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Running title: Effect of lifestyle on juvenile bone density

Keywords Peak bone mass · Juvenile · Bone mineral density · Lifestyle · Osteoporosis

Abstract

Objectives We explored the relationship between bone mineral density (BMD) and lifestyle in juveniles to identify factors leading to higher peak bone mass and prevention of osteoporosis in later life.

Methods Juveniles (1364 students: 770 males and 594 females, aged 6–18 years) attending school in Hokkaido prefecture, Japan, were asked to complete a brief self-administered diet history questionnaire for 10-years old (BDHQ10y) providing information about personal history, lifestyle, and intake of nutritional elements. In addition, BMD and grip strength were measured. We analyzed the relationship between BMD and lifestyle factors.

Results The difference in BMD for males was larger among the junior and senior high school groups. The difference in BMD for females was larger among older elementary school children and later groups. Anthropometric variables and grip strength were strongly correlated with BMD. Having a nap time routine was significantly correlated with BMD, but sleep time and sports club activities were not. BMD among juveniles who attained secondary sexual characteristics was significantly higher than that of juveniles of the same age who had not attained these characteristics. Calcium intake was significantly lower in senior high school students compared with other grades. Consumption of milk by senior high school males and junior high school females was weakly correlated with BMD.

Conclusions Our findings encourage educational interventions to counsel students to avoid

weight loss and calcium deficiency. This effective intervention should begin before the higher elementary school, when juveniles have the greatest likelihood for preventing lower peak bone mass and osteoporosis.

Introduction

Osteoporosis is a common clinical condition affecting an estimated 7.8–11 million people in Japan [1]. Since bone fractures not only reduce quality of life but also lead to immobility in elderly people, prevention of osteoporosis is an important concern in health care. Three primary strategies for preventing osteoporotic fracture have been identified: (1) minimizing bone loss in postmenopausal women, (2) preventing falls in elderly adults, and (3) increasing peak bone mass in adolescents. Recently, calcium intake and moderate exercise have attracted attention as strategies for preventing the development of osteoporosis by increasing peak bone mass in adolescence [2–6]. It has been reported that a 10% increase in peak bone mass is predicted to delay the development of osteoporosis by 13 years [7].

There are several methods for measuring bone mass and bone mineral density (BMD) such as quantitative ultrasound and dual X-ray absorptiometry (DXA) [8]. BMD measurement using DXA is one of the important elements in diagnosing osteoporosis and in screening people at a high risk of fracture. DXA is a convenient method for measuring osteoporosis in many people because it is both quick and inexpensive. The age of peak bone mass differs at various skeletal sites (lumbar spine, femoral neck, trochanter, intertrochanter, Ward's triangle, total hip, distal one-third of the radius, and ultradistal forearm) [9]. The distal one-third of the radius has been used when measurement of the lumbar spine could not be obtained.

Peak bone mass in the distal one-third radial BMD by DXA has been reported to be achieved by the third decade of life [8, 9]. Recently, several reports investigating BMD in juveniles have added to the previously accumulated data obtained for elderly adults in Japan [10]. However, few reports have surveyed the correlation between BMD and lifestyle in elementary and junior high school students [11]. In particular, insufficient data on BMD have been obtained from young males since the subjects in most of the previous studies were females. Furthermore, the examination methods used to assess the relationship between daily diet and BMD have been simple. In most of the previous studies conducted in Japanese populations, a 3-day dietary record or simple questionnaire has been used for dietary assessment and it was difficult to examine the correlation between BMD and detailed nutrient factors [12, 13]. Therefore the present study used the brief self-administered diet history questionnaire for 10-years old (BDHQ10y) was a semiquantitative food frequency questionnaire that asked the dietary habits of the previous month for juveniles [14, 15].

The aim of the present study was to explore the relationship between BMD and lifestyle, including nutrient intake, sports club activity, and sleep time, in juveniles in order to identify educational intervention methods to prevent osteoporosis in later life by achieving high peak bone mass in juveniles.

Methods

Subjects

The subjects were 1392 students, aged 6–18 years, attending school in seven municipalities throughout Hokkaido prefecture, Japan. Informed consent to participate in the study, including measurement of BMD and completion of the questionnaire, was obtained from students and their guardians. Subjects for whom BMD data could not be obtained were excluded from the analysis, leaving a study group of 1364 subjects (770 males and 594 females). This survey was carried out from September 2002 to September 2005.

BMD measurement

BMD was measured by DXA at the distal forearm of the non-dominant side (DTX-200; Osteometer MediTech Inc., CA, USA) as previously described [9]. When the subjects had a history of bone fracture or any bone diseases in the non-dominant side, the other side was scanned.

Anthropometric measurement

Data for weight (kg) and standing height (cm) of each subject were obtained from the most recent anthropometric measurement records at their school. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

Grip strength measurement

The grip strength of the non-dominant side was measured twice using a digital grip dynamometer (Grip-D; Takei Scientific Instruments Co., Ltd., Niigata, Japan) and the stronger values were used for analysis.

Questionnaire

Lifestyle, participation in sports club activities, secondary sexual characteristics (data on change in voice and menstrual status), history of bone fracture at any site, personal medical history, current therapy, and milk consumption were assessed by use of a self-administered questionnaire. If the subjects could not fill in the questionnaire by themselves, their guardian was asked to complete it with them. Nutrient intake was assessed with the BDHQ10y. Total calories and five nutrients, i.e., calcium; phosphorus; and vitamins A, C, and D which previous studies [16–19] have suggested influence BMD, were calculated for each subject and included in the analysis.

Statistical analysis

The difference in BMD by calendar age was analyzed by one way ANOVA or, if necessary, by the Bonferroni method to identify differences. Spearman rank correlation coefficient with BMD was computed for consumption of milk, grip strength, and anthropometric variables such as body height, body weight, and BMI. Differences in BMD between two groups divided by lifestyle were analyzed by Student’s *t*-test. To investigate the relationship between secondary sexual characteristics and BMD, subjects were divided into two groups, as follows: Males were divided into “before” or “after” groups based on change in voice; females were divided into “before” or “after” groups based on menarche. We used data from males aged 12–14 years and females aged 11–13 years to analyze the correlation between secondary sexual characteristics and BMD. The subjects were divided into four groups according to their grade of school, i.e., lower elementary school student, higher elementary school student, junior high school student, and senior high school student, to analyze consumption of milk and total calorie and nutrient intake. The means among them were compared using Kruskal-Wallis rank test or, if necessary, by Mann-Whitney U test corrected by the

Bonferroni method to identify differences. Multivariate analysis of BMD, anthropometric, lifestyle, and nutrient data was performed by multiple linear regression analysis. *p* values less than 5% were considered statistically significant in all analyses. All the statistical analyses were performed using SPSS for Windows version 14.0 (SPSS, Chicago, IL, USA).

Ethical issues

The Ethics Committee of Asahikawa Medical College, Asahikawa, Japan, approved the study protocol.

Results

Mean values and standard deviations of BMD according to gender and calendar age are presented in Figure 1. The difference in BMD was compared the two adjacent calendar ages in each gender. The difference in BMD for males was larger among the junior and senior high school groups. The standard deviation of male BMD after age 12–13 years was larger than before. On the other hand, although the difference in BMD for females was larger among older elementary school children and later groups, the difference was moderate among junior high school and almost plateaued in senior high school subjects, aged 16–18 years. The standard deviation of female BMD after 12–13 years was larger than before.

Each anthropometric variable was strongly correlated with BMD [body height (males and females): $r = 0.701$ and $r = 0.689$; body weight: $r = 0.823$, $r = 0.826$; BMI: $r = 0.716$, $r = 0.763$; all correlations were $p < 0.001$] . Grip strength was strongly correlated with BMD (males: $r = 0.811$, females: $r = 0.758$; $p < 0.001$ respectively). Multiple linear regression analysis was performed with BMD as the dependent variable and sex, calendar age, BMI, and grip strength as independent variables. The regression coefficient of any independent variable was significant ($p < 0.001$) (Table 1).

Multiple linear regression analysis was then performed with BMD as the dependent variable and sex, calendar age, BMI, and sports club activities as independent variables. The regression coefficient of sports club activities was not significant ($p = 0.360$) (Table 2). Similarly, sleep time, having a nap time routine, and history of bone fracture were analyzed by multiple linear regression analysis. The regression coefficient of having a nap time routine was significant ($p < 0.01$), but the regression coefficient of sleep time was not significant ($p = 0.059$). The regression coefficient of history of bone fracture was not significant ($p = 0.330$).

The relationship between secondary sexual characteristics and BMD is shown in Figure 2. Among males aged 12–14 years, BMD in the “after change in voice” group was higher than that in the “before change in voice” group, and significance was detected at the age of 12 and 13 years. Among females aged 11–13 years, BMD in the “after menarche” group was significantly higher than that in the “before menarche” group.

Milk is a common food that provides a ready source of calcium. The consumption of milk was significantly lower in senior high school students compared with other grades for both genders (Fig. 3). The correlation between consumption of milk and BMD was analyzed. The consumption of milk in senior high school males and junior high school females correlated weakly with BMD (Table 3).

Figure 4 shows means ± standard deviations of daily total calorie and calcium intakes based on the BDHQ10y. Total calorie intake of males in junior high school students was significantly higher than that in lower elementary school children ($p < 0.05$). Total calorie intake of females in senior high school students was significantly lower than that in lower elementary school children ($p < 0.05$) and higher elementary school children ($p < 0.01$). Calcium intake was significantly lower in senior high school students compared with other grades for both genders. Other nutrient elements showed a similar tendency as total calories. Intake of total calories; calcium; phosphorus; and vitamins A, C, and D were independent of BMD by multiple linear regression analysis (Table 4).

Discussion

Few reference data on forearm BMD obtained using the DXA method are available for Japanese juveniles [20]. Most previous studies of BMD in juveniles included only females and did not include elementary school children [13]. This study reports general population-based data on forearm BMD in Japanese juveniles.

We used a self-administered semiquantitative dietary assessment questionnaire, the BDHQ10y, which asked participants to report their dietary habits for the previous month. The BDHQ10y was easy to research and standardize and made it possible to investigate comparatively long-term dietary habits. The BDHQ10y had the advantage of not causing a day-to-day variation. The use of BDHQ10y was useful for the clarification of the correlation between BMD and detailed nutrient factors. The responses to the BDHQ10y provided detailed information on nutritional elements and total calories, enabling us to analyze the correlation between BMD and specific nutritional elements. Therefore, the data from this study are important for the prevention of osteoporosis.

The difference in BMD for males peaked among junior to senior high school groups, and the standard deviation after age 12–13 years was larger than before. The difference in BMD for females peaked among higher elementary school children and declined at age 17–18 years, and the standard deviation of BMD after 11–12 years was larger than before.

The widening standard deviation for BMD indicates a time lag in the growth spurt of BMD among individuals and that time is a critical period for increasing BMD. In other words, the period before 12–13 years of age in males and before 11–12 years of age in females is an important time for intervention to increase peak bone mass. Matsukura et al. reported that BMD increased steadily with age in males and increased with age and then plateaued in females among Japanese children and adolescents [20]. Our results indicate a similar tendency, although the mean value for BMD was different. This difference could be caused by differences in the measurement site; specifically, other studies measured at one-third of the forearm length proximal to the ulnar end plate and the ultradistal forearm, and we measured at the distal forearm.

It is agreed that BMD and anthropometric variables are strongly correlated. A significant relationship between BMD and anthropometric variables such as body weight has been reported [21, 22], and low body weight was reported to be the major risk factor for low peak bone mass [6, 23, 24]. Our results support this correlation. The strongest factor that correlated with BMD was body weight.

The grip strength was strongly correlated with BMD after adjusting for sex, calendar age, and BMI. There is ample evidence that mechanical stimuli can increase bone strength. Accordingly, physical exercise seems to prevent bone loss and possibly induce increases in bone mass even at older ages [25]. However, our results show that the regression coefficient of sports club activities and BMD are not significant. There are two possible explanations: (1) Physical activities other than sports club activities largely affected BMD increase, or (2) the difference among sporting events affected BMD increase. Maimoun et al. reported that BMD in triathletes was higher than in controls and swimmers, and no differences in BMD were found among cyclists, swimmers, and controls [26]. There have been many reports on the effects of exercise on BMD [6, 27, 28]. These correlations in juvenile require further exploration.

The supine position in bed places a light load on bones, and during nighttime sleep hormonal secretion varies from the waking state [29–31]. Therefore, it is thought that sleep periods may provide a chance to change bone remodeling. Specker reported that sleep deprivation is associated with lower BMD outcomes in some, but not all, individuals [32]. Our results show that frequent or habitual napping was associated with BMD increase, but that nighttime sleeping was not associated with such increase. Students (individuals) who had a habit of napping might spend longer periods sleeping than other students. Sports club activities might also be a confounding factor because students who participate in sports club activities are estimated to sleep longer and nap more often than other students since they get more tired. Detailed data are required to determine the effects of sleep.

BMD in juveniles in the period after the development of secondary sexual characteristics, identified as change in voice in males and menarche in females, was higher than that in juveniles of the same age in the period before development of these characteristics. This indicates that juveniles who developed secondary sexual characteristics at an early age had higher BMD. In other words, BMD was markedly increased at the secondary sexual characteristic period. However, this study could not clarify the relationship between secondary sexual characteristics and peak bone mass because the study was cross-sectional and BMD increase continued after 17–18 years of age. Iki et al. suggested that marked BMD increase occurred in males after pubic hair appeared and before menarche in females [3]. Therefore, it is recommended that educational intervention, to prevent osteoporosis, begin before puberty.

Although this study shows no significant correlation between BMD and any nutritional elements, we have identified some problems related to dietary habits in adolescence. The mean total calorie level in senior high school students was equivalent to that in junior high school students. This might suggest that senior high school students ate food that was not included in the BDHQ10y to obtain the necessary calories. Calcium intake was lower than the dietary reference value for all ages and noticeably lower among senior high school students. In addition, they might try to lose weight, which results in lack of calcium. The relationship between weight change and BMD change was significant [33]. This report supports that weight loss would cause adverse effect for BMD increase. Moreover, calcium intake tends to be less in ordinary daily meals in Japan because consumption of dairy products is lower than Europe or the United States [34]. Consequently, osteoporosis risk in later life for Japanese could be increased since they did not have enough calcium in adolescence, when additional calcium is needed to obtain greater BMD.

Milk is a readily available source of calcium. Milk and dairy products are excellent foods due to not only their high calcium content but also due to their high absorption rates [35]. Since drinking milk has been recommended as a means for preventing calcium deficiency, we investigated the effectiveness of drinking milk on BMD increase in adolescents. We found a relationship between the consumption of milk and BMD in the some age group. Cadogan reported that increased milk consumption significantly enhances bone mineral acquisition in adolescent girls [36]. However, intake of calcium has been shown to decrease significantly in senior high school students compared with other age groups. At the same time, the consumption of milk has been shown to decrease significantly in senior high school students compared with other age groups. In Japan, junior high schools and elementary schools have provided lunch services that include milk. However, senior high schools do not have a school-provided lunch service, resulting in decreased consumption of milk. The decrease in the consumption of milk has been suggested as a reason for a decrease in the calcium intake. Xueqin reported that a school-milk intervention trial enhanced bone mineral accretion in Chinese girls [37]. That report suggests milk service in school is an effective way to increase BMD. Although our results do not indicate that BMD was dependent on intake of calcium, it was a problem that calcium intake particularly among senior high school students was less than the dietary reference value. The calcium intake that supplements this decrease might be necessary because BMD increase had continued after junior high school.

This study has several limitations. First, this is a cross-sectional study and the present BMD data may have been affected by past lifestyle habits. The questionnaire asked mainly about present habits and the results could probably be influenced by a changed lifestyle habit of the past. Second, although the BDHQ10y was adapted for juveniles, it could not support all age groups. In particular, dietary consumption among senior high school students tended to be underrated. Although these limitations made the analysis more difficult, it is still possible to compare nutritional elements among the same grade groups.

In conclusion, this study elucidates several factors related to an increase of BMD in Japanese juveniles. Maintaining adequate body weight for obtaining higher peak bone mass. The calcium intake particularly among senior high school students was low level. Considering the dietary reference value aged 15-17 years (male 800 mg/day and female 700 mg/day), this calcium intake level was a problem. It has been suggested that the avoidance of weight loss and calcium deficiency may prevent lower peak bone mass and osteoporosis. Moreover, effective educational intervention to obtain higher peak bone mass must begin before the higher elementary school.

Acknowledgments

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Table 1 Multiple linear regression analysis with BMD as the dependent variable, and sex, calendar age, BMI, and grip strength as independent variables

Independent variable	Standardized regression coefficient	*p* value
Sex (male vs. female)	0.063	<0.001
Calendar age (years)	0.262	<0.001
BMI (kg/m^2)	0.348	<0.001
Grip strength (kg)	0.380	<0.001

Table 2 Multiple linear regression analysis of BMD with lifestyle and history of bone fracture

Independent variable	Standardized regression coefficient	p value
Sports club activities	−0.015	0.360
Sleep time at night	0.036	0.059
Habitual napping	0.059	<0.01
History of bone fracture	0.016	0.330

Adjusted for sex, calendar age (years), and BMI.

Table 3 Correlation between consumption of milk and BMD

	Male		Female	
Elementary school student	r = 0.045	$p = 0.458$	r = 0.041	$p = 0.506$
	(n = 245)		(n = 265)	
Junior high school student	r = 0.003	$p = 0.966$	r = 0.228	$p < 0.01$
	(n = 227)		(n = 148)	
Senior high school student	r = 0.149	$p < 0.05$	r = 0.137	$p = 0.065$
	(n = 268)		(n = 181)	

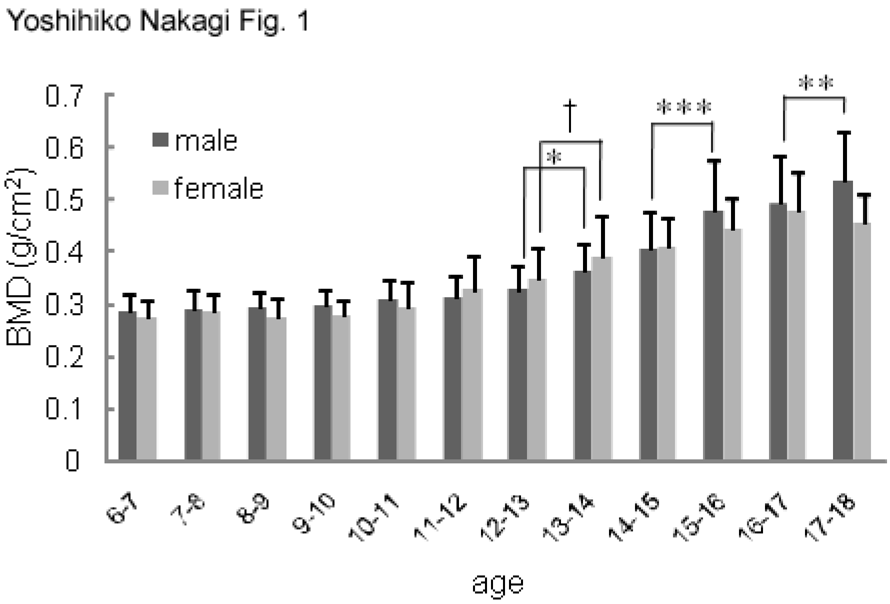
r: Spearman rank correlation coefficient

Table 4 Multiple linear regression analysis of BMD with intake of nutritional elements.

Independent variable	Standardized regression coefficient	*p* value
Total calorie	0.075	0.059
Calcium	-0.007	0.887
Phosphorus	-0.129	0.116
Vitamin A	0.038	0.070
Vitamin C	0.015	0.491
Vitamin D	0.045	0.068

Adjusted for sex, calendar age (years), and BMI.

Figure 1


Yoshihiko Nakagi Fig. 1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMD (g/cm2)
male
female
†
*
***
**
6-7
7-8
8-9
9-10
10-11
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14-15
15-16
16-17
17-18
age

Figure 2

Yoshihiko Nakagi Fig. 2

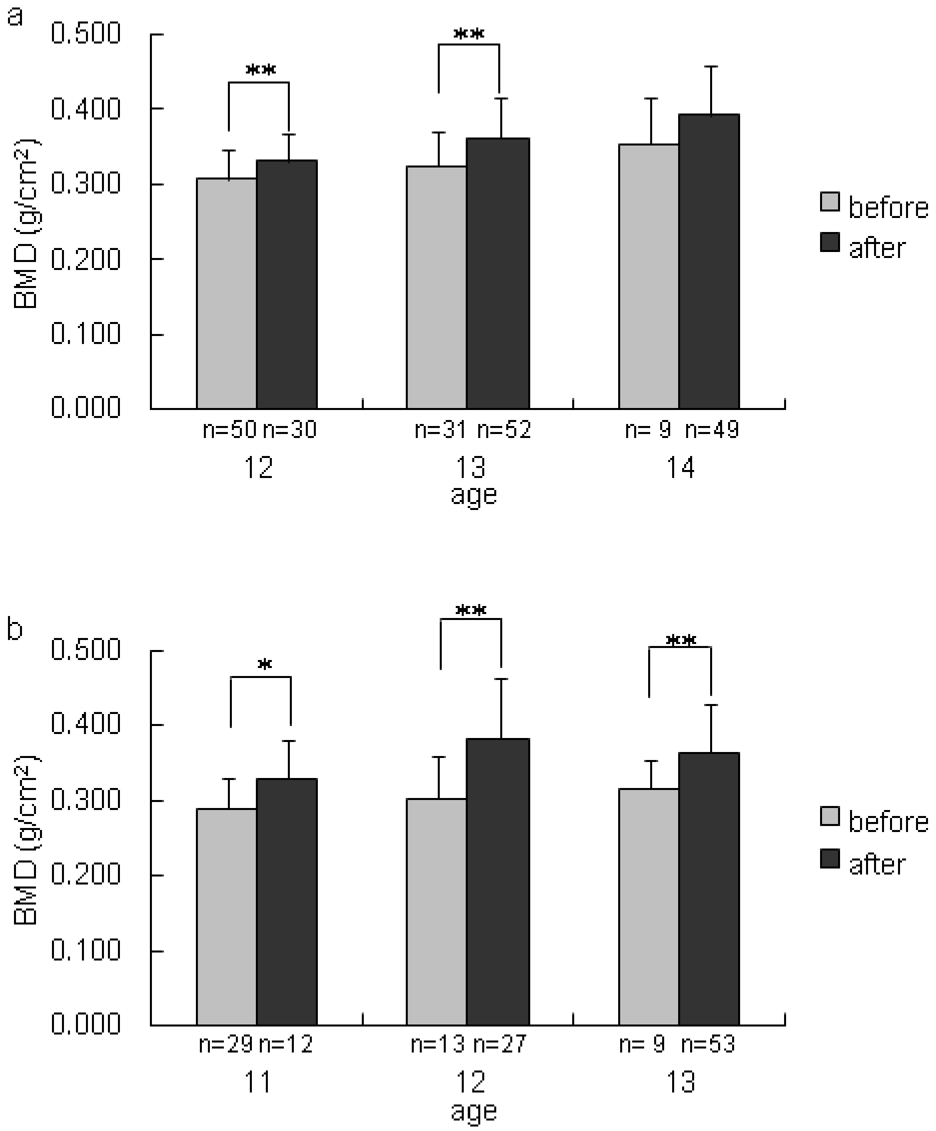

Figure 3

Yoshihiko Nakagi Fig. 3

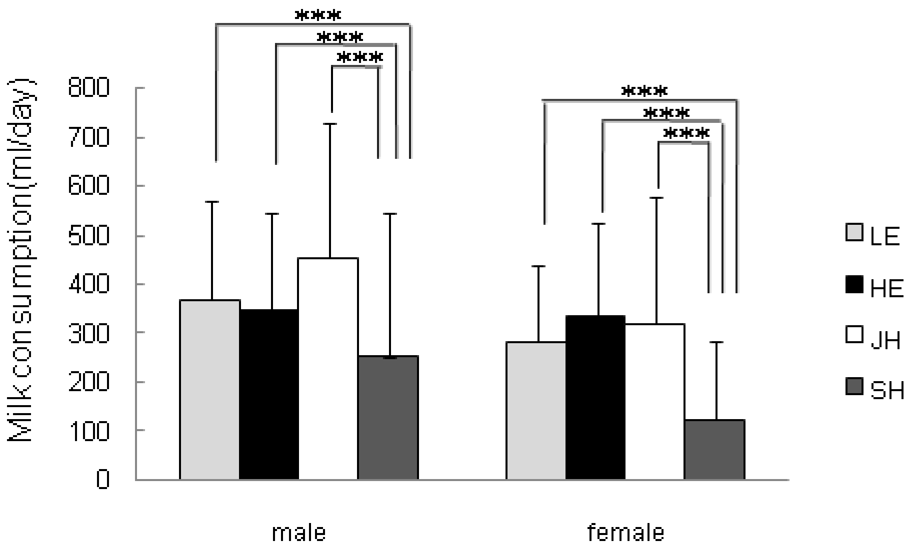

Figure 4

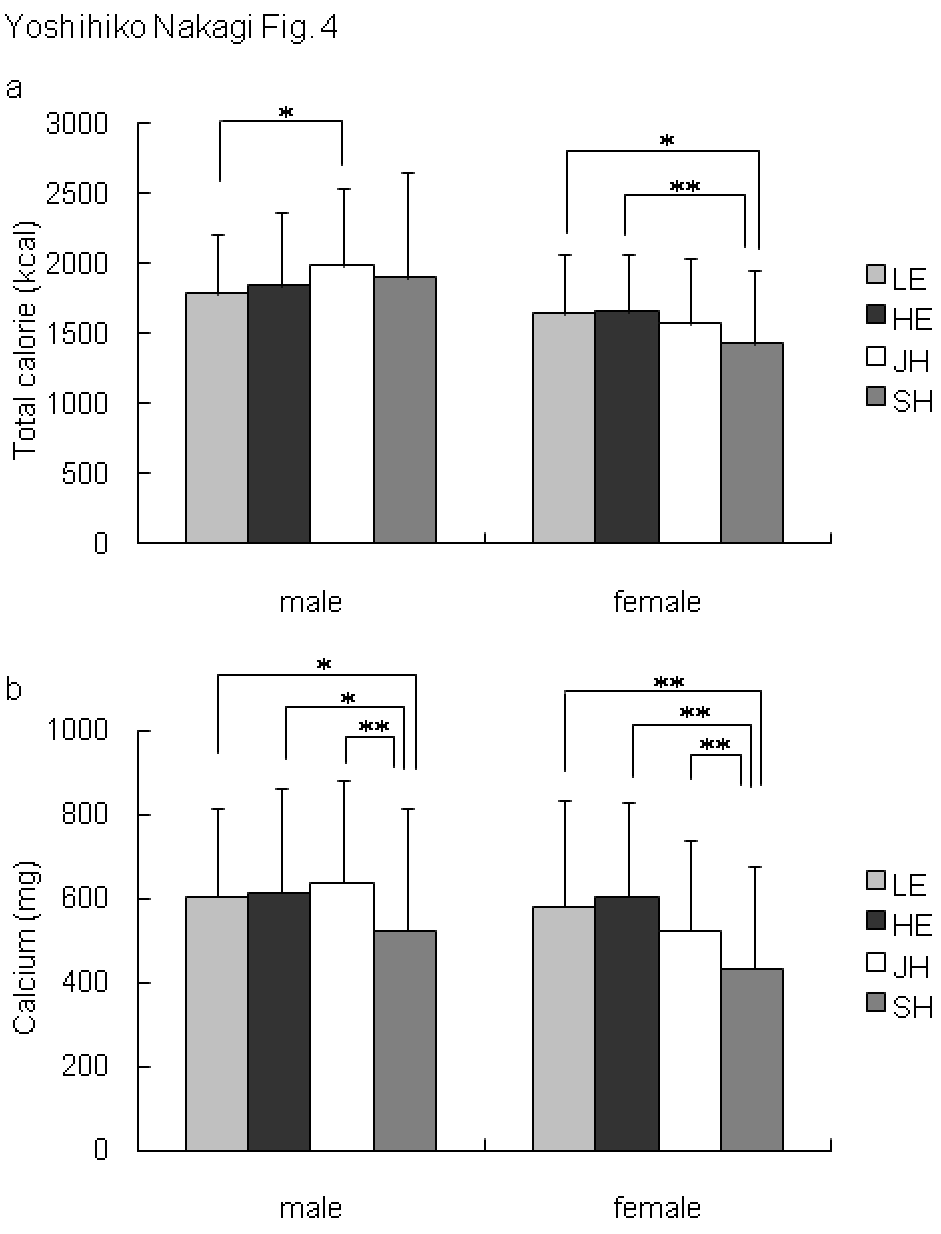
Yoshihiko Nakagi Fig. 4
a
Total calorie (kcal)
3000
2500
2000
1500
1000
500
0
*
*
**
male
female
LE
HE
JH
SH
b
Calcium (mg)
1000
800
600
400
200
0
*
*
**
**
**
**
male
female
LE
HE
JH
SH

Figure legends

Fig. 1 BMD of subjects by calendar age in each gender. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ according to Bonferroni method for multiple comparison (for males only); $^{\dagger}p < 0.05$ according to Bonferroni method for multiple comparison (for females only)

Fig. 2 The relationship between secondary sexual characteristics and BMD. (a) Comparison of BMD between the “before change in voice” and “after change in voice” groups in males. (b) Comparison of BMD between the “before menarche” and “after menarche” groups in female. $^{*}p < 0.05$, $^{**}p < 0.01$

Fig. 3 Mean values for daily consumption of milk. LE: Lower elementary student; HE: Higher elementary student; JH: Junior high school student; SH: Senior high school student. $^{***}p < 0.001$ according to Bonferroni method for multiple comparison

Fig. 4 Mean values for daily total calorie and calcium intakes based on the BDHQ10y. (a) Total calorie and (b) Calcium. LE: Lower elementary student; HE: Higher elementary student; JH: Junior high school student; SH: Senior high school student. $^{*}p < 0.05$, $^{**}p < 0.01$ according to Bonferroni method for multiple comparison