取量の評価，④身体活動の評価，⑤代謝状態の把握などからなる．

身体組成については，日本栄養アセスメント研究会が日本人の新身体計測基準値（JARD2001）を発表しており[7]，70～85歳までの5歳刻みおよび85歳以上の身長・体重や皮下脂肪厚等の基準値が算出されている．上腕の身体計測値などは，寝たままでも測定し比較することができるので，非常に有用である．ただしJARD2001策定のための調査対象者人数は，国民健康・栄養調査の約半分程度であるので，国民健康・栄養調査から求められている食事摂取基準（2010年版）の基準体位なども参考にするのがよい．身体組成や血液データは，ある一時点での結果ではなく，一定期間に指標がどの程度変化したかを評価することが重要である．例えば体重で，1か月に5%以上，1週間に2%以上の減少などが観察された場合は，値にかかわらず要注意である．

食事摂取量は，高齢者本人やその家族への聞き取り調査や，入院入所者では給与食事量と残食量などから，栄養計算によって把握する．摂取量の変化や，摂取状況－咀嚼・嚥下困難，食欲（拒食，過食），食物の認知－などに留意する必要がある．

身体活動の評価は，基本的な日常生活動作（(basic) activity of daily living：ADL），手段的日常生活動作（instrumental ADL）など身体機能の評価をはじめとして，ある程度自立した人の場合には，歩数の測定や運動・生活活動の聞き取りによって把握することができる．障害や疾病を有している場合は，代謝性ストレスによる消費エネルギーの増加，消化・吸収・代謝機能の変化，薬剤の服用による影響など，栄養に関連する代謝状態の把握も非常に重要である．

栄養状態の評価ツールとしては，高齢者用にMini Nutritional Assessment：MNAが開発されており，日本人でもその妥当性が検証されている[8]．初めに6項目からなるスクリーニングでリスクの高い者を割り出し，残り12項目で低栄養状態の評価を行うようになっている．計測項目も少なく簡便な評価ツールのひとつである．

5) 高齢者の栄養指導・栄養療法

高齢者への栄養指導・栄養療法を行うにあたっては，まず正確な栄養状態の評価を行い，対象者に必要でなおかつ摂取可能な栄養（食事）の提案および提供を行わなければならない．

自立高齢者については，食事摂取基準（2010年版）の値を参考にして，対象者の健康状態，身体活動量，生活環境なども考慮した上で，エネルギーなど必要な栄養量を算出する．高齢者で一般的に不足しやすい栄養素に注意するとともに，軽微な疾患を持つ場合には服薬の影響や代謝特性も念頭に入れて，対象者の食生活を考慮し，過剰や不足の栄養素がないよう計画を立てて指導することが必要である．

要介護・要医療の高齢者については，「不足」を中心とする栄養障害の予防・改善が，基礎疾患の治療，合併症の予防，生命予後などに大きく影響を及ぼす．従って，健常高齢者と同様に，正確な栄養評価を行い必要な栄養量を算出することはもちろんだが，栄養提供方法も主要な検討事項となる．経口摂取が基本ではあるが，必要に応じて経腸栄養や経静脈栄養を適切に実施し，特別用途食品（総合栄養食品やえん下困難者用食品）なども利用して，効率的に早期に栄養状態を改善することが，非常に重要である．

［森田明美］

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5. 食　育

5.1　食育基本法と食育推進基本計画

今日，栄養の過剰や不足，不規則な食生活による生活習慣病の増加，家族との共食の減少，健康食品やサプリメントへの依存，食の安全・安心の揺らぎ，依然として先進国中最低の水準にある食料自給率など，食生活をめぐって多くの課題がある．こうした課題解決に向けて，2007年6月食育基本法が制定され，翌7月から施行された．個人の食生活は，どのような食物が入手可能かといった地域社会のフードシステムや，健康・食情報の提供など，食環境の影響により規定される部分が少なくない．したがって，「食」を単に個人の問題としてだけ考えていては課題解決に至らない場合が多い．そこで，社会として「食」のあるべき方向を議論し，取り組む必要性が生じてきた．

「食育」ということばは，明治時代の軍医である石塚左玄が記した書物で使われたのが最初とされる．食育基本法の前文の中では，「食育」は以下のとおり，人間形成にとって最も基本的なこととして位置づけられた．

食育基本法　前文より
子どもたちが豊かな人間性をはぐくみ，生きる力を身につけていくためには，何よりも「食」が重要である．今，改めて，食育を，生きる上での基本であって，知育，徳育及び体育の基礎となるべきものと位置付けるとともに，さまざまな体験を通じて「食」に関する知識と「食」を選択する力を習得し，健全な食生活を実践することができる人間を育てる食育を推進することが求められている．

また，食育の具体的な内容として，以下の点が挙げられている．

- 国民の心身の健康と豊かな人間性をはぐくむ（第2条）
- 食に関する感謝の念と理解をはぐくむ（第3条）
- 食育推進運動の展開：自発性の尊重，地域特性への配慮，主体的参加（第4条）
- 子どもの食育推進における保護者（家庭），教育関係者等の役割（第5条）
- 食料の生産から消費に至るまでの食に関するさまざまな体験活動の重視（第6条）
- 伝統的な食文化，環境と調和した生産等への配慮，農産漁村の活性化，食料自給率の向上への貢献（第7条）
- 食品の安全性の確保，食の安心（第8条）

また，平成18年3月には，内閣総理大臣を会長とする食育推進会議において食育推進基本計画が策定され，食育推進の基本方針や，食育としてめざす数値目標が示された．表3－11は9つの目標項目に関する策定時の数値，平成21年の現状値，平成22年度迄に達成をめざす目標値である[1]．このような数値目標を食育に

Original Article

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

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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

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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

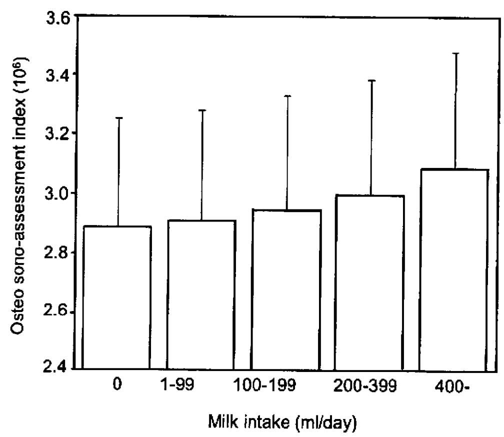

Figure 1. Mean osteo sono-assessment index (OSI) by levels of milk intake. Mean OSIs in milk intake groups were higher than the no milk intake group.

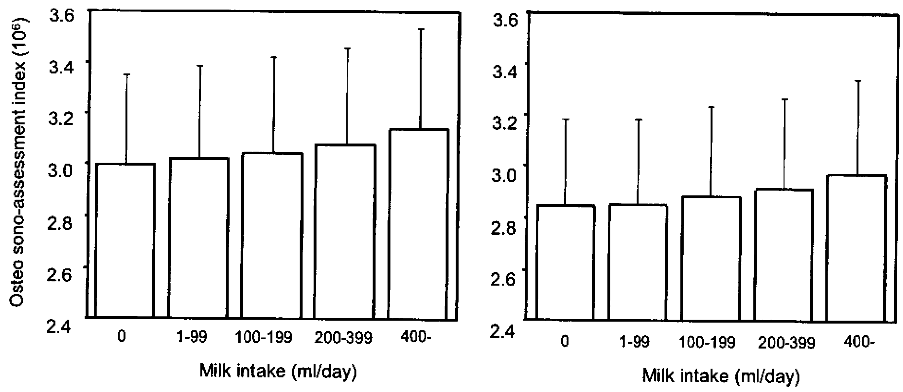

Figure 2. Mean osteo sono-assessment index (OSI) by levels of milk intake in males (left) and females (right). The pattern of association between OSI and milk intake was similar between genders, with R^2 vales of milk intake being 1.6 % for males and 1.1% for females.

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

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

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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

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Article

Fractional Absorption of Active Absorbable Algal Calcium (AAACa) and Calcium Carbonate Measured by a Dual Stable-Isotope Method

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Abstract: With the use of stable isotopes, this study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), obtained from oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient, HAI), with that of calcium carbonate. In 10 postmenopausal women volunteers aged 59 to 77 years (mean ± S.D., 67 ± 5.3), the fractional calcium absorption of AAACa and $CaCO_3$ was measured by a dual stable isotope method. ^{44}Ca-enriched $CaCO_3$ and AAACa were administered in all subjects one month apart. After a fixed-menu breakfast and pre-test urine collection (Urine 0), ^{42}Ca-enriched $CaCl_2$ was intravenously injected, followed by oral administration of ^{44}Ca-enriched $CaCO_3$ without carrier 15 minutes later, and complete urine collection for the next 24 hours (Urine 24). The fractional calcium absorption was calculated as the ratio of Augmentation of ^{44}Ca from Urine 0 to Urine 24/ augmentation of ^{42}Ca from Urine 0 to Urine 24. Differences and changes of ^{44}Ca and ^{42}Ca were corrected by comparing each with ^{43}Ca. Fractional absorption of AAACa (mean ± S.D., 23.1 ± 6.4), was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean fractional absorption was approximately 1.57-times higher for AAACa than for $CaCO_3$. The serum 25(OH) vitamin D level was low (mean ± S.D., 14.2 ± 4.95 ng/ml), as is common in this age group in Japan. Among the parameters of the bone and mineral metabolism measured, none displayed a significant correlation with the fractional absorption of $CaCO_3$ and AAACa. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ for osteoporosis.

Keywords: active absorbable algal calcium (AAACa); calcium carbonate; dual stable Ca isotope method; fractional absorption (FA); parathyroid hormone (PTH)

1. Introduction

Active absorbable algal calcium (AAACa) prepared from heated oyster shell and seaweed is a unique calcium supplement with high bioavailability, with a characteristic lamellar crystalline structure quite unlike that of calcium oxide and calcium carbonate ($CaCO_3$) [1]. In the Katsuragi Calcium study, a prospective, randomized, double blind and placebo-controlled study compared the effect of AAACa on osteoporosis with that of $CaCO_3$ in hospitalized women with a mean age of 80 years. It was found that AAACa alone increased spinal bone mineral density significantly over the level in subjects given a placebo, whereas $CaCO_3$ did not [2,3]. Fracture occurrence over the two year test period from among 58 subjects was 0 of 5 in the AAACa Group, 2 of 7 in the $CaCO_3$ Group and 3 of 5 in the Placebo Group, on evaluation of all X-rays available at the beginning and end of the test period. The AAACa Group exhibited a significantly lower rate of fracture occurrence than the placebo group, but the $CaCO_3$ Group showed no significant difference from placebo group. Serum parathyroid hormone (PTH) was also suppressed more efficiently by AAACa than $CaCO_3$.

Despite all these indirect lines of evidence indicating a high bioavailability of AAACa, a direct absorption test by a dual isotope method has not been conducted to date. We have therefore attempted to measure the fractional absorption of AAACa by using the dual stable-isotope method [4,5] to compare it with $CaCO_3$ in subjects in the age group most likely to need effective calcium supplementation to maintain their bone health: postmenopausal women.

2. Experimental Section

2.1. Subjects

Ten postmenopausal women between 59 and 77 years of age (mean ± SD, 67 ± 5.3 years) leading a normal healthy daily life without any known disease possibly affecting bone and mineral metabolism volunteered to participate as test subjects in the present study by providing written consent (Table 1). One subject, shown in parenthesis in Tables 1 and 2, was dropped from analysis because of a measured fractional absorption (FA) value of 0% on giving $CaCO_3$. The Institutional Review Board of the Fujii Medical Clinic approved the study.

Table 1. Background of the test subjects.

No.	**Age**	**Years after menopause**	**Height (cm)**	**Weight (kg)**	**Systolic blood pressure (mmHg)**	**Diastolic blood pressure (mmHg)**
1	68	19	154	54	138	80
2	72	23	147	50	142	62
3	65	15	157	63	148	70
4	65	13	148	43	125	70
(5) *	(59)	(9)	(153)	(60)	(133)	(88)
6	59	8	152	58	152	85
7	65	13	151	56	150	85
8	77	28	150	48	142	80
9	64	15	145	50	140	90
10	71	19	148	48	122	70
Mean	67	17	150	52	139	76
SD	5.3	6.0	3.7	6.1	10.4	9.3

* Case No. 5 was not included in the statistical analysis.

2.2. Background Data of the Test Subjects

In order to assess the metabolic background of the test subjects, serum Ca, P, albumin, creatinine, BUN, 25(OH)vitamin D, intact parathyroid hormone (PTH), bone specific alkaline phosphatase (BAP), urinary N-terminal type I collagen fragments (NTx) and urinary calcium/ creatinine ratio (UCa/ Cr) were measured prior to the test. The laboratory tests related to bone and calcium metabolism gave results approximately within the normal range, as shown in Table 2, except for one subject, who had a serum 25(OH) vitamin D level in the insufficiency range (7.6 ng/mL). This subject was without symptoms and signs of vitamin D insufficiency such as hypocalcemia, hypophosphatemia, high alkaline phosphatase, muscle weakness and bone pain.

Table 2. Parameters of mineral and bone metabolism of the test subjects.

No.	Serum Ca mg/dL	Serum P mg/dL	Serum albumin g/dL	Serum creatinine mg/dL	BUN mg/dL	25(OH) vitamin D ng/dL	Intact PTH pg/dL	BAP U/L	Urine NTx/Cr nMBCE/ mMCr	Urine Ca/Cr mg/mg
1	9.7	3.9	4.4	0.83	11.0	16.8	48	15.2	32.2	0.03
2	9.5	4.5	4.0	0.80	21.9	16.9	31	15.1	16.0	0.06
3	9.7	3.1	4.6	0.71	12.1	11.6	40	35.8	31.1	0.45
4	10.3	3.5	5.1	0.49	12.5	11.7	44	32.4	35.9	0.36
5 *	(9.5)	(3.4)	(4.5)	(0.74)	(14.2)	(21.8)	(61)	(21.6)	(29.0)	(0.08)
6	9.8	3.5	4.7	0.74	17.8	15.4	44	19.1	23.0	0.12
7	9.3	4.4	4.4	0.60	17.8	24.7	42	17.0	42.2	0.22
8	9.9	2.9	4.6	0.59	13.5	7.6	50	34.9	34.5	0.17
9	9.8	3.7	4.4	0.74	13.7	12.7	34	27.5	16.3	0.20
10	9.5	3.3	4.2	0.54	13.6	10.8	34	45.9	21.9	0.30
Mean	9.7	3.6	4.5	0.67	14.9	14.2	41	27.0	28.1	0.21
SD	0.29	0.55	0.31	0.119	3.53	4.95	6.59	11.02	9.20	0.138

Ca: calcium; P: phosphorus; BUN: Blood urea nitrogen; PTH: parathyroid hormone; BAP: Bone specific alkaline phosphatase; BCE: Bone collagen equivalent.

* Case No. 5 was not included in the statistical analysis

2.3. Materials

The first part of the test was performed on March 9, 2009, using ^{44}Ca-enriched $CaCO_3$ for oral load and ^{42}Ca in the form of $CaCl_2$, for intravenous injection (Table 3). On April 13, 2009, after one month, exactly the same procedure was repeated on the same test subjects, except for the use of ^{44}Ca-enriched AAACa in the place of $CaCO_3$ to ensure the stable isotope constituent of the body reached equilibrium. Intrinsic labeling is no doubt ideal, but it is impossible to label the shell of oysters abiding in the ocean, so an extrinsic labeling was adopted as the best substitute for it. The material for AAACa was obtained by heating oyster shell to 1,000 °C, resulting mostly in CaO powder after losing much of the organic components. To 5,082 mg of this CaO powder, 450.4 mg CaO Ca fraction was added that consisted of 95.9 ± 0.3% ^{44}Ca supplied by TRACE SCIENCES INTERNATIONAL (Ontario, Canada), and was thoroughly mixed in a melting pot. Aqueous solution of a small amount of algal component was pre-heated at a high temperature in a manner similar to the oyster shell to start a chemical reaction lasting for about 10 minutes. After sufficient stirring, it was divided into small portions for actual use and preserved in vacuum. The final product mostly consisted of $Ca(OH)_2$.

$CaCO_3$ labeled with ^{44}Ca was also obtained from the same source (the Ca fraction consisting of 95.9 ± 0.3% ^{44}Ca). To 781.6 mg of this material, 9,075 mg $CaCO_3$ (Japanese Pharmacopeia) was added, thoroughly mixed, and divided into small proportions and stored.

AAACa particle mean size was 5.8 microns; maximum size was 75 microns and $CaCO_3$ particle size ranged from 10 to 20 microns. As these values are based on different occasions of measurements they may not be directly comparable, but appears to lie over a similar range. If anything, a larger size is compatible with slower absorption.

For two subjects, part of the first urine sample was lost; in these cases, both parts of the test were repeated on July 30 and August 27, and the data from the uneventfully performed second set of tests were used to replace those of the first set.

The safety of the intravenous injection of $CaCl_2$ was verified before the study by the absence of any signs of toxicity such as chills, fever, neuromuscular irritability, skin eruptions, disturbance of consciousness, *etc.*

Table 3. Amount of isotope Ca (mg) per subject in 1 study.

Isotope	Oral						IV
	CaX			CaY			Total
	Supplied	Added	Total	Supplied	Added	Total	
^{42}Ca	0.01	1.79	1.80	0.00	1.79	1.79	3.192
^{43}Ca	0.01	0.39	0.40	0.00	0.39	0.39	0.0037
^{44}Ca	25.38	5.52	30.90	25.38	5.53	30.91	0.0334

The contents of Ca isotopes in the material used for the preparation of CaX and CaY on arrival from the supplier (Supplied), their contents in the material added to prepare samples for administration (Added) and the final total (Total) are indicated in Table 3.

A total of approximately 300 mg of Ca containing approximately 30 mg ^{44}Ca isotope (25 + 5) was orally administered to each subject and about 3 mg ^{42}Ca isotope was injected before the study and no symptoms and signs of toxicity were reported.

2.4. Test Procedure

After taking a fixed menu breakfast consisting of fruit juice, toast, eggs and coffee, a pre-test urine sample was collected (Urine 0) and ^{44}Ca-enriched $CaCO_3$ was orally administered followed by the intravenous injection of ^{42}Ca-enriched $CaCl_2$ 15 minutes later. A complete collection of 24 h urine followed (Urine 24). After one month to ensure clearance of the enriched isotope, exactly the same procedure, except for the use of ^{44}Ca-enriched AAACa instead of ^{44}Ca-enriched $CaCO_3$, was repeated.

2.4.1. Measurement of the Stable Isotope

Sample preparation for isotope enrichment measurement was conducted according to the method of Patterson *et al.* [6]. By using the inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 cs, Agilent Technologies, Inc., Tokyo), ^{42}Ca, ^{43}Ca, ^{43}Ca and other measurable stable Ca isotopes were measured in both Urine 0 and Urine 24. Utilizing ^{43}Ca as an internal standard of the stable Ca isotopes, the ratio of each stable Ca isotope to ^{43}Ca was calculated. The increase of the $^{42}Ca/^{43}Ca$ and $^{44}Ca/^{43}Ca$ in Urine 24 above the pretest natural abundance level for each test subject over the corresponding value in Urine 0 was then obtained. By dividing the ratio of the actual amount of the enrichment of ^{44}Ca by the corresponding amount of the enrichment of ^{42}Ca from Urine 0 to Urine 24, the FA of the ^{44}Ca-enriched material was obtained; for $CaCO_3$ in the first part of the test and AAACa in the second part (Table 3).

2.5. Statistical Analysis

The Excel Statistical Package was used to compare the FA of $CaCO_3$ and AAACa by paired t-test. A correlation matrix among the FA data, age and parameters of bone and mineral metabolism was constructed and evaluated by the Spearman method in view of the inclusion of variables with uncertain distribution. The p values < 0.05 were considered significant.

3. Results and Discussion

As shown in Table 4, the mean Fractional absorption (FA) of AAACa, 23.1 ± 6.4%, was 1.57-times higher than the corresponding value of $CaCO_3$, 14.7 ± 6.4%, with a significant difference at $p = 0.0060$ determined using paired t-test.

Table 4. Fractional absorption (FA) of CaCO3 and AAACa by dual stable isotope method.

Subject	FA CaCO3	FA AAACa
1	7.5	21.1
2	20.0	29.7
3	21.9	34.7
4	19.6	20.4
(5) *	(0.0)	(18.8)
6	6.1	22.7
7	14.3	24.9
8	11.7	11.9
9	8.7	22.1
10	22.2	20.4
Mean	14.7	23.1
SD	6.4	6.4

Paired comparison between FA CaCO3 and FA AAACa

$p = 0.0060$, $t = 3.708$ (paired t-Test)

* Subject 5 was not included in the statistical analysis.

According to the evaluation by means of the correlation coefficient matrix (Spearman) (Table 5) among the parameters of bone and mineral metabolism summarized in Table 2, no significant correlation was found between the FA of either calcium carbonate or AAACa and each parameter. In the subject with the lowest serum 25(OH) vitamin D of 7.6 ng/mL, the FA of $CaCO_3$ value was medium in the group, *i.e.*, 11.7%, fifth from the lowest, and the FA of AAACa, 11.9%, was the lowest in the group.

Until the advent of the dual isotope method, the true FA of calcium was extremely difficult to measure due to the complex behavior of calcium in living organisms, such as the rapid exchange through multiple Ca pools and various pathways of exit and reentrance [7,8]. Utilizing the presence of multiple stable isotopes in nature, the dual stable isotope method was developed to circumvent this complexity, and it is the only method of directly measuring the fractional intestinal Ca absorption.

Abrams and coworkers as well as other investigators [12-23] have used this method extensively to estimate calcium absorption, establishing it as the gold standard for calcium absorption. Since calcium absorption is influenced by age and the state of bone, as well as mineral metabolism, a correlation matrix was constructed and evaluated by Spearman's method (Table 5). None of the metabolic parameters tested exhibited significant correlation with FA of the calcium compounds. Absence of significant correlation between FA of calcium compound and age was expected because of the narrow age range of this group.

The FA of Ca compounds obtained in this study of postmenopausal women, with a mean age of 66 years and with a tendency of low 25(OH) vitamin D, appears to be much lower than those observed in children and younger subjects: FA; 54.8–63.1% [21], 58.2–64.3% [22], and also younger postmenopausal women with mean age of 56: FA; 34.6–39.1% [23]. In healthy volunteers between 25 to 45 years much lower values, yet still higher than the results in the present study, were reported: FA; 26–31% [24]. The reduced FA in the current study subjects could also be due to reduced estrogen level after menopause. FA is, thus, markedly influenced by age. The age range of the test subjects was quite narrow in this group of subjects, unsuitable for the assessment of the age-FA correlation. Statistically, the tendency of age-FA correlation was non-significant.

Table 5. Spearman's correlation matrix and correlation coefficients among fractional absorption (FA) and parameters of bone and mineral metabolism.

	FA CaCO3	FA AAACa	Age	SCa	SP	Salb	Cre	BUN	25D	PTH	BAP	UNTx	UCa/Cr
FA CaCO3	1.0000	0.1423	0.4238	−0.3882	−0.1925	−0.3405	−0.4854	−0.0335	−0.3167	−0.5630	−0.4833	−0.1333	0.6333
FA AAACa		1.0000	−0.3490	−0.5297	0.4328	−0.2137	0.5042	0.3445	0.5690	−0.5232	−0.3766	−0.2762	0.0418

SCa: serum calcium; SP: serum phosphate; Salb: serum albumin; Cre: creatinine; BUN: blood urea nitrogen; 25D: 25(OH)vitaminD; PTH: parathyroid hormone; BAP: bone specific alkaline phosphatase; UNtx: urine N-terminal type I collagen fragments; UCa/Cr: urinary Ca/creatinine ratio

Although these subjects are reasonably homogeneous and apparently free of any comorbidity, which could potentially influence the test results, the present study is limited by the small number of test subjects. Unlike similar studies conducted in this field in the past, post-menopausal women – who need calcium supplementation most because of high risk of osteoporosis – were asked to participate. A rather low intra-group variation was encouraging, and a clear-cut difference in FA between the two test materials may also add to the credibility of the conclusion.

It is possible that the difference in molecular weight and physicochemical properties of the ^{44}Ca-enriched $CaCO_3$ and AAACa, mostly consisting of $Ca(OH)_2$ as the result of oxidation of $CaCO_3$

obtained from oyster shell, cannot be completely ruled out. The similar molecular size and comparable particle size actually measured as 5.8 to 75 for AAACa and 10 to 20 for $CaCO_3$ and this is a limitation of the result but should not have affected the primary outcome. In view of the similar molecular size and physicochemical properties between $CaCO_3$ and AAACa, both much smaller than organic Ca salts, however, confounding effect exerted on the calculation of the absorptive rate is rather unlikely and the conclusion of difference in the absorption rate between the two compounds should be reasonably supported.

4. Conclusions

This study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient, HAI), with that of calcium carbonate. The Fractional absorption of AAACa, (mean ± S.D.; 23.1 ± 6.4) was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean was approximately 1.57-times higher for AAACa than $CaCO_3$. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ on osteoporosis.

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