Intakes of Calcium and Other Nutrients Related to Bone Health in Japanese Female College Students: A Study Using the Duplicate Portion Sampling Method

Kimiko Ueno, Kazutoshi Nakamura,¹ Tomoko Nishiwaki, Toshiko Saito,² Yoko Okuda¹ and Masaharu Yamamoto¹

Department of Nursing, School of Health Sciences, Niigata University, ¹ Department of Community Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, and ² Department of Health and Nutrition, Niigata University of Health and Welfare,

Niigata, Japan

UENO, K., NAKAMURA, K., NISHIWAKI, T., SAITO, T., OKUDA, Y. and YAMAMOTO, M. Intakes of Calcium and Other Nutrients Related to Bone Health in Japanese Female College Students: A Study Using the Duplicate Portion Sampling Method. Tohoku J. Exp. Med., 2005, **206** (4), 319-326 — The purposes of this study were to determine intakes of nutrients related to bone health, such as calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and protein, in Japanese female college students, using the duplicate portion sampling method, and to identify possible lifestyle factors explaining their calcium intakes. Subjects were 106 Japanese female college students aged 19-23 years. All foods in the duplicate portions, as eaten by the subjects during a three-weekday period, were collected. The minerals and protein in the food samples were analyzed. Life-style information was obtained by interview. Levels of cognitive eating restraint (CER) were assessed by the Three-Factor Eating Inventory. Average intakes of dietary Ca, P, Na, K, and protein were 380 (SD 209) mg/day, 649 (SD 212) mg/day, 2,535 (SD 847) mg/day, 1,108 (SD 429) mg/day, and 41.7 (SD 12.6) g/day, respectively. Ca intake was significantly associated with the frequency of milk intake ($R^2 = 0.278$, p < 0.001), intake of other dairy products ($R^2 = 0.338$, p < 0.001), and meal skipping ($R^2 = 0.161$, p < 0.001), but not with CER score (p = 0.378). Female college students are at high risk for poor Ca nutrition. Low intake of nutrients relevant to bone health is considered to adversely affect bone metabolism in young women. bone health; dietary calcium; duplication sampling method; female college student; minerals

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Calcium (Ca) is an essential nutrient for bone metabolism, and insufficient intake may result in weak bones, especially for women who have a greater risk of osteoporosis later in life. This point should be emphasized for young women who need to maximize their peak bone mass. Nonetheless, young Japanese women reportedly have lower Ca intakes than their older counter-

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Correspondence: Kimiko Ueno, RN, MA, Department of Nursing, School of Health Sciences, Niigata University 2-746, Asahimachi-dori, Niigata City 951-8518, Japan.

e-mail: uenok@clg.niigata-u.ac.jp

parts (Ministry of Health, Labour and Welfare, Japan 2003). Female college students are suggested to be at high risk of insufficient calcium intake. They have lifestyles that are often characterized by eating out, irregular food intake patterns, skipping meals, and dieting (Wong and Huang 1999). In addition, not a few college students live apart from their parents who once supervised their eating.

Accurate estimation of Ca intake is more difficult in Japanese than in Western populations, because Ca sources are more variable in the Japanese than in the Western diet. The National Nutrition Survey showed that 30% of Ca in the Japanese food supply is from milk products (Ministry of Health, Labour and Welfare, Japan 2003), while 73% of Ca intake in the United States is from dairy sources (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 1997). The National Nutrition Survey showed that beans, fish, grains, and green vegetables are also important Ca sources in Japan, the combination of which accounted for about 44% of Ca intake.

We used the duplicate portion sampling method, which is considered to be the most reliable way to accurately determine Ca consumption by young women. The duplicate portion sampling method also provides additional information on other nutrients related to bone metabolism, such as phosphorus (P) (Bales and Anderson 1995), sodium (Na) (Massey and Whiting 1996), potassium (K) (Tucker et al. 1999), and protein (Rizzoli et al. 1998).

The purpose of this study was to determine the intakes of Ca, P, Na, K, and protein in Japanese female college students using the duplicate portion sampling method. We also investigated possible lifestyle factors explaining their calcium intakes. These data will provide valuable information on bone health in female college students.

METHODS

Subjects were female second- and third-year students enrolled in the Nursing Course at Niigata University. Of the total 148 female students, 112 (75.7%) agreed to participate in this study. We excluded 6 subjects from analysis: three subjects who were older than 25 years were considered to be outliers; two subjects were on a strict diet involving special meals; and one subject consumed special meals with considerable calcium supplementation as a medication. Informed consent was obtained from all subjects. The protocol of this study was approved by the Ethics Committee of Niigata University School of Medicine.

This study was conducted between October 28 and December 20, 2002. We used the duplicate portion sampling method to estimate the subjects' dietary intakes. For the subjects to maintain a regular eating pattern, we had a 30-minute explanatory meeting prior to the study. This was to explain the overview and purposes of the present study. Thereafter the following instructions were given: 1) the subjects should provide a duplicate of all foods and drinks that they consumed except for tap water and tea without milk during the designated threeweekday period (Tap water in Japan is soft [Yasui et al. 1998; Nakamura et al. 2003], i.e., Ca intake from tap water is negligible.), 2) foods on one dish should be put in one plastic bag (liquid food was put in a freezer bag), 3) all daily meal samples should be put in an opaque bag, and submitted the next day in an opaque box to prevent its contents from being seen by others, 4) the subjects should provide routine meals, not special meals for a special occasion, because an unusual meal would not be of benefit to either the subjects or the researchers, 5) the subjects should not change their eating pattern especially for milk and milk products, small fish, beans and bean products, collared vegetables, which are important sources of dietary Ca, and 6) all of the provided meals were purchased by the researchers.

Before food analysis, we recorded the frequencies of skipped meals, milk consumption and eating dairy foods other than milk, such as cheese, yogurt, and vanilla ice cream, by checking food samples collected from each subject. Any inedible components were removed. Food samples were completely mixed and homogenized with a powerful food blender (Blender LBC-10, Waring Products, Inc., Torrington, CT, USA). These samples were dried at 110°C. Ca, Na, and K in the samples were determined with an atomic absorption spectrophotometer. Dietary P was determined by the spectrophotometric molybdovanadate method. Nitrogen was measured by the micro-Kjeldahl method, and protein intake was converted to the protein equivalent by multiplying the nitrogen content by 6.25. All of these nutritional measurements, including pre-treatment of the sample, were carried out using the standardized method of food analysis by the NAC Co., Ltd. (Tokyo). The intra- and inter-assay coefficients of variation were 2.6% and 1.2% for Ca, 0.8% and 1.4% for P, 1.3% and 1.6% for Na, 5.2% and 3.0% for K, and 0.7% and 1.8% for protein, respectively. We did not measure total energy, but obtained the total dry weight for a meal, which might well reflect total energy. To validate this, we directly measured the energy in 10 random samples from the mixed diets of the 106 subjects. The correlation coefficient between the total dry weight and the total energy directly measured was 0.995.

Subject age, age at menarche, lifestyle information, including smoking and drinking habits, and eating behaviors were recorded through interview. Tendency to restricted eating was evaluated by using the score for the cognitive eating restraint (CER) factor in the Three-Factor Eating Inventory (Stunkard and Messick 1985). The CER factor consists of 21 items (0 or 1 point for each item), the maximum CER level being 21 points and the minimum 0 points. Physical activity was quantified by the METS (ratio of working metabolic rate/resting metabolic rate) index using the 7-day-recall questionnaire (Sallis et al. 1985) through an interview. Body height and weight were measured and body mass index was calculated as weight (kg) divided by the square of height (m²).

Normality of distribution was assessed for all the continuous variables. Because the distributions of intakes of dietary calcium and protein were skewed to higher values, these two variables were logarithmically transformed for statistical tests. Single and multiple regression analyses were used to find determinants of calcium intake. PC - SAS version 6.12 for Windows was used for the computation.

RESULTS

Characteristics of the 106 subjects are shown in Table 1, in which mean values for demographic data, physical activity levels, and the CER score are presented. Other lifestyle characteristics are as follows. Eighty-five (80.2%) subjects were non-drinkers and the rest occasional drinkers. Ninety-four subjects (88.7%) were non-smokers, six (5.6%) were occasional smokers, and the other six (5.6%) smoked daily. Forty-four (41.5%) subjects were second-year and 62 (58.5%) were third-year nursing students. Eighty-five (80.2%) subjects lived alone apart from their families. Frequency distributions of skipped meals, milk intake, and other dairy foods intake during the three-weekday period of sampling (9 meals in total), are shown in Table 2. About one third of the subjects skipped meals at least once in the 3-day period. Sixty-two percent of the subjects did not have milk during that period.

Intakes of dietary Ca, P, Na, K, and protein and the Ca/P molar ratio in the 106 subjects are shown in Table 3. Three subjects took Ca supplements of 85, 250, and 95 mg/day. These were included in their total calcium intakes per day. Medians for Ca and protein were added because the two variables did not have a normal distribution. The mean total dry weight of 294 (g/day) was equivalent to 1,336 (kcal/day) of energy. Ca intakes did not differ significantly either between

	Mean	Mean S.D.	
Age (years)	20.5	0.7	19-23
Age at menarche (years)	12.0	1.3	8-16
Body height (cm)	158.8	5.0	145.2-169.4
Body weight (kg)	52.7	6.0	40.3-69.4
Body mass index (kg/m ²)	20.9	2.2	16.6-27.6
METS index [*]	233.8	9.9	216-286
CER [†] score	7.7	4.2	0-20
	(Median, 7)		

TABLE 1.	Characteristic	cs of the	106	subjects

*METS index is a multiple of the resting metabolic rate in the last 7 days. *CER, Cognitive eating restraint in the Three-Factor Eating Inventory.

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	0	1	2	3	4	5	6
Skipped meal	70	16	10	8	0	2	0
	(66.0%)	(15.1%)	(9.4%)	(7.6%)	(0%)	(1.9%)	(0%)
Milk	66	21	8	7	4	0	0
	(62.3%)	(19.8%)	(7.6%)	(6.6%)	(3.8%)	(0%)	(0%)
Dairy foods	23	22	18	20	12	6	5
	(21.7%)	(20.8%)	(17.0%)	(18.9%)	(11.3%)	(5.7%)	(4.7%)

 TABLE 2. Frequency distributions of numbers of skipped meals, milk intake, and other dairy food intakes in 9 sampled meals during the three-weekday period

TABLE 3. Intakes of dietary nutrients in the 106 subjects during a three-weekday period

	Mean	S.D.	Range
Calcium (Ca) (mg/day)	380 (Median, 332)	209	66-990
Phosphorus (P) (mg/day)	649	212	249-1,209
Ca/P molar ratio	0.431	0.133	0.189-0.958
Sodium (Na) (mg/day)	2,535	847	832-5,966
Potassium (K) (mg/day)	1,108	429	281-2,393
Protein (g/day)	41.7 (Median, 40.6)	12.6	20.2-76.9
Total dry weight (g/day)	294	72	144-488

TABLE 4. Correlation coefficients between dietary calcium (Ca), phosphorus (P), Ca/P ratio, sodium (Na), potassium (K), protein and the total dry weight

	Ca	Р	Ca/P ratio	Na	K	Protein
Р	0.865 [‡]					
Ca/P ratio	0.827^{\ddagger}	0.481‡				
Na	0.410‡	0.453‡	0.185			
Κ	0.728^{\ddagger}	0.805‡	0.404‡	0.459 [‡]		
Protein	0.736‡	0.908‡	0.303 [†]	0.498‡	0.740^{\ddagger}	
Dry weight	0.625*	0.770*	0.257^{\dagger}	0.592‡	0.660*	0.810‡

*Ca and protein are transformed logarithmically. $^{\dagger}p < 0.01$. $^{\ddagger}p < 0.001$.

the second- and third-year students (p = 0.083) or between those living with versus apart from their parents (p = 0.509). None of the nutrients correlated significantly with age, height, weight, BMI, or METS index.

A correlation matrix for dietary nutrients is shown in Table 4. Dietary calcium correlated highly with P (r = 0.865, p < 0.001), the Ca/P ratio (r = 0.827, p < 0.001), K (r = 0.728, p < 0.001), and protein (r = 0.736, p < 0.001), and moderately with total dry weight (r = 0.625, p < 0.001). Dietary P correlated highly with K (r = 0.805, p < 0.001) and protein (r = 0.908, p < 0.001).

Associations between dietary Ca and dietary habits were tested by single regression analysis. Dietary Ca was negatively associated with the frequency of skipping meals ($\mathbb{R}^2 = 0.161$, p < 0.001), and positively with the frequency of consuming milk ($\mathbb{R}^2 = 0.278$, p < 0.001) and eating dairy foods (except for milk) ($\mathbb{R}^2 = 0.338$, p < 0.001), during the three days of sampling, but not with the CER score (p = 0.378). When the associations between dietary Ca and the above three significant variables were tested using multiple regression analysis, consuming milk ($\mathbb{R}^2 = 0.200$, p < 0.001), eating dairy foods ($\mathbb{R}^2 = 0.183$, p < 0.001), and skipping meals ($\mathbb{R}^2 = 0.161$, p = 0.032) were all statistically significant.

DISCUSSION

To our knowledge, few studies using the duplicate sampling method have focused on dietary nutrients in young Japanese women. We should first discuss the validity of this method, despite its high analytical reliability. The estimated mean energy (1,336 kcal/day) in this study was lower than the national average (1,752 kcal/day, as assessed by the food record method) (Ministry of Health, Labour and Welfare, Japan 2003), raising the possibility of underestimation of dietary intake in this study. The duplicate sampling method is quite comparable to the weighed food record method. Both methods tend to underestimate usual dietary intake to some extent, especially energy (Gibson 1990), and the difference in energies obtained by the two methods may be about 7% (Dwyer 1999). Considering this information, the low mean energy found in this study would accurately be attributable to low dietary intake rather than an extreme underestimation.

Even considering the inherent underestimation of dietary intake by the present duplication sampling method, the observed mean Ca intake of 380 mg/day (median, 332) in Japanese college women is very low. This amount is much lower than both 457 mg/day in women aged 20-29 years determined by the National Nutrition Survey in 2001 (Ministry of Health, Labour and Welfare, Japan 2003), which used a dietary record method, and the recommended dietary allowance (RDA) of 600 mg/day suggested by the Ministry of Health, Labour and Welfare of Japan (2003). The National Nutrition Survey also showed that Ca intake in women 20-29 years old is the lowest of all age groups (Ministry of Health, Labour and Welfare, Japan 2003), suggesting women in their early twenties to be the lowest Ca intake generation in a woman's life.

Asians are generally considered to have lower Ca intakes than Europeans and North Americans. Investigators have reported mean Ca intakes of pre-menopausal adult women in Asian countries, such as Taiwan, Korea, and Thailand, to be 392-648 mg/day (Moon et al. 1997; Shimbo et al. 1997; Matsuda-Inoguchi et al. 2000). However, there have been a relatively few reports on Ca intake in young Asian women. One study reported Ca intake of Chinese women in their 20s, a value of 459 mg/day (Ho et al. 1997).

Ca intakes in young women in Europe and North America also appear to be insufficient. Despite an adequate Ca intake of young adults estimated by the Institute of Medicine to be 1,000 mg/day, the 1994 Continuing Survey of Food Intake of Individuals (CSFII) data and the Third National Health and Nutrition Examination Survey (NHANES III) showed the Ca intakes of women in their 20s in the United States to be only 647 (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 1997) and 768 mg/day (Bialostosky et al. 2002), respectively. Average Ca intakes of young women aged 20-23 years in six European countries ranged from 680 to 1,236 mg/day (Kardinaal et al. 1999). In comparison with these data, the Ca intakes of our subjects were much less; approximately half of that of young female populations with low Ca intake in Western countries.

The low calcium intake in this population would be due mainly to infrequent intake of dairy foods. Ninety percent of the subjects, on average, drank milk and 59% consumed some other dairy food less than once a day (i.e., < 3 times during the 3 days). In particular, those who consumed no milk during the three days accounted for 62% of the subjects. In addition, young women generally have a tendency for dieting, including skipping meals and/or restricted eating which seem to be more common than in other generations (Nakamura et al. 1999). The subjects of this study may not be an exception. One-third experienced skipping meals, which explained 16% of the variation in Ca intake. The total amount of foods eaten was considered to be unsatisfactorily small. This amount is comparable to that consumed by another group of individuals we previously studied using the same method (Nakamura et al. 2003). The mean total dry weight of foods consumed by the subjects in this study is calculated to be three fourths of that of elderly women living in a nearby community. Intakes of all the nutrients measured herein were also low, which indirectly supports the above finding (detailed discussion below). We believe that both infrequent intakes of dairy products and lower food intakes are responsible for the low Ca intake in this population.

Adequate intakes of P, Na, K, and protein are needed to maintain normal bone metabolism. Mean values of such nutrients as P (649 mg/day), Na (2,535 mg/day), K (1,108 mg/day), and protein (41.7 g/day) were apparently lower than the reference values (920 mg/day for P, 4173 mg/day for Na, 2,036 mg/day for K, and 65.8 g/day for protein) of Japanese women in their 20s provided by the National Nutrition Survey (Ministry of Health, Labour and Welfare, Japan 2003), although the analytical methods were not the same. They were also lower than those (1,142 mg/day)for P, 3,207 mg/day for Na, 2,321 mg/day for K, and 70 g/day for protein) of U.S. women in their 20s provided by NHANES III (Bialostosky et al. 2002). As shown in Table 4, most nutrients had high correlations with total dry weight, suggesting that the lower intakes of these nutrients may well be attributable to consumption of smaller amounts of food. Low intakes of P, K, and protein may have unfavorable effects on bone health. Especially, low protein intake, i.e., 41.7 g/day, may be more problematic and affect bone metabolism. Cooper et al. (1996) showed protein intake to be a determinant of bone mass in pre-menopausal women (mean protein intake, 75 g/day). The low protein intake in our subjects may be largely due to their eating smaller amounts of food in regular meals. Also, judging from the dietary samples collected, the subjects appeared to eat considerable amounts of snack foods rich in carbohydrates and fat as offsets.

Energy intake in the present study was apparently low compared to the national average of young women in their 20s (Ministry of Health, Labour and Welfare, Japan 2003). A nutrition survey using the dietary record to target Japanese second-year female college students reported that their mean energy and protein intake were low, at 80% and 65%, respectively, of the national average in 1997 (Wakamoto et al. 2000). The findings of their report are very similar to ours, suggesting low dietary intake to be prevalent in college female students.

There were some other limitations in this study. First, the subjects of this study were not a random sample of the entire female college population of Japan, and thus a selection bias may exist, although the subject participation rate in this study was acceptably high (76%). To assess this type of bias, the following eating-related parameters could be compared between this population and references. The mean BMI of the subjects was 20.9, whereas the national average of women aged 20-29 years is 20.5, indicating that the subjects of this study are unlikely to be slimmer. Additionally, the mean CER score of 7.7 for our subjects was similar to the 7.7 in college females reported previously (Kitagawa et al. 1996) and the 7.1 we recently found in another normal Japanese female college student population (unpublished data). These data suggest that there is no evidence that the present subjects had a greater tendency for restricted eating than other young female college students. Secondly, we collected meals in a three-weekday period, but did not assess meals at the weekends. The subjects in this study had a relatively low energy intake in comparison with their BMI, suggesting they might have a greater amount of food at the weekends. These limitations should be taken into account in the interpretation of this study.

It should be noted that the results of this study may not necessarily be generalizable to all women in their early 20s, because we have no information about possible discrepancies in eating patterns between students and non-students. Furthermore, because the general nutritional status in the present subjects was not in accordance with the data of the national survey, it may not be appropriate to generalize the results to all Japanese young women. Rather, it would be more appropriate to apply the results to female college students. Furthermore, the results are probably most applicable to those in professional schools, such as nursing schools.

In summary, the present study using the duplicate sampling method obtained the following findings. 1) The mean intakes of Ca, protein, P, and K in young Japanese female college students were low at 380 mg/day (median, 332), 41.7 g/day (median, 40.6), 649 mg/day, and 1,108, respectively. 2) The low Ca intake was due to a combination of infrequent dairy product consumption and smaller food intakes. It is concluded that female college students are a population who are at a high risk of low Ca nutrition. Additionally, the low nutrient intakes relevant to bone health may adversely affect bone metabolism in young women, and thus merit further investigation.

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