



Bone mineral density, vitamin D status, and calcium intake in healthy female university students from different socioeconomic groups in Turkey

Betül Ersoy¹ · Deniz Özalp Kizilay¹ · Seniha Kiremitci Yilmaz¹ · Fatma Taneli² · Gül Gümüşer³

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Abstract

Summary Peak bone mass is reached in late adolescence. Low peak bone mass is a well recognized risk factor for osteoporosis later in life. Our data do not support a link between vitamin D status, bone mineral density (BMD), and socioeconomic status (SES). However, there was a marked inadequacy of daily calcium intake and a high presence of osteopenia in females with low SES.

Purpose Our aims were to (1) examine the effects of different SES on BMD, vitamin D status, and daily calcium intake and (2) investigate any association between vitamin D status and BMD in female university students.

Subjects and methods A questionnaire was used to obtain information about SES, daily calcium intake, and physical activity in 138 healthy, female university students (age range 18–22 years). Subjects were stratified into lower, middle, and higher SES according to the educational and occupational levels of their parents. All serum samples were collected in spring for 25-hydroxyvitamin D concentration (25OHD). Lumbar spine and total body BMD was obtained by dual-energy X-ray absorptiometry (DXA) scan (Lunar DPX series). Osteopenia was defined as a BMD between -1.0 and -2.5 standard deviations (SDs) below the mean for healthy young adults on lumbar spine DXA.

Results No significant difference was found between the three socioeconomic groups in terms of serum 25OHD concentration, BMD levels, or BMD Z scores ($p > 0.05$). Both the daily intake of calcium was significantly lower ($p = 0.02$), and the frequency of osteopenia was significantly higher in girls with low SES ($p = 0.02$). There was no correlation between serum 25OHD concentration and calcium intake and BMD values and BMD Z scores ($p > 0.05$). The most important factor affecting BMD was weight ($\beta = 0.38$, $p < 0.001$).

Conclusions Low SES may be associated with sub-optimal bone health and predispose to osteopenia in later life, even in female university students.

Keywords Vitamin D · Osteopenia · Bone mineral density

Introduction

Activation of bone formation from infancy to late adolescence results in bone mass accumulation [1]. Puberty plays a fundamental role in bone mass accrual. Bone mass increases from early to late puberty in both sexes [2]. The age of attainment of peak bone mass is still disputed. It has been reported that peak bone mass occurs as early as 20 years of age [3, 4]. High peak bone mass in early life reduces fracture risk in later life [3, 5]. Dual-energy X-ray absorptiometry (DXA) has become the gold standard for assessment of bone mass. The most common sites for measurements of bone mass by DXA are lumbar spine and whole body [6].

✉ Betül Ersoy
betul.ersoy@cbu.edu.tr

¹ Division of Pediatric Endocrinology and Metabolism, School of Medicine, Celal Bayar University, Manisa, Turkey

² Department of Clinical Biochemistry, School of Medicine, Celal Bayar University, Manisa, Turkey

³ Department of Nuclear Medicine, School of Medicine, Celal Bayar University, Manisa, Turkey

Bone mass is determined by genetic, environmental, and lifestyle factors, such as physical activity and nutrition. Socioeconomic status (SES) is known to be strongly related with environmental factors including those affecting bone mass such as nutrition and physical activity [7, 8]. SES may therefore influence bone mass accrual. An association between SES and BMD has been reported in some adult studies, yet this association is still poorly understood [9]. Similar data in late teen or young adults are scarce.

Vitamin D status is known to be important for skeletal integrity [10]. Some adult studies have shown that 25OHD is associated positively with bone mineral density (BMD) [11]. In addition, studies from children and adolescents have reported that optimal vitamin D level is essential for attainment of peak bone mass [12]. Vitamin D is obtained from dietary sources and cutaneous synthesis by ultraviolet radiation on the skin. There are numerous studies documenting vitamin D status in healthy adolescents. Season, gender, and ethnicity have been reported as determinants of vitamin D status [13, 14]. SES can affect vitamin D levels in healthy individuals because of differences in life style including variations in the amount of exercise taken, nutrition, and sunlight exposure. There are a few studies examining the impact of SES on vitamin D levels which include school-age children [15, 16]. To our knowledge, no study is available examining the impact of SES on vitamin D levels in late teen females at the time of peak bone mass accrual.

In this study, we evaluated BMD and vitamin D status in female university students at an age when they should achieve peak bone mass. Our aims were to examine the effects of different socioeconomic status on BMD, vitamin D status, and daily calcium intake in healthy, female university students and to investigate any association between vitamin D status and BMD.

Participants and methods

Selection of participants

The study design is cross sectional. It was planned to evaluate BMD and vitamin D status in 200 healthy female university students aged between 18 and 22 years. Participants for this study were randomly selected. Initially, the proposed study was advertised among female university students. All volunteers were included in the study. Written informed consent was obtained from all participants. The study was conducted in April and May (spring). One hundred seventy-five young females agreed to participate in the study during this period. Blood samples were collected from these volunteers. However some of them did not come for DXA and were excluded from the study. In addition, it was noticed that some wore Muslim style clothing (covered). These were also

excluded from the study in order to provide group homogenization. Thus, the final study group comprised 138 participants.

The exclusion criteria for this study were the following: presence of diabetes mellitus; acute or chronic undernourishment; congenital or acquired bone diseases; gastrointestinal diseases accompanied by malabsorption; history of nephropathy with or without chronic renal failure; endocrinopathies; chronic drug consumption; cystic fibrosis; celiac disease; drug use that negatively affected bone metabolism (such as anticonvulsants and antacids with aluminum); more than 2 h per week of physical exercises, since excessive physical exercise interferes with bone mineralization; use of contraceptives; pregnancy at any time; history of fracture; or being a professional athlete. None of the participants took calcium or vitamin D supplements. Young female students were asked individually about their menarcheal ages and their menstrual characteristics. Those with amenorrhea or menstrual disorders were not included in the study.

This study was approved by the local Ethics Committee on Clinical Investigation.

Socioeconomic status evaluation

The educational and occupational status of subjects' parents was obtained from the participants before BMD measurement. Categorization of socioeconomic class was based on the occupation and education of the parents by applying the Hollingshead index as shown in Table 1 [17]. Five educational levels and five occupational categories were used to identify socioeconomic classes. A score of 0 was given to the lowest level of education and occupation and a score of 4 was given to the highest. Three socioeconomic classes were identified, ranging from lowest to the highest, on the basis of the sum of scores. Hollingshead scoring was modified according to national Turkish standards. The first and second socioeconomic classes in Hollingshead scoring were defined as low and middle socioeconomic classes, respectively. The third and fourth classes were defined as high socioeconomic class.

Anthropometric and BMD measurements

Anthropometric and DXA measurements were obtained from the subjects during the same visit. Weight was measured using digital scales with a precision of 0.1 kg, and height was measured to the nearest 0.1 cm with a stadiometer. A Lunar DPX-NT scanner (General Electric, Madison, Wisconsin, USA) was used for measurements of BMD. BMD (measured in grams per square centimeter) was calculated for the second, third, and fourth lumbar vertebrae and for the total body. During measurement of the lumbar spine, the subject was supine, and the physiological lumbar lordosis was flattened by elevation of the knees. All measurements were performed and analyzed by the same person. The person performing BMD

Table 1 Scoring of the educational level and occupation of both parents according to the Hollingshead index of socioeconomic groups

Educational level	Score	Occupation	Score
No education	0	No occupation	0
Elementary school	1	Workman	1
Junior school	2	Blue collar worker	2
High School	3	White collar worker	3
University	4	Professional	4

Educational and occupational scores of parents: 0–4: I; 5–8: II; 9–12: III; and 13–16: IV

measurements was blinded to the socioeconomic status of young women. Results were reported as BMD Z scores (BMDz). BMD Z scores are preferred in females prior to menopause [18].

The term “osteopenia” can be used instead of “decreased bone density” and describes reduced bone mass [19]. Osteopenia is defined as a BMD between -1.0 and -2.5 standard deviations (SDs) below the mean at the site of the lumbar spine for healthy young adults. Osteoporosis is a disease characterized by severe low bone mass. It is defined by a BMD SD score lower than -2.5 [20].

Nutritional assessment

Adequate calcium intake is essential for normal growth and development of the skeleton and teeth and for adequate bone mineralization, in addition to being important for the normal function of a number of systems including muscle, nerves, and cellular membranes. The recommended daily intake of calcium for young women is 1000 mg [21]. We assessed dietary calcium intake using a short questionnaire. Questions regarding frequency of intake of food containing calcium (number of times per day, number of days per week) were asked. Daily calcium intake was estimated according to food sources of calcium [22].

Physical activity

Adults should do at least 150 min a week of moderate-intensity or 75 min a week of vigorous-intensity aerobic, physical activity, according to physical activity guidelines [23]. Exercise frequency was assessed from a questionnaire inquiring about the number of hours spent on sports per week. Physical activity was assessed as regular or irregular.

Biochemical analysis

Measurement of serum 25OHD is a reliable assay for assessment of vitamin D status. Vitamin D deficiency is defined as a 25OHD concentration below 20 ng/ml (50 nmol/L) and

vitamin D insufficiency as 25OHD between 21 and 29 ng/ml (525–725 nmol/L) [24]. After an overnight fast, venous blood samples were collected into EDTA tubes (BD, Canada) between 9 a.m. and 10 a.m. Blood samples were centrifuged at $1500\times g$ for 10 min within 1 h of collection. Hemolytic, lipemic, and icteric serum samples and samples collected in inadequate test tubes or samples with insufficient volume were excluded from the study. Plasma samples were assessed using a Roche Cobas e411 (Roche Diagnostics GmbH, Mannheim, Germany) by electrochemiluminescence immunoassay using Roche Cobas Vitamin D total assay reagent (Roche Diagnostics GmbH, Mannheim, Germany). This method quantitatively determines plasma 25OHD concentrations. Samples were processed in a single batch according to the manufacturer’s instructions. Calibration curves were constructed using calibrators provided in the kits. Measurement ranges of the assays were 7.5–175 nmol/L for the Roche Cobas Vitamin D total assay. Internal quality control samples were included in the assay run, and intra-assay coefficients of variation (CVs) values were obtained from the measurements. For Cobas e411 analysis, intra-assay CVs were 4.8 and 2.7% for target values of 49.8, and 95.8 nmol/L respectively (PeciControl Varia 1 and 2, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was done using SPSS for Windows v13.0. Parametric data are reported as mean \pm SD. Participants were divided into three groups according to SES as described previously. Statistical differences across the three groups were analyzed using Pearson’s chi-square or Fisher’s exact test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Pearson’s correlation coefficients were computed to assess any linear relation between the variables.

Results

Mean age of participants was 20.1 ± 0.9 years (age range 18–22 years). Mean height, weight, and BMI were 164.2 ± 6.0 cm, 57.2 ± 8.1 kg, and 21.2 ± 2.7 kg/m², respectively. The SES grouping of the subjects were low (47.8%, n 66), middle (29.7%, n 41), or high (22.5%, n 31) ($p = 0.672$). The educational attainment level of the parents of subjects from the three SES cohorts is shown in Table 2.

Table 3 shows participants characteristics according to SES. There were no significant differences between weight, height, and BMI of female university students from different socioeconomic classes ($p > 0.05$). No significant differences were found in terms of BMD values and BMD Z scores between the three SES groups ($p > 0.05$) (Fig. 1). Female

Table 2 Distribution of female university students according to socio-demographic features

Socio-demographic features (<i>n</i> 138)	Number	Percent (%)
Educational levels of mothers		
No education	6	4.3
Elementary school	76	55.1
Junior school	14	10.1
High school	19	13.8
University	23	16.7
Educational levels of fathers		
No education	2	1.4
Elementary school	51	37.0
Junior school	12	8.7
High school	32	23.2
University	41	29.7

students from the low SES have lower serum 25OHD concentration than the other groups. However, there was no significant difference between the three groups ($p > 0.05$) (Fig. 2). Daily calcium intake was lower in females belonging low SES than those in middle and high SES. However, a significant difference was only found between females belonging the low and middle SES groups ($p = 0.025$) (Fig. 2). Daily calcium intake was below 1000 mg in all female students included in this study. Therefore, we redefined calcium intake as adequate if it was above 750 mg per day.

Frequency of vitamin D deficiency was higher in females belonging to the low SES group although this again was not significant ($p = 0.67$) (Fig. 3). The frequency of osteopenia was significantly higher in females belonging to the low SES group ($p = 0.02$). The presence of osteopenia demonstrated a linear association with SES. The frequency of osteopenia

decreased as the socioeconomic level rises (Fig. 3). Similar results were obtained for frequency of inadequate daily calcium intake ($p = 0.02$). However, none of the participants actually had osteoporosis, defined as a BMD *Z* score below -2.5 SD.

The physical activity levels of all groups were assessed as irregular (72.1% of low SES, 75.0% in the middle SES, and 80.0% in the high SES, 75.0% overall), and this was not significantly different between the groups ($p = 0.67$). BMD values were not influenced by either regular or irregular physical activity ($p > 0.05$).

No correlations were found between BMD, serum 25OHD concentration, and daily calcium intake. There was a weak correlation between BMD L2-L4 and weight ($r = 0.17$, $p = 0.046$). The body weight of the girls with osteopenia (54.9 ± 6.4 kg) was significantly lower than the girls without osteopenia (58.0 ± 8.4 kg) ($p = 0.04$). BMD values in participant with vitamin D deficiency did not differ significantly from those without vitamin D deficiency (data not shown). We were unable to assess the difference between adequate and inadequate calcium intake groups regarding BMD due to the small sample size of the adequate calcium consumer group.

Linear regression analysis was used to assess the independent effects of factors affecting BMD such as age, BMI, calcium intake, and weight. As a result of the analysis, weight was found to be a significant variable for both whole body BMD and BMD L2-L4 ($\beta = 0.38$, $p < 0.001$).

Discussion

In this study, we evaluated the impact of socioeconomic background on bone mineral density and vitamin D status in

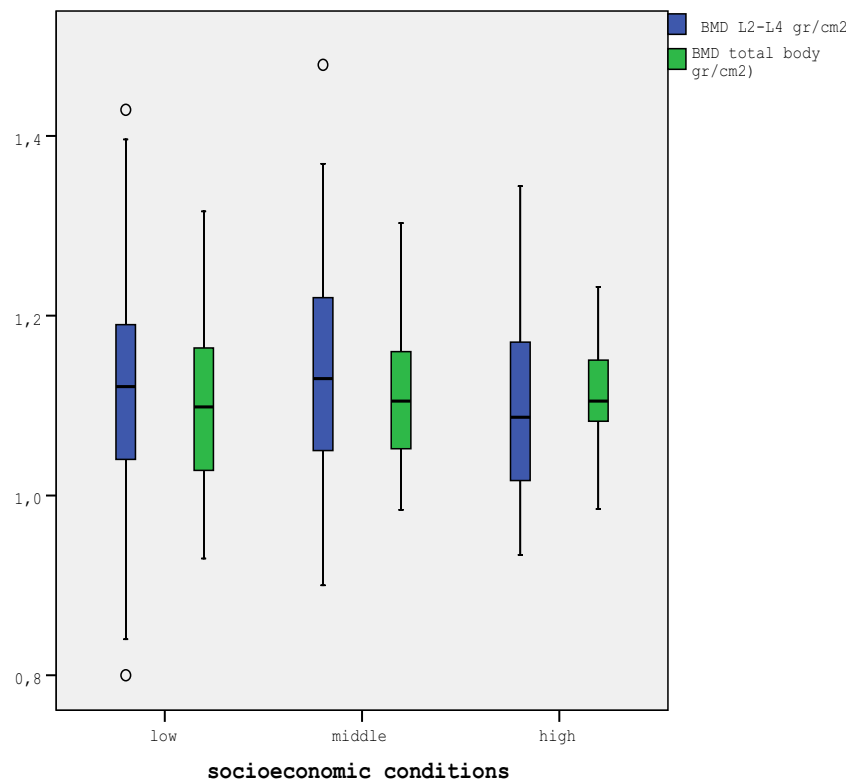
Table 3 Participants' characteristics according to SES

Characteristics	Socio-economic status (SES)			<i>p</i> value
	Low (<i>n</i> 66)	Middle (<i>n</i> 41)	High (<i>n</i> 31)	
Age (year)	20.28 ± 0.7	20.36 ± 0.9	20.5 ± 0.7	0.14
Height (cm)	163.7 ± 6.0	165.1 ± 6.1	163.7 ± 5.9	0.38
Weight (kg)	56.7 ± 7.2	58.5 ± 9.3	56.7 ± 8.1	0.60
BMI (kg/m ²)	21.2 ± 2.5	21.4 ± 2.9	21.2 ± 2.6	0.89
BMD <i>Z</i> score (total body)	-0.28 ± 1.0	1.1 ± 0.07	1.1 ± 0.05	0.12
BMD <i>Z</i> score (L2-L4)	-0.52 ± 1.0	1.1 ± 0.12	1.0 ± 0.1	0.47
BMD (gr/cm ²) (total body)	1.09 ± 0.08	1.11 ± 0.07	1.11 ± 0.05	0.41
BMD (gr/cm ²) (L2-L4)	1.12 ± 0.12	1.13 ± 0.12	1.09 ± 0.1	0.70
Daily calcium intake (mg/day)	144.84 ± 111.36	254.39 ± 194.48	212.93 ± 182.14	0.005*
25 OHD level (ng/dl)	32.48 ± 20.41	33.47 ± 17.94	35.17 ± 28.95	0.61

BMI body mass index, BMD bone mineral density, 25OHD 25-hydroxyvitamin D

*Daily calcium intake is lower in girls with low SES than in girls with medium and high SES

Fig. 1 BMD values according to SES. BMD L2-L4 and BMD total body values have not been shown significantly difference between groups



female university students at the time of peak bone mass accrual. To the best of our knowledge, this is the first study to examine this in young adult or late adolescent females. We determined that BMD values were not influenced by socioeconomic status in our subjects. Similar results were obtained for vitamin D status and the frequency of vitamin D deficiency with no difference between groups. However, participants belonging to the low socioeconomic groups have a higher frequency of osteopenia. One of the key results of our study was that all of the young females had inadequate daily calcium intake. This was more pronounced in young women from low socioeconomic level who had significantly lower daily calcium intake than those from the middle socioeconomic level.

SES has a significant role in musculoskeletal health as well as in a number of causes of morbidity and mortality. Studies showing the relationship between SES and bone health are mostly adult studies [9, 25, 26]. In a study of approximately 20,000 adults in the USA, an association between low income and low BMD in women has been reported [25]. Besides, it has been reported that socioeconomic advantage in childhood and adult education level were associated with higher adult lumbar spine BMD [26]. A meta-analysis of adult studies on the relationship between SES and BMD suggests that there is a link between low educational level and low BMD in women, but this has not yet been confirmed [9]. There are a limited number of studies on children and adolescents in this area.

We investigated whether bone mineral densities of female university students differed according to SES. We found that there was no significant difference in BMD in female university students belonging to different SES. One Spanish study in an adolescent age group has been published. Similarly to our study, the Spanish researchers reported that bone mineral density was not affected by SES in their adolescent subjects. However, their subjects were too young to have achieved peak bone mass with an age range of 12.5–17.5 years [8] while our subjects were assessed at the time of peak bone mass accrual.

In this study, we evaluated subjects individually and calculated the frequency of osteopenia and osteoporosis. None of the girls had osteoporosis. However, the frequency of osteopenia was significantly higher in girls with low SES. Osteopenia is defined as decreased or low bone density (BMD SD from -1.0 to -2.5). If not corrected, it can progress to (severe) osteoporosis (BMD SD lower than -2.5) and a risk for fractures [19]. Our finding led us to speculate whether osteopenia in this period of peak bone mass would increase the risk of fracture in later ages. In the literature, there is no longitudinal study that examines the effect of low BMD level during youth on fracture risk at older ages and the effect of SES. A Portuguese study has reported that hip fracture frequency increased in women at older ages in areas with low socioeconomic status [27]. Being underweight in women especially at 20 years is a significant risk factor for osteopenia [28]. Similarly, we found that girls with osteopenia had a

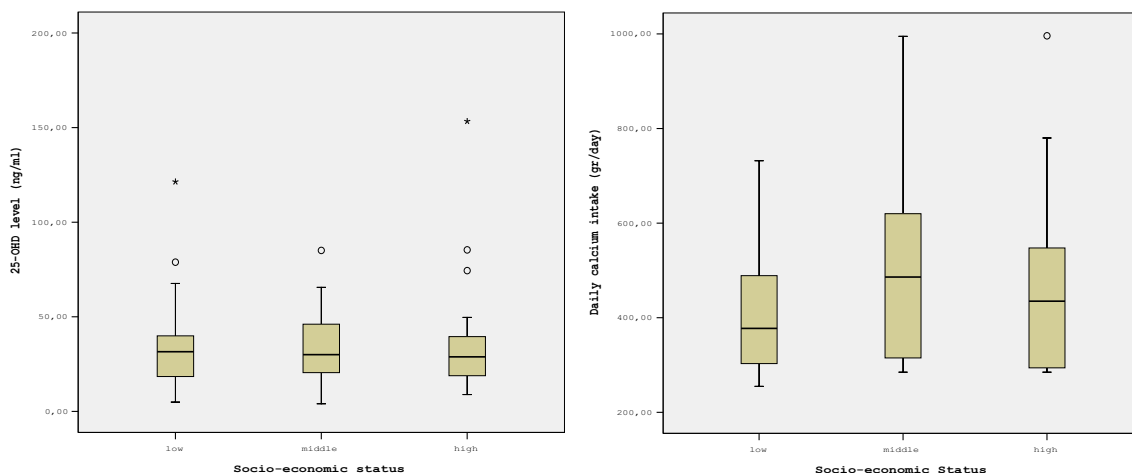


Fig. 2 Vitamin D levels (left panel) and daily calcium intake (right panel) according to SES. No significant difference was found in terms of 25-OH Vitamin D levels between groups. Daily calcium intake was lower in

females belonging to low SES than those in middle and high SES. This was only significant between females belonging the low and middle SES groups ($p < 0.025$)

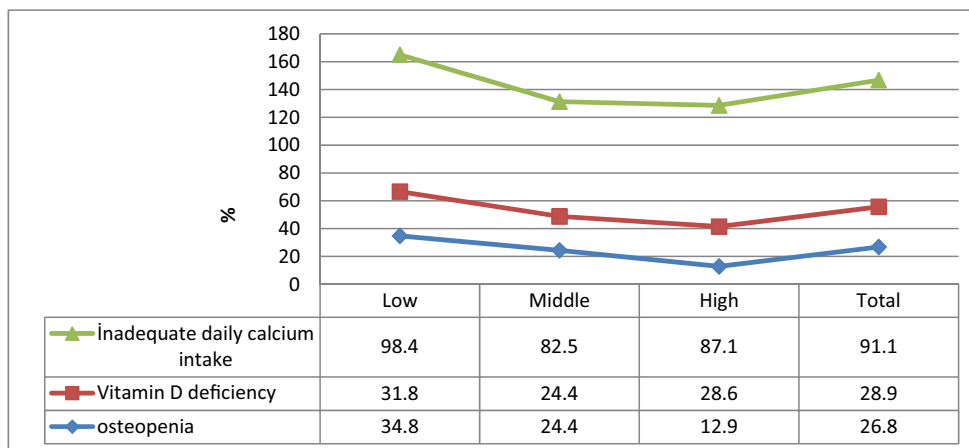
lower body weight than those without osteopenia. We also found a weak positive association between body weight and BMD.

Vitamin D has a pivotal role in bone health as well as the regulation of calcium and phosphorus metabolism [12]. One of the most important determinants of vitamin D status for children and adults and a major source of vitamin D is sun exposure [29]. We found that vitamin D status in young women did not differ significantly according to SES. When assessed individually, we showed that the frequency of vitamin D deficiency did not change significantly regardless of SES. This result indicates that SES in young female university students does not affect vitamin D status. This may be related to the fact that the region in which our study was conducted is sunny all year round. However, the frequency of vitamin D deficiency approached 30%, which indicates that a subset of young women, independently of SES, did not obtain enough sunlight exposure. The reason for this high rate may be related

with the assessment of vitamin D levels after winter months. Vitamin D levels exhibit seasonal variation and this is more pronounced in women [30]. In young, healthy Austrian women aged 18–30 years, vitamin D insufficiency was reported in up to 89% of subjects in the spring [31]. These results suggest that seasons should be taken into account when commenting on the vitamin D status.

We did not find any association between vitamin D level and BMD. In an Australian study in 400 young women aged 16 to 25 years, a similar result was obtained [32]. The contribution of vitamin D to peak bone mass and the relationship between vitamin D and BMD remain controversial. In a population-based study, a positive relationship between vitamin D levels and total hip BMD as measured by DXA has been demonstrated in younger and older adults [21]. In a systematic review and meta-analysis, it has been shown that vitamin D supplementation improves the lumbar spine BMD in children and adolescents with vitamin D deficiency [33].

Fig. 3 Osteopenia, vitamin D deficiency, and inadequate daily calcium (Ca) intake according to SES. The most striking finding is the almost universal inadequacy of dietary calcium intake. The incidence of vitamin D deficiency is not significantly different according to socioeconomic status. The incidence of osteopenia and inadequate daily calcium intake was significantly higher in female university students from the low SES group



Although calcium is one of the important factors in bone mass accrual, the association of higher calcium intake with better BMD is still unclear [34, 35]. Interestingly, we found that daily calcium intake of the vast majority of young female university students in this study was inadequate. The frequency of inadequate daily calcium intake was significantly higher in girls with low SES. A high frequency of osteopenia in conjunction with inadequate daily calcium intake in girls at low socioeconomic level suggests that daily calcium intake is important in increasing bone mass. However, we could not find any association between BMD and daily calcium intake. As we determined the amount of daily calcium intake by self-reported questionnaire, making such an association may be unreliable. Some studies have shown that there is no association between daily calcium intake and BMD, as in our study [36, 37]. Other studies have shown such an association [35, 38]. A significant interaction between the vitamin D receptor (VDR) and calcium intake was found in one study. It was thought that the VDR modulates the calcium effect on BMD [38]. Another study emphasizes that long-term and regular calcium consumption during childhood positively affects bone mineral density [35].

Our study had several limitations. Our study is a cross-sectional study. If we had assessed the bone mineral densities of female university students longitudinally from adolescence, we would have gained more valuable information about bone mass accrual. Our data on daily calcium intake and physical activity may not be very reliable as this was obtained through self-reported, subjective questionnaire survey. For this reason, we did not make a detailed statistical evaluation concerning self-reported physical activity.

In conclusion, our data do not support a link between vitamin D status, BMD, and SES. However, osteopenia is more frequent in young female university students belonging to low SES. Although there is no difference in bone health among young female university students belonging to different SES when assessed globally, low socioeconomic level poses a risk for bone health when assessed individually. Interestingly, in our population, daily dietary calcium intake was almost universally inadequate. This finding was even more pronounced in young women from low SES. However, we were unable to show an association between calcium intake and BMD. We recommend that adolescents and young adult women, especially at low socioeconomic levels, should be advised about sufficient daily dietary calcium intake.

Compliance with ethical standards

Conflicts of interest None.

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