# Factors affecting bone mineral density of young women and predictive factors of low bone mineral density

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**Summary**

*Purpose of investigation:* We investigated various factors affecting bone mineral density (BMD) in young women and predictive factors of low BMD. *Methods:* Subjects were 105 nursing school students aged from 19 to 24 years old. Body weight (BW), pituitary hormones, sex steroid hormone and bone turnover markers were selected as factors. BMD was measured at the lumbar spine at L2-L4 by dual-energy X-ray absorptiometry (DXA). *Results:* BW (p = 0.002), serum N-terminal telopeptide of type 1 collagen (NTx) (p = 0.006) and bone specific alkaline phosphatase (BAP) (p = 0.02) were significantly correlated with BMD. For identification of the low BMD group, all subjects were divided into four groups on the basis of BW and NTx concentrations. In the group with BW under 51 kg and Ntx concentrations over 11 nMBCE/l, BMD was significantly (p = 0.0013) decreased compared with the other three groups. In this group, the ratio of women with a low BMD was significantly higher (p = 0.004) than the other groups. *Conclusion:* In young women, BW and bone turnover markers significantly affected BMD. Low BMD can be indicated using BW and NTx concentrations without measurement by DXA.

*Key words*: Young women; Osteoporosis; Bone mineral density; Body weight; Serum N-terminal telopeptide of type 1 collagen; Predictive factor.

## Introduction

As the average life span in Japan continues to increase, the society will continue to become a more aged one. Due to these conditions, osteoporosis is becoming a serious social problem. Current strategies have focused on identifying postmenopausal women who have low bone mineral density (BMD) and who are thus already at risk for fracture. This approach is problematic, because intervention with proven efficacious therapies, while resulting in reduction in fractures over time, cannot eliminate fracture risk entirely if BMD is already significantly reduced at the time of first assessment.

An alternative approach is to focus on premenopausal women. An increase in peak bone mass may contribute to a decrease in the incidence of osteoporosis. Recent reports show that the peak bone mass in Caucasian females and Japanese females occurs at the age of 18 years [1, 2]. For prevention of osteoporosis, young women with a low BMD are identified and offered interventional health education.

We have already reported that body weight and age of menarche are well correlated with BMD [3]. The objective of this study was to evaluate the osteoporosis risk factor among young women. We evaluated the influence of body weight, pituitary hormones, sex hormones and bone turnover markers on BMD. Further, we attempted to identify factors that might indicate low bone mass in young females without measurement of dual-energy X-ray absorptiometry (DXA).

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## Subjects and Methods

### *Subjects*

The subjects were 105 female students enrolled at the nursing school of Wakayama Medical University. Characteristics of the subjects are shown in Table 1.

Table 1. — *Demographic and clinical characteristics of the 105 young women in this study.*

| | Age 1 | BMD 2 | BW 3 | PRL 4 | FSH 5 | E2 6 | NTX 7 | BAP 8 |
|---|---|---|---|---|---|---|---|---|
| Mean | 19.5 | 1.10 | 51.6 | 26.2 | 5.91 | 77.8 | 10.2 | 26.2 |
| SD | 0.878 | 0.117 | 7.54 | 6.48 | 3.06 | 61.3 | 2.52 | 6.48 |
| Range | 19~ 24 | 0.828~ 1.41 | 36.1~ 93.9 | 6.90~ 44.6 | 1.50~ 18.7 | 10.0~ 26.4 | 6.90~ 20.4 | 6.90~ 20.4 |
| Median | 19 | 1.104 | 50.2 | 25.8 | 5.90 | 55.7 | 9.60 | 9.60 |

1 year old; 2 g/cm$^2$; 3 kg; 4 ng/ml; 5 mIU/ml; 6 pg/ml;7 nM BCE/L; 8 U/l.

### *Measurements of BMD*

The BMD of the posterior-anterior lumbar spine at L2-4 was measured by DXA (DPX-NT, GE Co. Utah, USA).

### *Measurements of sex steroids, pituitary hormones and bone turnover markers*

Serum estradiol (E2) levels were measured by electrochemiluminescence immunoassay (ECLIA) and serum prolactin and serum follicular stimulating hormone (FSH) concentrations were measured by chemiluminescent immunoassay (CLIA). We selected serum bone-specific alkaline phosphatase (BAP) and serum N-terminal telopeptide of type 1 collagen (NTx) as bone turnover markers. Serum NTx concentration was measured by an enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody directed against the N-telopeptide intermolecular cross-linked domain of type 1 collagen of bone (Osteomark; Mochida Pharmaceutical Co., Tokyo, Japan).

Serum BAP concentration was measured by the ELISA kit (Osteolinks-BAP; Sumitomo Pharmaceutical Inc., Tokyo, Japan). Blood samples were collected once on a given day for each subject at 12 a.m.

*Identification of the low BMD group*

For selection of the low BMD group, we divided the subjects into the following four groups on the basis of the mean of BW and NTx concentrations. A group: BW over 51 kg, NTx under 10 nMlBCE/l; B group: BW over 51 kg, NTx over ten nMlBCE/l; C group: BW under 51 kg, NTx under ten nMlBCE/l; D group: BW under 51 kg, NTx over ten nMlBCE/l. BMD was compared among the four groups. Further, the ratio of young women who had a low BMD in the various groups was evaluated. Low BMD was identified as being under 0.986 g/m$^2$ (mean-1SD).

*Statistical analysis*

Data analysis was performed using Stat View software (ver. 5, Hulinks, Tokyo, Japan) and a p value of less than 0.05 was considered statistically significant. Correlations between BMD or NTx concentrations and various factors were assessed by univariate analysis using Pearson's correlation coefficients. Independent variables related to BMD or NTx were analyzed by multivariate analysis. Comparison of the four groups (A, B, C, and D) was analyzed by one-way factorial ANOVA and multiple comparison tests. Comparisons between the D group and A, B, and C groups were analyzed by Fisher's exact method.

## Results

The correlation between BMD and various factors by univariate analysis is shown in Table 2. A scatter gram and regression between BW and BMD and NTx and BMD are shown in Figures 1 and 2. BW was significantly ($p < 0.005$) positively correlated with BMD and Ntx was significantly ($p < 0.05$) negatively correlated with BMD. Independent variables significantly related to BMD were evaluated by multivariate analysis and were as follows: BW ($p < 0.005$), NTx ($p < 0.01$) and BAP ($p < 0.05$). Correlation of NTx with various factors by univariate analysis is shown in Table 4. Only prolactin was significantly ($p < 0.05$) negatively correlated with NTx. However, we could not find various factors significantly related to NTx by using multivariate analysis (Table 5).

A significant difference ($p < 0.005$) was found when comparing BMD among the four groups (A, B, C, and D) by using one-way factorial ANOVA. Using a multiple comparison test with Fisher's PLSD, A vs D ($p < 0.001$), B vs D ($p < 0.005$) and C vs D ($p < 0.05$) groups were found to be significantly different. Therefore, the BMD of the D group was significantly lower than the BMD of the other groups (Figure 3).

The ratio of young women with a low BMD was evaluated among the four groups (Table 6). Using Fisher's exact method, the D group had a significantly higher ratio ($p < 0.005$) compared with the other groups. In the C and D groups, 87.5% of young women had a low BMD.

Table 2. — *Correlation between BMD and various factors in univariate analysis.*

| Factors | r* | *p* value |
|---|---|---|
| BW | 0.28 | < 0.005 |
| FSH | 0.096 | n.s. |
| Prolactin | 0.077 | n.s. |
| E2 | 0.13 | n.s. |
| NTx | 0.28 | < 0.005 |
| BAP | 0.179 | n.s. |

*r: correlation coefficient; n.s. = non significant.

Table 3. — *Correlation between BMD and various factors in multivariate analysis.*

| Factors | *p* value |
|---|---|
| BW | < 0.005 |
| FSH | n.s. |
| Prolactin | n.s. |
| E2 | n.s. |
| NTx | < 0.01 |
| BAP | < 0.05 |

n.s. = non significant.

Table 4. — *Correlation of NTx and various factors in univariate analysis.*

| Factors | r* | *p* value |
|---|---|---|
| BW | 0.060 | n.s. |
| FSH | 0.008 | n.s. |
| Prolactin | 0.20 | < 0.05 |
| E2 | 0.14 | n.s. |
| BAP | 0.048 | n.s. |

*r: correlation coefficient; n.s. = non significant.

Table 5. — *Correlation of NTx and various factors in multivariate analysis.*

| Factors | *p* value |
|---|---|
| BW | n.s. |
| FSH | n.s. |
| Prolactin | n.s. |
| E2 | n.s. |
| BAP | n.s. |

n.s. = non significant.

Table 6. — *Ratio of young women with low BMD in the various groups.*

| BMD g/m$^2$ | Group | | | |
|---|---|---|---|---|
| | A | B | C | D |
| ≤ 0.986 (%) | 1 (4.5) | 1 (4) | 5 (14) | 9 (43) |
| > 0.986 | 21 | 24 | 32 | 12 |
| | 22 | 25 | 37 | 21 |

## Discussion

In order to prevent osteoporosis in old age, maintenance of a high BMD is very important. We have previously reported that maintaining an appropriate body weight is crucial for maintaining a high BMD [3].

In this study, serum factors such as pituitary hormones, sex hormone and bone turnover markers were evaluated to determine their association with BMD. E2 is known to be a factor involved in increasing BMD. BMD loss had been attributed to relative estrogen deficiency in postmenopausal women [4], a view reinforced by studies of bone loss after oophorectomy [5] and minimization of

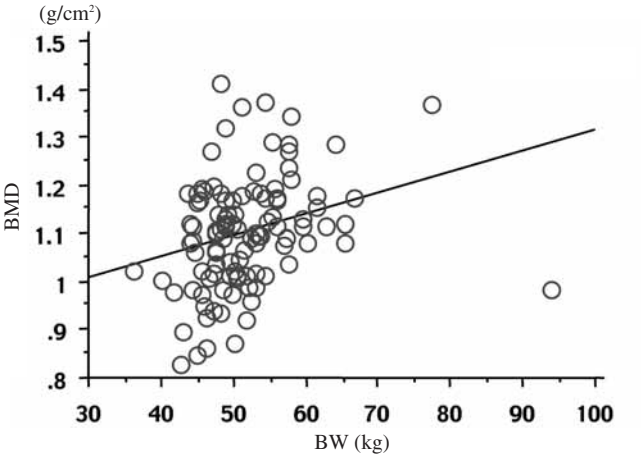


Figure 1. — Correlation between BMD and BW in univariate analysis ($p < 0.005$, R = 0.28).

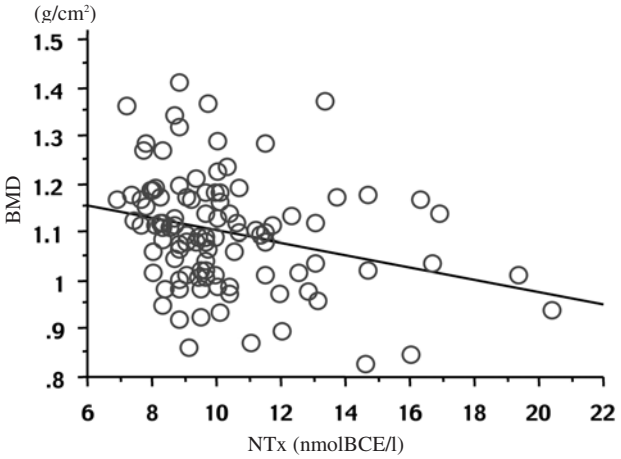


Figure 2. — The correlation between BMD and serum NTx in univariate analysis ($p < 0.005$, $R^2 = 0.28$).

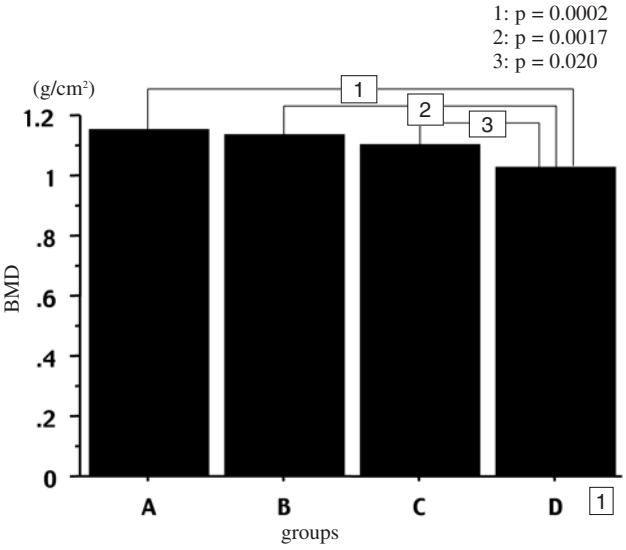


Figure 3. — Comparison of BMD among the four groups.

bone loss with estrogen replacement [6]. On the other hand, prolactin is associated with decreasing BMD; high prolactin levels have been shown to be correlated with a low BMD [7]. FSH is also related to BMD. FSHβ (ligand) and FSH receptor null mice have normal bone mass despite severe hypogonadism, and therefore, FSH might have direct negative effects on bone [8]. However, in our study, FSH, prolactin and E2 were not correlated to BMD by univariate and multivariate analysis. The highest level (44.1 ng/ml) of prolactin in this study did not affect BMD. FSH levels above 26 mIU/ml have been reported to decrease BMD [9]. Because the highest FSH level in our study was only 18.7 mIU/ml, this could be the reason why FSH did not affect BMD in our subjects. BMD loss was reported to be detectable in women whose E2 levels were less than 35 pg/ml [10]. In these women, E2 levels of 28 cases were less than 35 pg/ml. However, in our study, E2 did not influence BMD, which could have been due to the short exposure period to E2.

Serum BAP and NTx were selected as bone turnover markers in our study. BAP levels correspond well with osteoblast activity. The half life of BAP is 3.5 days. Therefore, no diurnal variation has been observed, and it is considered to be a very stable marker. NTx is produced by osteoblasts during bone resorption. Therefore, it is considered to be a bone resorption marker. Previously, NTx was measured in the urine; however, recently, measurement of NTx in serum has become possible. Urinary NTx and serum NTx concentrations are well correlated [11]. In this study, E2, prolactin, FSH and BAP were measured in serum, and therefore, NTx was also measured in serum. The concentration of serum NTx has a diurnal variation, which is highest at 8:00 a.m. and lowest at 12:00 a.m. to 8:00 p.m. [11]. The time of collection of blood in our study was fixed at 12:00 noon when morning school lessons were finished.

The normal concentrations of serum NTx are 7.5-16.5 nM BCE/L in 40-44-year-old women (premenopausal age) and 10.7-24.0 nM BCE/L in 45-79-year-old women (postmenopausal age). However, normal concentrations of NTx in young adult females have not been reported. In this study, we found that NTx concentrations in young females were 5.26 ± 15.1 nM BCE/L (mean, ± 1.96). These NTx concentrations are similar to concentrations in premenopausal females. Serum NTx concentrations are high in patients with Paget's disease and primary parathyroid disease, except in those patients with osteoporosis. In this study, NTx was significantly ($p < 0.005$) negatively correlated to BMD by univariate analysis. However, BW ($p < 0.05$), serum NTx ($p < 0.01$) and BAP ($p < 0.05$) were significantly correlated to BMD by multivariate analysis. The correlation between BMD and NTx in postmenopausal females has been previously reported [12]. To our knowledge, this is the first report of a good correlation of both BMD and NTx in young women. We also investigated factors, which correlated with NTx. Using univariate analysis, a significant correlation ($p < 0.005$) was found between NTx and prolactin.

However, there was no correlation between prolactin NTx by multivariate analysis. Unfortunately, we could not find any factors that affected NTx in this study.

For identifying the low BMD group, we selected two factors, BW and NTx, which show a good correlation with BMD. In this study, BAP was excluded for the sake of simplicity of clinical use. All subjects were divided into four groups according to the mean values of two factors. In subjects whose BW was less than 51 kg and NTx concentrations were over 11 nM BCE/L, BMD was significantly decreased. Further, the ratio of women who had a low BMD in the four groups was evaluated. Low BMD was identified as a BMD reduction of 1 SD from the mean of the study group, because this value is believed to represent a 2-3 fold increase in fracture risk [13]. Women with a low BMD made up 43% of the D group. In addition, in the C group and D group, 88% of women had a low BMD. Measurement of BMD by DXA was required in these groups (C and D) for precise evaluation of BMD. On the other hand, measurement of DXA for the A and B groups was not necessary. In health examinations at school, measurement of BMD for all students (young women) is very difficult. The factors we found in this study are considered to be useful predictors for the selection of students who need measurement of BMD. Even if measurement of BMD is not available, assessment of these factors accurately identifies a group of young women at potentially high risk for subsequent development of postmenopausal osteoporosis. For these young women, health education about osteoporosis, such as lifestyle intervention and risk factor modification, should be given.

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