894 ± 0.095

BMI, body mass index; Alb, albumin; Alp, alkaline phosphatase; P, phosphorus; Ca, calcium; BUN, blood urea nitrogen; U, urinary; Cr, creatinine; L, lumbar; F, femoral neck; 1/3R, mid-radial; UDR, ultradistal radial

Table 2

Simple regression analysis between each IGF system component: numbers in cells describe correlation coefficients

	IGFBP-3	IGFBP-4	IGFBP-5
IGF	0.468 ^a	0.011	0.305 ^a
IGFBP-3		0.415 ^a	0.597 ^a
IGFBP-4			0.524 ^a

^a $P < 0.0001$

Table 3

Multiple regression analysis between BMD at each skeletal site and serum levels of IGF-I and IGFBPs adjusted for age, body weight, height, and Cr as independent variables: numbers in cells describe correlation coefficients

BMD region	Independent variables			
	IGF-I	IGFBP-3	IGFBP-4	IGFBP-5
L (g/cm ²)	0.198 ^b	0.120	0.001	-0.059
F (g/cm ²)	0.053	-0.010	-0.036	-0.101
1/3R (g/cm ²)	0.199 ^b	0.264 ^c	0.188 ^b	0.094
UDR (g/cm ²)	0.228 ^b	0.307 ^d	0.257 ^d	0.215 ^c
Total (g/cm ²)	0.227 ^b	0.011	0.014	-0.160 ^a

^a $P < 0.05$,

^b $P < 0.01$,

^c $P < 0.001$, and

^d $P < 0.0001$

Table 4

Comparison of subjects with and without vertebral fractures

	Vertebral fractures		<i>P</i>
	Yes	No	
Number of subjects	61	132	
Age (years)	68.7 ± 6.6	59.7 ± 7.0	<0.0001 ^a
Age at menopause (years)	48.9 ± 4.1	48.7 ± 3.9	0.7406
Body weight (kg)	45.8 ± 6.7	52.2 ± 7.5	<0.0001 ^a
Height (cm)	148.2 ± 5.4	153.4 ± 5.3	<0.0001 ^a
BMI (kg/m ²)	20.8 ± 2.7	22.2 ± 2.9	0.0023 ^a
Alb (g/dl)	4.04 ± 0.30	4.30 ± 0.25	<0.0001 ^a
Ca (mg/dl)	9.08 ± 0.37	9.18 ± 0.35	0.1663
P (mg/dl)	3.76 ± 0.46	3.69 ± 0.44	0.3959
Alp (IU/l)	248 ± 85	240 ± 66	0.5310
BUN (mg/dl)	16.6 ± 4.5	16.6 ± 3.7	0.9273
Cr (mg/dl)	0.64 ± 0.10	0.63 ± 0.10	0.2144
U-Ca/U-Cr	0.22 ± 0.15	0.26 ± 0.19	0.3783
IGF-I (ng/ml)	97.1 ± 32.1	143.9 ± 40.9	<0.0001 ^a
IGFBP-3 (μg/ml)	2.18 ± 1.02	3.23 ± 1.07	<0.0001 ^a
IGFBP-4 (ng/ml)	322.1 ± 110.4	316.4 ± 108.8	0.7408
IGFBP-5 (ng/ml)	223.6 ± 63.3	246.5 ± 71.5	0.0330 ^a
L BMD (g/cm ²)	0.623 ± 0.079	0.792 ± 0.123	<0.0001 ^a
L BMD (Z score)	-0.582 ± 0.813	0.382 ± 1.221	<0.0001 ^a
F BMD (g/cm ²)	0.521 ± 0.082	0.642 ± 0.105	<0.0001 ^a
F BMD (Z score)	-0.263 ± 1.233	0.502 ± 1.495	0.0007 ^a
1/3R BMD (g/cm ²)	0.463 ± 0.077	0.562 ± 0.087	<0.0001 ^a
1/3R BMD (Z score)	-1.298 ± 1.170	-0.981 ± 1.187	0.0850
UDR BMD (g/cm ²)	0.264 ± 0.048	0.337 ± 0.073	<0.0001 ^a
UDR BMD (Z score)	-1.427 ± 0.932	-1.222 ± 0.967	0.1678
Total BMD (g/cm ²)	0.826 ± 0.070	0.926 ± 0.089	<0.0001 ^a

BMI, body mass index; Alb, albumin; Alp, alkaline phosphatase; P, phosphorus; Ca, calcium; BUN, blood urea nitrogen; U, urinary; Cr, creatinine; L, lumbar; F, femoral neck; 1/3R, mid-radial; UDR, ultradistal radial

^a*P* < 0.05

Table 5Associations between the presence of vertebral fractures and serum levels of IGF-I and IGFBPs^a

	Presence of vertebral fractures	
	OR (95% CI)	P
IGF-I	0.29 (0.15–0.57)	0.0003 ^b
IGFBP-3	0.31 (0.16–0.61)	0.0007 ^b
IGFBP-4	0.88 (0.55–1.42)	0.6042
IGFBP-5	0.85 (0.54–1.35)	0.4947

^a Multivariate analysis adjusted for age, body weight, height, creatinine, and albumin; unit of change: per SD increase. CI, confidence interval^b $P < 0.05$