Original Article

Intake of dairy products and bone ultrasound measurement in late adolescents: a nationwide cross-sectional study in Japan

Kazuhiro Uenishi PhD[1], Kazutoshi Nakamura MD[2]

[1]*Laboratory of Physiological Nutrition, Kagawa Nutrition University, Saitama, Japan*
[2]*Department of Community Preventive Medicine, Niigata University, Graduate School of Medical and Dental Sciences, Niigata, Japan*

Introduction: There is little evidence regarding the effects of dairy product intake on bone mineralization among late adolescents, especially in Asians. The aim of this study was to determine the association between dairy product intake and bone strength as measured by quantitative ultrasound (QUS) in a large Japanese population. Methods: Subjects were 38,719 high school students (14,996 males and 23,723 females) across 33 prefectures in Japan. Bone stiffness of the calcaneus was measured by QUS densitometry (AOS-100, Aloka). Subjects were given a self-administered questionnaire, which included questions on gender, age, height, weight, consumption of dairy products, and levels of physical activity. Intake of milk and yogurt were classified as none, 1-99, 100-199, 200-399, and ≥400 ml/day. Results: The proportion of subjects who consumed milk 400 ml/day or more was 21% in males and 7.3% in females, while 24% of males and 41.1% of females did not consume milk. After adjusting for physical activity, weight, gender, age, and area of residence, milk intake (R^2=2.8%, p<0.001) and yogurt intake (R^2=0.1%, p<0.001) were independently associated with the QUS measurement. Similar associations were found in males and females when a gender-stratified analysis was conducted. Conclusion: We found a positive dose-effect relationship between milk intake and bone strength in late adolescents, to whom we recommend milk intake of 400 ml/day or more to obtain greater bone mass.

Key Words: adolescent, bone density, calcium, dairy products, quantitative ultrasound measurement

INTRODUCTION

Dairy products, especially milk from cows, are an important source of calcium (Ca), which is essential for maintaining normal skeletal growth in children and adolescents. Many observational and interventional studies have shown that adequate Ca intake is required to attain maximal peak bone mass.[1,2] In addition, several researchers have demonstrated a favorable effect of increased milk and other dairy intake on bone parameters.[3-8] However, Lanou et al.[9] conducted a systematic review that revealed insufficient evidence to support the idea that increased intake of milk or other dairy products has a favorable effect on promoting bone mineralization in children and adolescents. They also pointed out that such evidence is scarce in non-Caucasian children and adolescents.

Dairy products are not popular in Japan. However, almost all elementary schools and most junior high schools provide students with a bottle of milk (200 ml) as part of the school lunches, which is supervised by a registered dietician. This enables students to maintain a relatively high Ca intake. The median Ca intake of students in the age groups 6-8, 9-11, and 12-14 years old were 614, 706, and 788 mg/day in males and 586, 659, and 656 mg/day for females, respectively.[10] However, high school students are rarely provided with school lunch (i.e., milk) and, therefore, their Ca intake is lower. The median Ca intake of students 15-17 years old was 570 mg/day in males and 467 mg/day for females.[10]

Despite ample evidence regarding the positive effect of increased Ca intake on bone mass in pre- and peripubertal children, less is known about the influence of Ca intake on bone mineral accretion in the late teenage years.[11] We hypothesized that increased Ca intake from dairy products is important for Japanese high school students whose Ca intake is low. The aim of this study was to determine the association between intake of dairy products and bone strength, as measured by quantitative ultrasound (QUS) in a large population of late Japanese adolescents.

MATERIALS AND METHODS

Subjects

We targeted high school students aged 15-18 years. We

Corresponding Author: Dr Kazuhiro Uenishi, Laboratory of Physiological Nutrition, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado City, Saitama 350-0288, Japan.
Tel: +81-49-284-3895; Fax: +81-49-284-3895
Email: uenishi@eiyo.ac.jp
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contacted 33 prefectures (of 47 prefectures) in Japan and invited high schools to join our study. In total, 236 high schools participated on a voluntary basis. Among the targeted 40,111 students, 38,719 students (96.5%, 14,996 males and 23,723 females) agreed to participate in this study. This study was conducted according to the guidelines established by the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of Kagawa Nutrition University. Written informed consent was obtained from all subjects. QUS measurements and surveys using self-administered questionnaires were conducted in each high school from summer of 2006 to fall of 2007.

QUS Measurement

The strength of the calcaneus, or heel bone, of the right foot was evaluated with QUS densitometry using AOS-100 (Aloka, Tokyo). A QUS device measures elasticity and bone strength, which is dependent on both bone mass and bone architecture. The AOS-100 measures the speed of sound (SOS; in m/second) and the transmission index (TI). TI values are related to frequency-dependent attenuation. SOS and TI values measured with the AOS-100 are highly correlated with the SOS (r=0.89) and broadband ultrasound attenuation (BUA) (r=0.88) measured with a conventional QUS device (UBA575+, Hologic Inc.).[12] Osteo sono-assessment index (OSI), which is an estimate of elastic modules equal to the product of physical density and the square of SOS, was calculated as a combined parameter of SOS and TI by multiplying TI by the square of SOS.[12] OSI showed better reproducibility than BUA. The validity of the present QUS measurement has been previously published and coefficients of variation of SOS, TI, and OSI measurements were 0.3%, 1.2%, and 1.6%, respectively, [12] which is within the standard value provided by the manufacturer.

Demographic and Lifestyle Characteristics

The self-administered questionnaire included questions regarding the subject's gender, age, height, weight, current consumption of dairy products, and current physical activity level. Subjects reported their height and weight to the nearest 1 cm and 1 kg, respectively, and body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m^2). Intake of milk and yogurt was classified as none, 1-99 ml/day, 100-199 ml/day, 200-399 ml/day, and ≥400 ml/day; the intake of cheese was classified as none, 1-19 g/day, 20-39 g/day, 40-59 g/day, and

Table 1. Subject characteristics

	Mean or Proportion		
	Male (n=14,996)	Female (n=23,723)	*p* value
Age (years)	16.3 (SD 0.9)	16.4 (SD 0.9)	<0.001
Height (cm)	170.2 (SD 5.9)	157.8 (SD 5.4)	<0.001
Weight (kg)	61.4 (SD 10.6)	51.5 (SD 7.7)	<0.001
Body mass index (kg/m^2)	21.2 (SD 3.2)	20.7 (SD 2.8)	<0.001
Speed of sound (m/sec)	1577 (SD 174)	1592 (SD 164)	<0.001
Osteo sono-assessment index (10^6)	3.06 (SD 0.40)	2.88 (SD 0.35)	<0.001
Milk intake (ml/day)			
0	24.0%	41.1%	<0.001
>0 to <100	14.4%	19.1%	χ^2=2597
≥100 to <200	14.8%	15.4%	
≥200 to <400	25.8%	17.2%	
≥400	21.0%	7.3%	
Yogurt intake (ml/day)			
0	43.0%	37.7%	<0.001
>0 to <100	35.0%	38.7%	χ^2=193
≥100 to <200	14.2%	17.2%	
≥200 to <400	5.8%	5.0%	
≥400	2.0%	1.3%	
Cheese intake (g/day)			
0	62.0%	61.8%	<0.001
>0 to <20	19.9%	21.3%	χ^2=78
≥20 to <40	9.6%	10.4%	
≥40 to <60	4.1%	3.5%	
≥60	4.4%	3.0%	
Frequency of exercise (times/week)			
≤1	36.1%	61.4%	<0.001
2- 3	10.6%	11.3%	χ^2=2836
4- 5	10.3%	6.3%	
≥6	43.0%	21.0%	

≥60 g/day. These classifications are easy to understand for most Japanese because a bottle or a pack of milk has historically corresponded to approximately 200 ml, a cup of yogurt has corresponded to approximately 100 ml, and a standard serving size of cheese corresponds to 20 g. As for physical activity, subjects were asked to report the frequency of their current, weekly exercise regime.

Statistical analysis

All continuous variables were checked for normality. Weight and BMI were skewed to higher values, and were transformed logarithmically prior to conducting statistical tests. Student's *t*-test was used to test for differences between two groups. The chi-square test was used to test for independence of categorical data. Regression analyses were used to assess the linear association between predictor variables and the QUS value. In multiple regression analyses, exercise habit, body statue, gender, age, and area of residence (either in Tohoku, Kanto, Chubu, Kinki, Chugoku, Shikoku, or Kyushu area in Japan) were considered as covariates. The parameter "area" was treated as a dummy variable. SAS statistical package (version 9.13, SAS Institute, Cary, NC, USA) was used for all analyses. A *p*-value <0.05 was considered significant.

RESULTS

Table 1 shows the characteristics of all subjects. Mean values of age, height, weight, BMI, and OSI were significantly different between males and females. Males had higher milk intake and exercised more frequently than females. There was a slight but significant difference in yogurt and cheese intake between males and females.

Table 2 shows the results of simple linear regression analyses with OSI as the outcome. All predictor variables were significantly associated with OSI. Weight was the strongest predictor of OSI, followed by exercise frequency, BMI, gender, milk intake, and age. Table 2 also shows results according to gender. Figure 1 illustrates mean OSI by levels of milk intake. The mean OSI of the milk intake groups were higher than the no milk intake group, by 0.7% in the 1-99ml-group, 2% in the 100-199ml-group, 3.8% in the 200-399ml-group, and 6.7% in the ≥400ml-group. Figure 2 illustrates mean OSI by levels of milk intake according to gender. The pattern of association between OSI and milk intake was similar between genders.

Table 2. Simple linear regression analyses with OSI as the dependent variable by gender

Predictor variable	Regression coefficient (b)	R^2 (%)	*p*-value
Total			
Milk intake*	0.044	2.8	<0.001
Yogurt intake*	0.025	0.4	<0.001
Cheese intake†	0.01	0.1	<0.001
Frequency of exercise‡	0.075	6.8	<0.001
Weight (kg)§	0.746	11.2	<0.001
Body mass index (kg/m^2)§	0.709	6	<0.001
Gender (1, male; 0, female)	0.184	5.6	<0.001
Age (years)	0.043	1.1	<0.001
Males			
Milk intake*	0.034	1.6	<0.001
Yogurt intake*	0.028	0.5	<0.001
Cheese intake†	0.009	0.1	0.003
Frequency of exercise‡	0.048	2.7	<0.001
Weight (kg)§	0.587	5.6	<0.001
Body mass index (kg/m^2)§	0.64	5.1	<0.001
Age (years)	0.074	3.1	<0.001
Females			
Milk intake*	0.027	1.1	<0.001
Yogurt intake*	0.026	0.5	<0.001
Cheese intake†	0.008	0.1	<0.001
Frequency of exercise‡	0.071	6.2	<0.001
Weight (kg)§	0.689	7.6	<0.001
Body mass index (kg/m^2)§	0.676	5.8	<0.001
Age (years)	0.029	0.6	<0.001

*Coded as 1: 0 ml/day; 2: >0 to <100 ml/day; 3: ≥100 to <200 ml/day; 4: ≥200 to <400 ml/day; 5: ≥400 ml/day
†Coded as 1: 0 g/day; 2: >0 to <20 g/day; 3: ≥20 to <40 g/day; 4: ≥40 to <60 g/day; 5: ≥60 g/day
‡Coded as 1: ≤1 time/week; 2: 2-3 times/week; 3: 4-5 times/week; 4: ≥6 times/week
§Logarithmically transformed

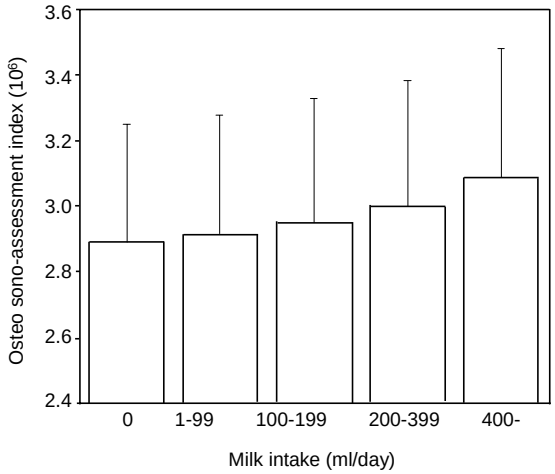

Figure 1. Mean osteo sono-assessment index (OSI) by levels of milk intake. Mean OSIs in milk intake groups were higher than the no milk intake group.

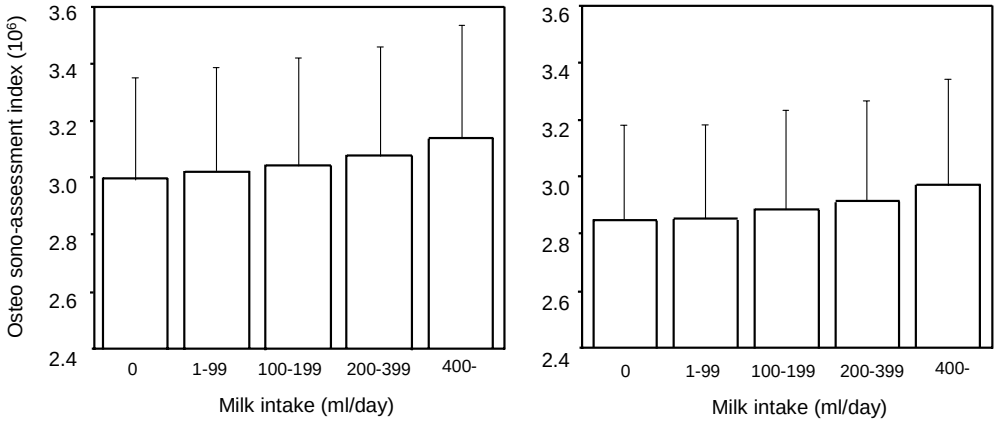

Figure 2. Mean osteo sono-assessment index (OSI) by levels of milk intake in males (left) and females (right). The pattern of association between OSI and milk intake was similar between genders, with R^2 vales of milk intake being 1.6 % for males and 1.1% for females.

Table 3 shows the results of multiple regression analyses. In this multivariate model, we included weight as the index of body statue, but did not include BMI because BMI was highly correlated with weight (r=0.821). After adjusting for exercise frequency, weight, gender, age, and area of residence, regression analyses indicated that milk and yogurt intake were independently associated with OSI, while cheese intake was not. The interaction of milk intake and exercise frequency was not significant (p=0.424) when the interaction term was added to the multivariate model. Table 3 shows these results according to gender. R^2 values of milk intake in males (1.6%) and females (1.1%) were lower than that in males and females combined (2.8%).

DISCUSSION

The relationship between Ca intake and bone health in children and adolescents is an area of interest for clinicians and epidemiologists. Most recently, Winzenberg *et al*.[13] demonstrated in their meta-analysis of randomized clinical trials that Ca supplementation has a small effect on the bone mineral density (BMD) of upper arm limbs and total body, but no effect on hip or vertebral BMD. Their study, however, has several limitations. The baseline Ca intake in most studies enrolled in the meta-analysis ranged from moderate to high. Another meta-analysis has revealed that Ca supplementation among subjects with low Ca intake has a positive effect on bone mineral content (BMC), much larger than that seen among children with normal Ca intake.[14] Therefore, it is important to demonstrate this association in low-Ca-intake populations. The present study did not evaluate total Ca intake. However, the results of the National Health and Nutrition Survey can be applied to the present study because we used a nationwide sample. The 2004 National Health and Nutrition Survey reported that the median Ca intake among 15-17 year old adolescents was 570 mg/day in males and 467 mg/day in females, which are both lower than the recommended dietary reference intakes for Japanese of 1100 mg/day and 850 mg/day, respectively.[15] The subjects in the present study represent a low Ca intake population.

The present study demonstrated a clear dose-effect relationship between milk intake and calcaneal QUS values in adolescents aged 15-18 years, thereby demonstrating the importance of milk for bone health in Japanese adolescents. Specifically, milk intake of 400 ml/day or more is recommended to attain maximal peak bone mass. Sev-

Table 3. Multiple linear regression analyses with OSI as the dependent variable by gender

Predictor variable*	Regression coefficient (b)	R^2 (%)	*p*-value
Total	Overall R^2=20.0		
Milk intake†	0.016	2.8	<0.001
Yogurt intake†	0.011	0.1	<0.001
Cheese intake‡	0.00005	0	0.978
Frequency of exercise§	0.064	5.3	<0.001
Weight (kg)¶	0.599	8.1	<0.001
Gender (1, male; 0, female)	0.035	0.1	<0.001
Age (years)	0.052	1.7	<0.001
Males	Overall R^2=15.2		
Milk intake†	0.02	1.6	<0.001
Yogurt intake†	0.01	0.2	0.002
Cheese intake‡	-0.003	0	0.372
Frequency of exercise§	0.059	1.9	<0.001
Weight (kg)¶	0.512	5.1	<0.001
Age (years)	0.082	3.7	<0.001
Females	Overall R^2=16.4		
Milk intake†	0.013	1.1	<0.001
Yogurt intake†	0.012	0.2	<0.001
Cheese intake‡	0.002	0	0.453
Frequency of exercise§	0.07	5.5	<0.001
Weight (kg)¶	0.659	7	<0.001
Age (years)	0.033	0.9	<0.001

*Dummy variable of "area of residence" is also included in the multivariate model in addition to the variables shown in this table
†Coded as 1: 0 ml/day; 2: >0 to <100 ml/day; 3: ≥100 to <200 ml/day; 4: ≥200 to <400 ml/day; 5: ≥400 ml/day
‡Coded as 1: 0 g/day; 2: >0 to <20 g/day; 3: ≥20 to <40 g/day; 4: ≥40 to, <60 g/day; 5: ≥60 g/day
§Coded as 1: ≤1 time/week; 2: 2-3 times/week; 3: 4-5 times/week; 4: ≥6 times/week
¶Logarithmically transformed

eral studies have tried to determine the association between milk intake and bone mass in adolescents. In one study, dairy food supplements, including 300-400 mg/day of Ca, increased the bone mass of various bone sites among Caucasian adolescents.[3,5] Matkovic *et al*.[16] showed that Ca and dairy products influence bone mass acquisition in 15-18 year old females, leading to a higher peak bone mass. They also determined that Ca exerts its action on bone accretion during growth, primarily by influencing volumetric bone mineral density, while dairy products may have an additional impact on bone growth and periosteal bone expansion. One study has specifically been conducted among East Asians, and showed that the high-milk intake group (128 mg/day of milk) had significantly higher BMC at the distal radius than the no-milk intake group of Chinese girls aged 12-14 years.[6] These previous studies are all in line with the results of our study.

Studies conducted on the association between Ca intake and bone mass in late teens are much fewer than in pre- and peripubertal children.[11,13,14] Two interventional studies targeting late adolescents showed that Ca supplementation was effective for bone mass accrual.[17,18] An observational study using a QUS device showed that Ca intake is associated with calcaneal QUS values in American girls aged 14-18 years.[19] All of these studies, including the present study, suggest that a favorable effect of Ca and dairy foods on bone mass or bone growth is consistently observed in late adolescents.

Yogurt intake was also associated with QUS values independently, but its contribution was small (R^2=0.1%), while cheese intake was not associated with QUS. This finding can be explained partly by the fact that intake of yogurt and cheese was less than that of milk. In general, dairy products do not appear to be common foods in the dietary habits of the Japanese. Yogurt was not consumed by 40% of the subjects in this study, and cheese was not consumed by 60%. Furthermore, milk was not consumed in 40% of female subjects. The National Health and Nutrition Survey demonstrated that dairy products only account for 30% of total Ca intake.[10] Low Ca intake due to low dairy product intake is common in Asians.[6,20] Therefore, increased intake of dairy products may be an effective strategy to maximize peak bone mass in late Asian adolescents.

The prevalence of lactose intolerance in the Japanese, like that in other Eastern Asians,[6] is high; the prevalence in Japanese high school students is reported to be 33.8%.[21] As the relatively lower consumption of dairy products in Japanese girls may be due in part to the symptoms of lactose intolerance, strategies other than simply encouraging

the consumption of dairy products may be needed to increase their calcium intake.

Physical activity has been shown to be another key factor associated with bone mass in late adolescents.[19,22,23] In the present study, physical activity was found to be a more influential factor on QUS values than milk intake, especially for females. This finding was partly explained by the fact that many subjects exercised frequently; 43% of males and 21% of females reported exercising six or more times a week. Many high school students in Japan enthusiastically participate in school sports club activities, and thus both the frequency and intensity of their physical activity is considered to be high.

This study has many strengths. The sample size of this study was large, such that we had sufficient statistical power to detect a possible association between dairy product intake and QUS values. Furthermore, subjects were recruited from various regions of Japan, which makes the results generalizable to all Japanese late adolescents. This study also has several limitations. First, we measured bone stiffness of only the calcaneus using a QUS device. Although this technique is useful in estimating bone mass or bone growth in adolescents,[19,24,25] we did not obtain information on other bone sites of interest, such as the proximal femur and vertebrae. The effect of dairy products on these bones may not be the same as that seen with the calcaneus, and thus this needs to be clarified in future studies. Second, we did not evaluate pubertal status, which would have had an effect on bone status, especially for younger subjects (only a few years after puberty onset). Third, information on consumption of dairy products was self-reported. One may drink milk by family- or economic-packs rather than from individual packages or bottles. This method of evaluation is associated with potential measurement bias, which may have attenuated the association between milk intake and QUS values. Finally, the design of this study was cross-sectional, which does not necessarily indicate causal relationships. Intervention studies are needed to remedy these problems.

The present study demonstrated a dose-effect relationship between milk intake and bone strength in late adolescents, to whom we recommend an intake of 400 ml/day or more to obtain greater bone mass. These findings may be generalizable to populations whose dairy product intake is generally low.

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AUTHOR DISCLOSURES

The authors have no conflicts of interest.

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Original Article

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Kazuhiro Uenishi PhD[1], Kazutoshi Nakamura MD[2]

[1]*Laboratory of Physiological Nutrition, Kagawa Nutrition University, Saitama, Japan*
[2]*Department of Community Preventive Medicine, Niigata University, Graduate School of Medical and Dental Sciences, Niigata, Japan*

乳製品攝取與青少年晚期骨骼超音波測量：日本全國橫斷性研究

前言：目前很少，特別是對亞洲人，關於乳製品的攝取與青少年晚期骨骼礦化影響的實證資料。本篇研究之目的在於評估日本大型族群乳製品的攝取與定量超音波儀(QUS)測量之骨骼強度間的關係。方法：共有遍及 33 縣的 38,719 位高級中學的學生為個案(14,996 位男生及 23,723 位女生)。跟骨硬度以定量超音波儀(AOS-100，Aloka)測量。給予個案一份自評問卷，包含性別、年齡、身高、體重、乳製品攝取及體能活動情形。鮮奶及酸奶的攝取被分類為無攝取、每天攝取 1-99、100-199、200-399 及≥400 mL。結果：鮮奶每天攝取≥400 mL 的比例在男生為 21%，女生為 7.3%；另外有 24%的男生及 41.1%的女生無攝取鮮奶。在校正體能活動、體重、性別、年齡及居住區域後，QUS 測量值與鮮奶攝取(R^2=2.8%，p<0.001)及酸奶攝取(R^2=0.1%，p<0.001)分別有相關。將男生與女生按性別分層後，仍有類似的結果。結論：青少年晚期的鮮奶攝取量與骨骼強度之間呈現正向的劑量效應，因此我們建議，此段年齡的青少年每天至少攝取鮮奶 400 mL，以獲得較好的骨質。

關鍵字：青少年、骨密度、鈣、乳製品、定量超音波測量