— **Regular Article** —

Lumbar Bone Mineral Density and Insulin-like Growth Factor Binding Protein-3 in Japanese Collegiate Women

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The purpose of this study was to investigate the relationship between lumbar bone mineral density (BMD) and insulin-like growth factor binding protein-3 (IGFBP-3) level in 55 collegiate women. In univariate analyses, body weight, body mass index (BMI), waist circumference, maximal oxygen uptake ($\dot{V}O_2$max) in $L \cdot min^{-1}$, and IGFBP-3 level were significantly positively correlated, while serum calcium (Ca) level was significantly negatively correlated with lumbar BMD. Multiple regression analysis was performed with lumbar BMD as a dependent variable and body weight, BMI, waist circumference, $\dot{V}O_2$max in $L \cdot min^{-1}$, and serum levels of IGFBP-3 and Ca as independent variables. Lumbar BMD was significantly positively correlated with body weight, $\dot{V}O_2$max in $L \cdot min^{-1}$, and IGFBP-3 level, while negatively correlated with serum Ca level. The subjects were divided into 3 groups in accordance with IGFBP-3 level. After adjusting for body weight, $\dot{V}O_2$max in $L \cdot min^{-1}$, and serum Ca level in the analysis of covariance, the group with the highest IGFBP-3 had significantly higher lumbar BMD than the lowest group. The results indicate that the known association of IGFBP-3 with lumbar BMD in older adults is already apparent in young women.

Key words —— lumbar bone mineral density, growth hormone, insulin-like growth factor-1, insulin-like growth factor binding protein-3, sex hormone

INTRODUCTION

Growth hormone (GH) is secreted by the pituitary gland and stimulates the synthesis of insulin-like growth factor-1 (IGF-1) by the liver. IGF-1 is also produced locally and has autocrine and paracrine activities. The circulating IGF-1 is the major effector of bone growth and exhibits its mitogenic activity by mediating most of the physiological actions of GH.[1,2] The majority of circulating IGF-1 is bound to IGF binding protein-3 (IGFBP-3), which is the quantitatively predominant IGFBP in circulation. Decreased levels of IGF-1 and IGFBP-3 have been reported in osteoporosis patients.[3,4] Levels of IGF-1 and IGFBP-3 decline with age, however, IGFBP-3 is more strongly associated with the presence of vertebral fractures than IGF-1 and age in postmenopausal women.[5] In some studies, no association has been found between IGF-1 level and bone mineral density (BMD) in men or women,[6–8] while in other studies positive relationships were found between IGF-1 and/or IGFBP-3 level and BMD in men.[9–14] However, these data are mainly based on studies of middle-aged or older individuals conducted in Western countries. Thus, it is difficult to extrapolate these findings to young Japanese women at an age when bone mass reaches its peak.[15]

Many factors related to BMD and/or IGF-1 level have been identified, which include physical fitness,[16] physical activity,[17–19] dietary and nutrient intake,[17–20] body weight,[21,22] cigarette smoking,[22] alcohol intake,[23] and sex hormone.[24] The purpose of this study was to investigate the relationship between IGFBP-3 level and lumbar BMD in young women, controlling or statistically adjusting for these confounding factors.

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MATERIALS AND METHODS

Subjects and Self-administered Questionnaire —— Fifty-five collegiate women were recruited from one university and consented to undergo the study procedure after explanation of the purpose of the study. To be included in the study, they had to meet the following criteria: they were menstruating at normal intervals, ranging from 26–31 days, which falls within the normally accepted range;[25] they drank alcohol less than once a week and even then only had a small amount; they were not on any medication at the time of their participation in the study; and they had never taken birth control pills. The study protocol was approved by the Ethics Committee of Nakamura Gakuen University and informed consent was obtained from each subject.

A few days before blood sampling, information on physical activity was obtained via a self-administered questionnaire. The accuracy of the questionnaire was checked through individual interviews. The frequency, duration, and mode of physical activity were checked, and scores of 1–5 were given according to Young and Steinhardt,[26] who reported a significant correlation ($r = 0.57$) between maximal time on the treadmill and activity scores of 1–5. In brief, the scores 1–5 were as follows: 1 = low activity, 2 = moderate activity, 3 = less than 60 min per week of vigorous activity, 4 = 1–6 hr per week of vigorous activity, and 5 = more than 6 hr per week of vigorous activity.

Measurements —— Body weight and height were measured with the subjects in underwear to the nearest 0.1 kg and 0.1 cm, respectively. The body mass index (BMI) was expressed as weight/height^2 (kg/m^2).

Lumbar BMD (L2–L4) was measured with dual-energy X-ray absorptiometry (Lunar Radiation Co., WI, U.S.A.).

Maximal oxygen uptake ($\dot{V}O_2max$) was measured with a continuous multistage exercise test to volitional exhaustion on a Monark bicycle ergometer. The test was conducted in air-conditioned facilities with the temperature set at 25°C. Ventilatory measurements were made by standard open-circuit calorimetry (Wyvern Software Physiologic Exercise Testing System; P.K. Morgan Instruments, Inc., Andover, MA, U.S.A.) with 30 s sampling intervals. Details of the methods have been presented elsewhere.[27]

Dietary information was collected using a 3-weekday diet record. Each diet was analyzed using a computer program. Each food item was coded according to the Tables of Japanese Foodstuff Composition.[28]

Blood Analysis —— Because GH and IGF-1 levels change throughout the menstrual cycle,[29, 30] blood samples were obtained between days 7 and 9 of the menstrual cycle when estrogen levels were relatively low.[25] Physical exercise was not allowed 48 hr prior to the blood sampling, and beverages other than water could not be consumed 24 hr prior to the blood sampling. Subjects arrived at the laboratory by 08:00. The temperature of the laboratory was set at 25°C. Fasting (12 hr) blood samples were drawn from the antecubital vein after each subject had been seated quietly for at least 20 min. The samples were immediately stored on ice, and kept on ice until centrifugation within 10 min in a refrigerated centrifuge at 4°C. Samples were stored in a freezer at −80°C and were analyzed within 10 days by a local commercial laboratory (SRL Inc., Tokyo, Japan). All measurements were duplicated.

GH,[31] estradiol,[32] progesterone,[33] luteinizing hormone (LH),[34] testosterone,[35] thyroid-stimulating hormone (TSH),[36] IGFBP-3,[37] and IGF-1[38] in serum were measured by radioimmunoassay. Serum sex hormone binding globulin (SHBG), calcium (Ca), inorganic phosphate (IP), and magnesium were measured with immunoradiometric assay,[39] Orthocresolphthalein Complexone,[40] direct molybdenum,[41] and Xylidyl Blue methods,[42] respectively.

Statistical Analysis —— Statistical analyses were performed with SPSS for Windows (Version 17.0J; SPSS Inc., Chicago, IL, U.S.A.). Descriptive statistics included means and S.D. or S.E. Pearson correlation coefficients were used to examine simple correlations between 2 variables. Multiple regression analyses were performed. Because of the small sample size, the number of independent variables included in each model was limited to variables that showed significant correlation in the univariate analyses ($p < 0.05$) with lumbar BMD. In the multiple regression analysis, only variables that significantly ($p < 0.05$) contributed to the R^2 were considered independent determinants of lumbar BMD. The subjects were divided into 3 groups in accordance with IGFBP-3 level in which approximately equal numbers of subjects were included in each group, and then analysis of covariance was performed to adjust mean values for possible influences of confounding factors. Two-sided $p < 0.05$ was considered to be

Table 1. Characteristics of Subjects and Biochemical Values (n = 55)

	Mean ± S.D.	Standard value
Age (year)	20.7 ± 0.9	
Height (cm)	159.6 ± 4.5	158.9 ± 5.3[a)]
Weight (cm)	52.3 ± 5.2	52.3 ± 6.0[a)]
BMI	20.5 ± 1.7	20.7 ± 2.2[a)]
% Fat	26.2 ± 4.0	28.4 ± 8.3[b)]
Waist (cm)	70.4 ± 5.1	65.0 ± 5.9[a)]
$\dot{V}O_2$max ($ml \cdot min^{-1}$)	2129 ± 481	2050 ± 240[a)]
$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.9 ± 9.3	36.4 ± 5.0[a)]
Lumbar BMD (g/cm^2)	1.162 ± 0.116	1.192 ± 0.146[c)]
TSH (μIU/ml)	1.84 ± 2.06	0.34 ~ 3.50[d)]
GH (ng/ml)	10.37 ± 8.28	0.66 ~ 3.68[d)]
IGF-l (ng/ml)	278 ± 63	121 ~ 436[d)]
LH (mIU/ml)	4.0 ± 2.4	1.8 ~ 7.6[d)]
Estradiol (E_2, pg/ml)*	46.0 ± 16.0	11 ~ 82[d)]
Progesterone (P, ng/ml)**	0.6 ± 0.2	(below 1.7)[d)]
Testosterone (TS, ng/dl)***	34.3 ± 13.4	10 ~ 60[d)]
SHBG (nmol/l)	60 ± 21	18.6 ~ 117[d)]
IGFBP-3 (μg/ml)	2.90 ± 0.43	2.29 ~ 4.17[d)]
Ca (mg/dl)	9.2 ± 0.3	8.7 ~ 10.1[d)]
IP (mg/dl)	3.8 ± 0.4	2.4 ~ 4.3[d)]
Mg (mg/dl)	2.2 ± 0.2	1.8 ~ 2.6[d)]

Standard values were taken from *a*) Ref. 44); *b*) Ref. 45); *c*) Ref. 46); *d*) the values reported by SRL Inc.

Table 2. Nutrient Intake of Subjects (n = 55)

Energy (kcal)	1679 ± 407
Protein (g)	56.1 ± 12.3
Fat (g)	56.4 ± 19.4
Carbohydrate (g)	229.5 ± 53.7
Ca (mg)	464 ± 176
Iron (mg)	6.3 ± 2.4
Vitamin A (μg)	428 ± 316
Vitamin B_1 (mg)	0.80 ± 0.30
Vitamin B_2 (mg)	1.10 ± 0.30
Vitamin C (mg)	81 ± 49
Dietary Fiber (g)	10.9 ± 4.4

Mean ± S.D.

Table 3. Pearson Correlation Coefficients between Lumbar BMD and Other Variables (n = 55)

Age	0.072
Height	0.232
Weight	0.452**
BMI	0.384**
Waist	0.323*
$\dot{V}O_2$max ($L \cdot min^{-1}$)	0.332**
$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)	0.135
TSH	−0.009
GH	0.03
IGF-l	0.155
LH	0.003
Estradiol	0.016
Progesterone	−0.11
Testosterone	−0.119
SHBG	0.019
IGFBP-3	0.327*
Ca	−0.411**
IP	−0.235
Magnesium	0.154

*$p < 0.05$, **$p < 0.01$.

statistically significant.

RESULTS

For each physical activity score from 1 to 5, there were 26, 3, 3, 10, and 13 subjects, respectively.

The mean characteristics and biochemical values are shown in Table 1. The subjects were very thin, and none of the subjects had BMI > 25.

The mean nutrient intakes are shown in Table 2.

The Pearson correlation coefficients between lumbar BMD and other variables are shown in Table 3 and Fig. 1. Lumbar BMD was significantly positively correlated with body weight, BMI, waist circumference, $\dot{V}O_2$max in $ml \cdot min^{-1}$, and IGFBP-3 level and negatively correlated with serum Ca

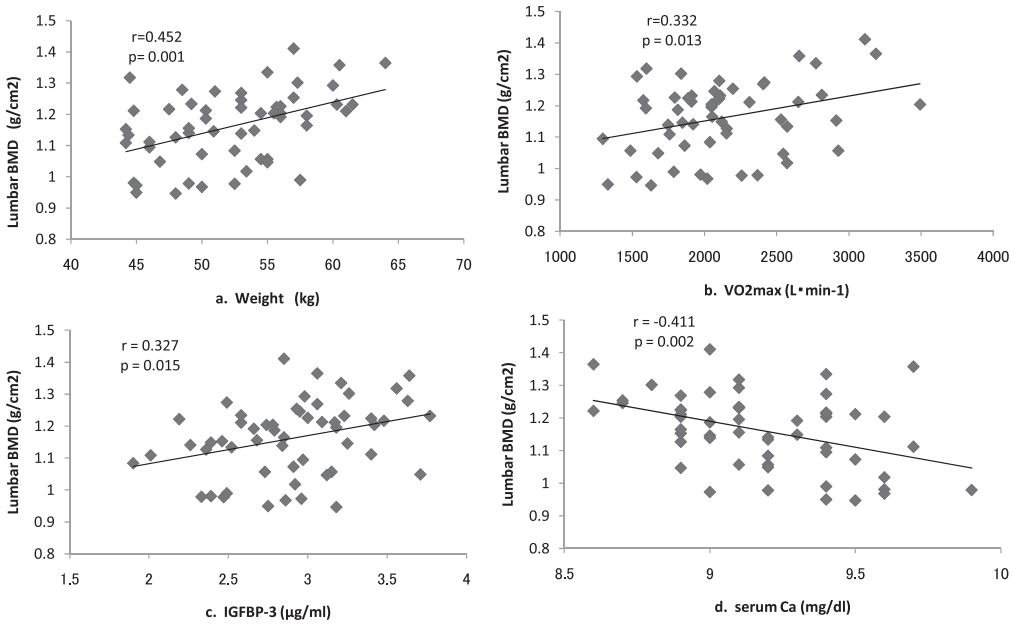

Fig. 1. The Pearson Correlation Coefficients between Lumbar BMD and Body Weight, $\dot{V}O_2$max in $L \cdot min^{-1}$, IGFBP-3, and Serum Ca in 55 Collegiate Women

Table 4. Standardised Partial Regression Coefficient of Determinants of Lumbar BMD (n = 55)

	Body weight	0.214
	$\dot{V}O_2$max ($L \cdot min^{-1}$)	0.288
	IGFBP-3	0.307
	serum Ca	−0.334
R^2		0.430

level. Dietary and nutrient intakes did not significantly correlate with lumbar BMD (data not shown).

When we entered body weight, BMI, waist circumference, $\dot{V}O_2$max in $L \cdot min^{-1}$, IGFBP-3 level, and serum Ca level into the multiple regression analyses as independent variables using lumbar BMD as a dependent variable, body weight, $\dot{V}O_2$max in $L \cdot min^{-1}$, IGFBP-3 level, and serum Ca level remained as significant predictors of lumbar BMD (Table 4).

The subjects were divided into 3 groups in accordance with IGFBP-3 level. After adjusting for body weight, $\dot{V}O_2$max in $L \cdot min^{-1}$, and serum Ca level in the analysis of covariance, the group with the highest IGFBP-3 had significantly higher lumbar BMD than the lowest group (Table 5).

DISCUSSION

Two limitations of our study need to be mentioned. First, the small sample size and the cross-sectional nature of the study do not permit the assessment of causality owing to the uncertain temporality of the association. Second, approximately 99% of circulating IGFs are bound to 6 specific IGFBPs, which modulate IGF action in a positive or negative manner. For example, IGFBP-4 inhibits while IGFBP-5 stimulates IGF actions in bone cells.[3,5] However, we only measured IGFBP-3 level because the majority of circulating IGF-1 is bound to IGFBP-3, which is the quantitatively predominant IGFBP in circulation.

It has been reported that smoking and alcohol intake were inversely associated with IGF-1 levels,[22,23] and smokers had significantly lower BMD.[23] In the present study, to avoid the confounding influence of these factors, only collegiate women who had never smoked and who drank alcohol less than once a week were admitted to the study. In addition, physical exercise was not allowed 48 hr prior to the blood sampling to avoid the acute effects of exercise, and beverages other than water could not be consumed 24 hr prior to the blood sampling. Furthermore, because GH, IGF-1, and estrogen levels change throughout the menstrual cycle,[29,30] blood samples were obtained between days 7 and 9 of the menstrual cycle when estrogen levels were relatively low.[25]

Physical activity,[17–19] and dietary and nutrient intakes, such as Ca, phosphorus, protein, vitamin D, fruit, vegetables, and milk intakes,[17–20] have been reported to influence BMD. It has also been reported

Table 5. Adjusted Mean Values of Lumbar BMD

	IGFBP-3		
	Low (n = 19)	Middle (n = 18)	High (n = 18)
mean	2.43	2.92	3.38
range	(1.90 ~ 2.74)	(2.75 ~ 3.09)	(3.12 ~ 3.77)
Lumber BMD (g/cm^2)[a)]	1.128 ± 0.022	1.160 ± 0.023	1.198 ± 0.022*
range	(0.978 ~ 1.274)	(0.950 ~ 1.411)	(0.947 ~ 1.358)

Mean ± S.E. *a*) Adjusted for body weight, $\dot{V}O_2$max (L · min^{-1}), and serum Ca. $^*p < 0.05$ *vs*. Low.

that androgens and estrogen functioned as independent and additive determinants of trabecular BMD in 30 young women aged 18–22. In the present study, however, activity scores, dietary and nutrient intakes, serum sex hormone, and TSH were not significantly correlated with lumbar BMD. Thus, the influences of these variables appear to be limited. These results may be due, at least in part, to the fact that the subjects had a narrow range of these confounding factors.

Physical fitness[16] and body weight[21,22] are two of the established factors known to be positively correlated with BMD. The results of the present study are in agreement with these studies.

In the present study, serum Ca level was negatively correlated with lumbar BMD even after adjusting for body weight, $\dot{V}O_2$max in L · min^{-1}, and IGFBP-3 level in the multiple regression analyses. This could be due to increased osteoclast activity; however, unfortunately, we did not measure markers of bone resorption, and Ca homeostasis is not well understood.[43]

In some studies on Caucasian populations, no association was found between IGF-1 level and BMD in men or women,[6–8] while in other studies on Caucasian men, positive correlations were found between IGF-1 and/or IGFBP-3 level and BMD.[9–14] Szulc *et al*.[9] reported that IGF-1 level was positively correlated with total hip BMD in men aged 19–65 years. In two other studies, a positive correlation was found between IGF-1 and IGFBP-3 levels and lumbar and/or femoral neck BMD.[10,11] Seck *et al*.[12] conducted a 3.3 year follow-up study on 173 elderly men and 107 postmenopausal women and found an association between IGF-1 level and change in femoral neck BMD in women more than 10 years past menopause. Kim *et al*.[13] reported that IGF-1 and IGFBP-3 levels were positively correlated with lumbar and Ward's triangle BMD in Korean postmenopausal women aged 47–60. Although the different results obtained in these studies could be due, at least in part, to racial and sexual differences, these studies are mainly based on middle-aged or older individuals. The results of the present study, focusing on young Japanese women at an age when bone mass reaches its peak,[15] show that IGF-1 is not significantly correlated with lumbar BMD. However, IGFBP-3 level was positively correlated with lumbar BMD even after adjusting for body weight, $\dot{V}O_2$max in L · min^{-1}, and serum Ca level in the multiple regression analyses. Furthermore, after adjusting for body weight, $\dot{V}O_2$max in L · min^{-1}, and serum Ca level in the analysis of covariance, the group with the highest IGFBP-3 had significantly higher lumbar BMD than the lowest group. These results are in agreement with a previous study[14] that focused on healthy men between 25 and 59 years of age. In that study, it was reported that despite the inclusion of established bone mass determinants in both mechanical forces (muscle strength, weight, and physical capacity) and systemic factors [IGF-1, IGFBP-3, GH, parathyroid hormone (PTH), and testosterone], IGFBP-3 level was the dominating explanatory factor for BMD.

In conclusion, IGFBP-3 level was positively correlated with lumbar BMD even after adjusting for confounding factors. Furthermore, after adjusting for body weight, $\dot{V}O_2$max in L · min^{-1}, and serum Ca level in the analysis of covariance, the group with the highest IGFBP-3 had significantly higher lumbar BMD than the lowest group. The results indicate that the known association of IGFBP-3 with lumbar BMD in older adults is already apparent in young women.

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