Association between Current Nutrient Intakes and Bone Mineral Density at Calcaneus in Pre- and Postmenopausal Japanese Women

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(Received January 9, 2001)

Summary The association between nutrient intake and bone mineral density (BMD) at calcaneus was cross-sectionally examined in 243 pre- (aged 29-60 years) and 137 postmenopausal (aged 39-60 years) Japanese women who participated in a BMD checkup and have kept a stable diet for at least 3 previous years and had no dietary therapy. Nutrient intakes were assessed with a self-administered diet history questionnaire. BMD at calcaneus was measured with dual-energy X-ray absorptiometry. In a multiple regression analysis with adjustments for nondietary factors such as age, body height, fat body weight, nonfat body weight, and number of deliveries, calcium (p<0.01) and niacin (p<0.05) significantly and positively, and phosphorus and dietary fiber (p<0.05 for both) significantly and negatively correlated in premenopausal women, and only potassium (p<0.05) significantly and positively correlated in postmenopausal women with BMD. The results suggest that several nutrients correlate with BMD, and the associations differ depending on menopausal status.

Key Words epidemiology, diet, nutrients, bone mineral density, Japanese women

Several epidemiological studies have reported associations between bone mineral density (BMD) and intakes of several nutrients such as calcium (1), protein (2, 3), phosphorus (2, 3), sodium (4), potassium (5, 6), magnesium (5, 6), vitamin C (5, 7), vitamin D (8), niacin (7), dietary fiber (4), alcohol (9), and caffeine (10). The influence of cations such as potassium and magnesium on calcium retention in kidney is of great interest in recent epidemiological studies on nutrient-bone mass association (5, 6). However, most studies conducted in Oriental populations have focused only on calcium (11, 12), and little attention has been paid to other nutrients (13). Moreover few studies have examined a possible differential effect of nutrient intakes to BMD by menopausal status except for calcium (14).

A short-day, mostly 3-day dietary record or simple questionnaires have been used for a dietary assessment in most previous studies conducted in Japanese populations in this issue (11, 13). However, measurement errors caused by the wide day-to-day variation of diets (15) and a lack of validity are serious problems in the former and latter assessment methods, respectively. Some recent studies have used a validated semiquantitative food frequency questionnaire to examine nutrient-bone mass association, avoiding these problems in Western populations (3, 5, 6, 10). We therefore performed a cross-sectional study to examine the associations between BMD and the intakes of several nutrients in Japanese women separated by menopausal status, using a validated semiquantitative food frequency questionnaire.

SUBJECTS AND METHODS

Subjects. Women for this study were recipients of a health checkup on BMD conducted by Hikone City, Japan, in June and July, 1997–99. Women aged 30, 35, 40, 45, 50, 55, and 60 years living in the city were invited to participate in the health checkup each year. A total of 709 women participated during the 3 years. After excluding an elderly subject over the study age range (n=1), a pregnant subject (n=1), subjects after delivery (n=3), and subjects with missing values for percent of body fat (n=15) and number of deliveries (n=216), 473 subjects remained. To include only subjects with stable diets, subjects who had changed their diets within 3 previous years (n=76) and who were taking dietary therapy under the supervision of professionals (n=26) were further excluded. A final count showed that 380 subjects remained and were included in the analysis. Among them, 171, 51, and 158 reported regular menstruation, irregular menstruation, and no menstruation, respectively. A total of 137 subjects who had reported no menstruation for more than or equal to 12 months were considered “postmenopausal,” and the remaining 243 subjects were considered “premenopausal” in this study.

Measurement of BMD. The BMD at right calcaneus was measured by using dual-energy X-ray absorptiometry on a Heel Scan 2000 bone densitometer (ARKRAY Co., Kyoto, Japan). The coefficient of variation of this densitometer was reported to be less than 1.0% (personal communication with ARKRAY Co., 2000).

Anthropometric measurement. Each subject was weighed to the nearest 0.1 kg while wearing normal in-
door clothing, but no shoes. Standing height was recorded as a self-reported value. The percent of body fat was assessed by using a bioelectrical impedance method on a body fat analyzer (TBF-51, TANITA Co. Ltd., Tokyo, Japan). The body impedance was measured by putting the soles of the feet on the two electric poles. This method has a significant correlation with an underwater weighing method and causes minimal measurement errors (16). Fat body weight and nonfat body weight were determined by using the following equations:

\[
\text{Fat body weight (kg)} = \text{body weight (kg)} \times \text{percent body fat/100}
\]

\[
\text{Nonfat body weight (kg)} = \text{body weight (kg)} - \text{fat body weight (kg)}
\]

Assessment of nondietary variables. Age on the day of the health checkup was used in this study. Menstrual status (regular, irregular, or no menstruation), history of bone fracture at any site, current hormonal therapy, number of deliveries, and smoking status were assessed by use of a self-administered questionnaire.

Assessment of nutrient intake. Nutrient intake was assessed with a self-administered diet history questionnaire (DHQ) (17, 18). The subjects were asked to answer the DHQs that were mailed about 2 weeks before the date of the checkup and to submit them at the checkup site. The DHQs were checked by trained public health nurses at the checkup site. Information about the use of vitamin and mineral supplements was included in the DHQ, but it was excluded from the nutrient computation because no reliable food composition table existed for supplements in Japan. Energy and 8 nutrients, i.e., calcium, phosphorus, protein, sodium, potassium, vitamin C, niacin, and dietary fiber, of which the influence to BMD has been suggested in previous studies (1-7), were calculated for each subject which the influence to BMD has been suggested in previous studies (1-7), were calculated for each subject and included in the analysis. Although other nutrients such as vitamin D, magnesium, and caffeine have also been suggested as influences on BMD in previous studies (5, 6, 8), they were not included in the computation because a reliable food composition table was lacking for these nutrients in Japan.

Statistical analysis. First, simple and age-adjusted Pearson correlation coefficients with BMD were computed for nondietary numerical variables such as age (simple correlation only), body height, body weight, fat body weight, and nonfat body weight. For categorical, i.e., dichotomous, variables such as history of bone fracture at any site (yes/no), current hormonal therapy (yes/no), current smoking (yes/no), current alcohol consumption (yes [23 g ethanol [1 “go” in “sake”] or more a day]/no (other)), and current calcium supplement use (yes [once a week or more]/no (other)), mean BMD was compared between the two groups for each variable by t-test and analysis of covariance (ANCOVA), which was used for age-adjusted comparison.

Second, simple and partial Pearson correlation coefficients with BMD were computed for energy and 8 nutrient intakes for which a significant correlation with BMD has been reported in previous epidemiological studies (1-7). Age, body height, fat body weight, nonfat body weight, and number of deliveries that showed a significant correlation with BMD in simple correlation analysis were adjusted for partial correlation coefficients. Energy-adjusted intakes by residual method were used for nutrient intakes (19).

Third, to determine whether any of the nutrients correlate independently with BMD, the 8 nutrients were entered into a linear multiple regression model with backward elimination procedure. The 5 nondiagnostic variables such as age, body height, fat body weight, nonfat body weight, and number of deliveries were entered in the model for adjustment. The nutrients that correlated with BMD at p value, less than 5% error, were remained at each step.

All analyses were performed separately by menopausal status. All statistical analyses were performed with SAS software, release 6.12 (SAS Institute Inc., Cary, NC, USA). P values with less than 5% error were considered statistically significant in all analyses.

RESULTS

Means ± standard deviations for numerical nondietary variables and BMD were shown in Table 1. The mean BMD was 0.80 and 0.74 g/cm² in pre- and postmenopausal women, respectively. Among the variables examined, the mean was significantly different by menopausal status for BMD, age, and body height. A significant correlation with BMD was observed for body weight, fat body weight, nonfat body weight (p<0.001), and number of deliveries (p<0.01) in premenopausal women, and for age, body weight, fat body weight, nonfat body weight (p<0.001), body height, and number of deliveries (p<0.01) in postmenopausal women. The highest degree of correlation was observed for body weight (r=0.40 and 0.52 in pre- and postmenopausal women, respectively). Mean BMD was not statistically different between the two groups, as determined by all categorical variables examined. Age adjustment did not materially alter the results. No categorical variables significantly correlated with BMD.

Table 2 shows means ± standard deviations of daily energy and nutrient intakes. The mean intake was significantly higher in postmenopausal women for protein, calcium, phosphorus, potassium, and dietary fiber (p<0.05). Only potassium in postmenopausal women showed a significantly positive correlation with BMD after adjustment for nondietary variables (r=0.21, p<0.05).

Table 3 shows the results of multiple regression analysis. Calcium (p<0.01) and niacin (p<0.05) significantly and positively and phosphorus and dietary fiber (p<0.05) significantly and negatively correlated with BMD in premenopausal women. Only potassium showed a significantly positive correlation with BMD in postmenopausal women (p<0.05).
### Table 1. Basic characteristics of subjects and their correlates with BMD in pre- and postmenopausal women (n=243 and 137, respectively).

| Numerical variable                        | Premenopausal | Postmenopausal | Pearson correlation coefficient with BMD for numerical and mean BMD difference for categorical variables |
|-------------------------------------------|---------------|----------------|-----------------------------------------------------------------------------------------|
| BMD (g/cm²)                               | 0.80±0.08     | 0.74±0.09***   | —                                                                                     |
| Age (y)                                   | 41.8±7.5      | 55.5±4.0***    | —                                                                                     |
| Age-range (y)                             | (29–60)       | (39–60)        | —                                                                                     |
| Body height (cm)                          | 156.4±3.3     | 154.3±3.8***   | 0.12 ns                                                                 |
| Body weight (kg)                          | 52.5±6.7      | 53.8±6.1 ns    | 0.40***                                                                 |
| Fat body weight (kg)                      | 13.2±4.1      | 14.4±3.8 ns    | 0.38***                                                                 |
| Nonfat body weight (kg)                   | 39.4±3.9      | 39.3±3.5 ns    | 0.32***                                                                 |
| Number of deliveries (times)              | 2.0±0.9       | 2.0±0.9 ns     | 0.17***                                                                 |
| Categorical variable                      |               |                |                                                                                       |
| History of bone fracture (yes/no)         | 18/224        | 12/124 ns      | —                                                                                     |
| Current hormonal therapy (yes/no)         | 14/229        | 6/131 ns       | —                                                                                     |
| Current smoking (yes/no)                  | 15/227        | 2/135 ns       | —                                                                                     |
| Habitual alcohol drinking (yes/no)        | 3/240         | 1/136 ns       | —                                                                                     |
| Current calcium supplement use (yes/no)   | 40/203        | 33/104 ns      | —                                                                                     |

1 2.3 g ethanol (1 "go" in "sake") a day or more.
2 Once a week or more.
3 No mean difference was significant by t-test or analysis of covariance for simple and age-adjusted comparisons, respectively.
4 Abbreviation: BMD=bone mineral density.

Difference between pre- and postmenopausal women by t-test: ns not significant, ***p<0.001.
Significance from null correlation: ns not significant, #p<0.05, ##p<0.01, ###p<0.001.

### Table 2. Means±standard deviations of daily energy and nutrient intakes and their correlation coefficients with bone mineral density in pre- and postmenopausal women (n=243 and 137, respectively).

| Nutrient                  | Mean±standard deviation | Pearson correlation coefficient |
|--------------------------|-------------------------|--------------------------------|
|                          | Premenopausal           | Postmenopausal                 | Premenopausal | Postmenopausal | Partial² |
| Energy (kJ)              | 7.949±2.031             | 7.952±2.064 ns                 | 0.04 ns       | 0.05 ns        | 0.03 ns   | 0.06 ns   |
| Protein (g)              | 70.7±10.9               | 77.3±13.1***                   | −0.05 ns      | −0.01 ns       | 0.10 ns   | 0.14 ns   |
| Calcium (mg)             | 687±216                 | 816±206***                     | 0.03 ns       | 0.07 ns        | 0.14 ns   | 0.15 ns   |
| Phosphorus (mg)          | 1,095±211               | 1,233±228***                   | −0.03 ns      | 0.01 ns        | 0.12 ns   | 0.13 ns   |
| Sodium (mg)              | 3,814±964               | 3,940±1,156 ns                 | −0.08 ns      | −0.02 ns       | 0.07 ns   | 0.03 ns   |
| Potassium (mg)           | 2,527±592               | 2,875±649***                   | −0.01 ns      | 0.01 ns        | 0.16 ns   | 0.21 #    |
| Vitamin C (mg)           | 129±83                  | 154±73***                      | 0.03 ns       | 0.04 ns        | 0.07 ns   | 0.10 ns   |
| Niacin (mg)              | 14.6±3.5                | 16.2±4.4***                    | −0.01 ns      | 0.03 ns        | 0.06 ns   | 0.11 ns   |
| Dietary fiber (g)        | 13.2±3.5                | 15.5±4.3***                    | −0.10 ns      | −0.10 ns       | 0.03 ns   | 0.07 ns   |

1 Nutrient intake was adjusted for total energy intake by the residual method.
2 Adjusted for age, body height, fat body weight, nonfat body weight, and number of deliveries.

Difference between pre- and postmenopausal women by t-test: ns not significant, **p<0.01, ***p<0.001.
Significance from null correlation: ns not significant, #p<0.05, **p<0.01, ***p<0.001.
significant associations of urinary excretion of sodium and calcium excretion. Some previous studies have reported minerals that have shown a relationship with urinary intake level is a determinant factor of BMD, as observed only in premenopausal women, among whom calcium in the guts, dietary fiber may be an important factor women.

intake to bone mass between pre- and postmenopausal this study supported the differential role of calcium intake to BMD observed only in premenopausal women (22-24). A significantly positive association of calcium BMD in postmenopausal women remains controversial the relationship between current calcium intake and peak bone mass in and/or current calcium intake and BMD observed in a multiple regression analysis in premenopausal women. Although the observed association is biologically plausible and some previous epidemiological studies in Western populations reported the association (2, 3), to our knowledge this study is the first to observe the significant association after the adjustment of confounding factors in an Oriental population.

A significantly positive correlation between niacin and BMD was observed in premenopausal women. One study reported a similar positive correlation in postmenopausal women (7). Since niacin has received little attention in other studies, evidence is insufficient to determine whether the observed association was causal or by chance.

Some nutrients such as protein, sodium, and vitamin C did not significantly correlate with BMD either in correlation or multiple regression analyses. A very high level of correlation was observed between the intakes of protein and phosphorus (correlation coefficient = 0.84 in pre- and 0.90 in postmenopausal women). This might have made difficult the examination of an independent association of protein, or phosphorus, to BMD in this population. Urinary calcium loss induced by high sodium intake is of great interest (25). However, a relatively poor validity of sodium intake in the questionnaire used in this study (18) might have obscured the results. A correlation between vitamin C intake and BMD is inconsistent in previous studies (5, 7, 28). More study is needed for this nutrient.

DISCUSSION

This is, to our knowledge, the first cross-sectional study that has shown differential associations of several nutrient intakes to BMD between pre- and postmenopausal Japanese women.

In contrast to a positive association between teenage and/or current calcium intake and peak bone mass in premenopausal women (14, 20, 21) and a significant association of a retrospectively assessed calcium intake during adolescence with BMD after menopause (22), the relationship between current calcium intake and BMD in postmenopausal women remains controversial (22-24). A significantly positive association of calcium intake to BMD observed only in premenopausal women in this study supported the differential role of calcium intake to bone mass between pre- and postmenopausal women.

Since dietary fiber interferes with calcium absorption in the guts, dietary fiber may be an important factor only in premenopausal women, among whom calcium intake level is a determinant factor of BMD, as observed in the present and previous studies (5).

Sodium (25), potassium, and magnesium (26) are minerals that have shown a relationship with urinary calcium excretion. Some previous studies have reported significant associations of urinary excretion of sodium (4) and intakes of magnesium (5, 6) to BMD. Three cross-sectional studies have reported a significant association between potassium intake and BMD (5, 6, 27). The present study observed a significant association with potassium intake only in postmenopausal women.

A significantly negative correlation of phosphorus intake to BMD was observed in a multiple regression analysis in premenopausal women. Although the observed association is biologically plausible and some previous epidemiological studies in Western populations reported the association (5, 6), in our knowledge this study is the first to observe the significant association after the adjustment of confounding factors in an Oriental population.

Table 3. Multiple regression analysis with backward elimination procedure for bone mineral density (g/cm²).

|                        | Pre-menopausal women | Post-menopausal women |
|------------------------|----------------------|-----------------------|
|                        | (n=243)              | (n=137)               |
| **R²**                 | 0.24                 | 0.42                  |
| Intercept              | 0.793                | 0.978                 |
| Age (y)                | -0.002*              | -0.006***             |
| Body height (cm)       | -0.001               | -0.004*               |
| Fat body weight (kg)   | 0.006***             | 0.002                 |
| Nonfat body weight (kg)| 0.004*               | 0.015***              |
| Number of deliveries   | 0.016**              | 0.016*                |
| (times)                |                      |                       |
| Calcium (mg/d)         | 0.0002**             | 0.0002*               |
| Phosphorus (mg/d)      | -0.0002*             |                       |
| Potassium (mg/d)       | -0.005*              | 0.0002*               |
| Niacin (mg/d)          | -0.004*              |                       |
| Dietary fiber (g/d)    |                      |                       |

1 Partial regression coefficient.
2 Protein, sodium, and vitamin C were also entered in the first models and did not remain in the final models.
3 Only nutrients of which partial regression coefficients were significant (p<0.05) were included in the final models.
4 Five nondietary variables were included in the models for adjustment.
5 Nutrient intake was adjusted for total energy intake by the residual method.
Abbreviation: R² = determination coefficient.
Significance level: *p<0.05, **p<0.01, ***p<0.001.

Several important factors such as physical activity level (2, 11) and past calcium intake level (22) were not included in this study, and physical activity level with reasonable validity was not obtained. However, since a highly positive correlation between lean body mass and daily total energy expenditure (r=0.53, n=167) observed in one study suggested a possibility of nonfat body weight as a surrogate of physical activity level
In conclusion, this study reported the differential associations of several types of nutrients to BMD between pre- and postmenopausal women, which were generally in agreement with the theoretical roles of each nutrient to calcium balance in the body. But several possible risk and preventive factors were not included in the analysis, and the number of subjects in the study was relatively small. Further studies using a better study design and a larger sample size are necessary to examine the possible associations of several nutrients with BMD that may differ by menopausal status.

Acknowledgments

We are grateful to Ms. Yoshiko Imamura and Ms. Miwae Deguchi for the data collection, to Drs. Kelko Amano and Akane Katagiri for the data processing, and to Ms. Yoshiko Takahashi for the manuscript preparation.

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