

# Bone mineral density and lifestyle among female students aged 16–24 years

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## ABSTRACT

The objective of the study was to investigate bone mineral density and bone turnover among female students aged 16–24 years in relation to lifestyle factors, such as dietary habits and physical activity, as well as physiological factors, such as age, body weight, and menstrual pattern.

Female college and university students ( $n = 218$ ) were given a validated questionnaire with 34 questions concerning diet, recreational physical activity, alcohol, smoking, menstrual pattern, weight gain and loss. Bone mineral density (BMD) measurements were performed using a heel bone scanner (DEXA). Deoxypyridinoline (DPD) levels were measured in urine samples. The data were analyzed by linear regression and multiple regression analysis. The mean BMD was  $0.568 \text{ g/cm}^2$ . Multiple regression showed that hormonal age was a better predictor of high BMD and low bone mineral turnover than chronological age. The best model predicting high BMD was composed of physical activity, regular menstruation, hormonal age and body weight. Smoking, alcohol consumption and current calcium intake did not contribute to the model. A negative association between BMD and DPD was found, indicating an enhanced bone remodeling. A correlation was found between DPD and hormonal age, chronological age, sugar intake and time with irregular menses. In multiple regression analysis, hormonal age, high sugar intake and weight loss were the

factors best predicting DPD. BMD was positively influenced by a healthy lifestyle, including a physically active life and healthy dietary habits without dieting.

Our study shows that hormonal age is a stronger BMD predictor than chronological age. Menstrual disturbances might be an indication of a risk for low BMD and might therefore be a reason for measuring BMD among young females.

## INTRODUCTION

The risk of osteoporotic fractures among women in later life is determined by the peak bone mass achieved during adolescence and early adulthood and the rate of bone loss related to menopause. Women have a lower peak bone mass than men and in connection with the menopause women lose bone faster than men<sup>1</sup>. As a consequence, women are at an increased risk of osteoporosis beginning in middle-age and early old-age. It has been suggested that up to 85% of the population variance in peak bone mass is attributable to genetic factors and that lifestyle factors make only a modest contribution to the variance<sup>2</sup>. However, it is by their lifestyles that individuals have the possibility to affect their peak bone mass and it

seems important to outline the impact that different lifestyle factors, e.g. physical activity, bodyweight, dieting, smoking and alcohol consumption, have on the accrual of peak bone mass during different stages of adolescence and early adulthood.

Lifestyle and physiological factors, for example age, bone turnover and influence of gonadal hormones, are major determinants of bone mineral density (BMD) at the end of adolescence and are likely to interact. The impact of calcium intake on BMD among adolescent and young adult females is a matter of debate<sup>3,4</sup> and it has been suggested by Uusi-Rasi *et al.*<sup>5</sup> that correlation between calcium intake and BMD might be associated if the bone is weight-bearing. High body mass index (BMI) and body weight are established predictors of BMD. Grimston and colleagues<sup>6</sup> argue that body weight and exercise seem to interact in contributing to BMD since weight-bearing exercises, such as walking, have a greater effect than non-weight-bearing activities, such as swimming. It has also been suggested that a late menarche is associated with a low BMD<sup>7</sup> and bone loss occurs in young women who experience amenorrhea or ovulatory disturbances<sup>8</sup>. Preventive strategies against osteoporosis require a clear understanding of the factors influencing bone gain in early life. This calls for a wide perspective which considers hormonal and bone mineral metabolisms as well as lifestyle factors.

Most research on BMD among females aged 16–24 years, has focused on a couple of specific areas, for example calcium intake, physical activity, hormonal disturbances or bone turnover. But accrual of BMD among young women is conceivably related to both lifestyle and physiological factors. The aim of this study was to investigate BMD and bone turnover in relation to both lifestyle factors, e.g. dietary habits and physical activity, and physiological factors, e.g. age, body weight and menstrual patterns, among female students aged 16–24 years, and to obtain a baseline data set for a follow-up study.

## MATERIAL AND METHODS

### Participants

Female students, aged 16–24 years, were recruited from the Health Care Program at Upper

Secondary School and the Nurse Education Program at Lund University, Sweden. Student nurses are frequently used as study subjects because as a group they are thought to be socio-economically representative of the population as a whole and they show less attrition. Participants under the age of 18 years had written consent from their legal guardians. The study was conducted from January to May 1999 after approval by the local Research Ethics Committee (LU 548-98).

According to the school registry there were 306 students aged 16–24 years. The response rate was 73%: 13% refused to take part in the study; 7% agreed to participate but later refused for a variety of reasons; and 7% did not reply. BMD was measured in 218 of the students and 211 agreed to a deoxy pyridinoline (DPD) analysis.

### Questionnaire

With the invitation to participate, students also received a structured questionnaire comprising 34 questions on diet, recreational physical activity, alcohol consumption, smoking, menstrual pattern, weight gain and loss. The questionnaire was a short version of the one used in the Women's Health in Lund Area (WHILA) study<sup>9</sup>: reliability and validity had not been calculated. Since the questionnaire had not previously been used among young women a test-retest was conducted to validate the reliability of the questionnaire.

Age at menarche is defined as the recalled chronological age at which menstruation started, rounded to the nearest completed year. Hormonal age is years with hormonal influence counted from the recalled age at menarche up to present chronological age, in complete years.

In conjunction with the densitometry test, one investigator (CE) asked all students if they had any problems in answering the questionnaire, and any potential problems were addressed. Intake of milk and other dairy products was specified. Height and body weight were measured according to standard procedures. Time spent outdoors was estimated in minutes/day during both summer (April to September) and winter (October to March).

### Bone mass measurements

All measurements were performed using a heel bone (calcaneus) DEXA scanner<sup>10</sup>. External

radiation is minimal, hence the equipment can be used in any environment without specific precautions. Internal validity controls were carried out daily by calibrating the calcaneus scanner against a reference phantom. *In vivo* reproducibility of the method was established at 1.5% and accuracy at 2%. The methodology was further validated by 10 measurements on one individual. Coefficient of variation was 1.5%, which was in line with the information provided by the manufacturer (CalScan, Demitech AB, Sweden).

### Biochemical markers

Urine samples were collected in conjunction with the densitometry test (between 0800 to 1500 hours). Deoxypyridinoline (DPD) was determined in urine by a commercial competitive immunoassay Pylilinks™-D Kit (Metra Biosystems Inc. Palo Alto, CA, USA) a standard routine procedure. Results of DPD concentration are given in nmol/l and the concentration of U-creatinine in mmol/l. From these values DPD concentration was calculated in relation to creatinine.

### Statistical methods

SPSS 8.0 for Windows® was used for the statistical analyses. Linear regression coefficients between BMD/DPD and anthropometric/lifestyle data

were calculated. Predictors for BMD and for DPD were determined by means of multiple regression analysis; potential predictors were included separately into the model<sup>11</sup>, but chronological age was forced in. When including sugar and fiber in multiple regression and linear regression analysis these variables were dichotomized with the two lower levels used as references. A high fiber intake was defined as consisting of bread and cereals with high fiber content, potatoes and other root crops. A high intake of sugar was defined as eating candy daily, preferring sweet cookies, lemonade and juice and having a taste for sweet desserts and ice-cream. With physical activity, the three lower levels were used for reference where high physical activity was defined as heavy exercise or competitive sport, e.g. running, gymnastics or skiing on a regular basis and several times a week. Other potential predictors such as smoking, weight loss, regular menses and use of contraceptive pills were dichotomized and negative answers were used as references. A *p* value less than 0.05 was considered statistically significant.

### RESULTS

Table 1 depicts anthropometric data, bone turnover and bone mineral data. The number of students smoking on a regular daily basis was 23%, with 'partysmokers' comprising 22%, and

**Table 1** Anthropometric characteristics and correlation coefficient (*r*-value) and *p* value between bone mineral density (BMD), deoxypyridinoline (DPD) and anthropometric characteristics with linear regression (*n* = 218)

	<i>n</i>	Mean (± SD)	BMD <i>r</i> -value	DPD <i>r</i> -value
DPD (nmol/l)	211	6.79 (± 1.94)	-0.23***	
BMD (g/cm <sup>2</sup> )	218	0.568 (± 0.088)		-0.23***
Chronological age	218	19.7 (± 2.25)	0.15*	-0.45***
Hormonal age	217	7.2 (± 2.7)	0.16*	-0.50***
Age at menarche	217	12.6 (± 1.30)	-0.06	0.22**
Height (m)	218	1.66 (± 0.06)	0.10	0.02
Weight (kg)	217	62.2 (± 9.9)	0.33***	0.05
BMI (kg/m <sup>2</sup> )	217	22.50 (± 3.25)	0.32***	0.06
Current calcium intake (mg/day)	216	808 (± 405)	-0.05	0.02
Alcohol (g/month)	218	16.6 (± 14.0)	-0.05	0.04
Months with irregular menstruation	98	19.0 (19.5)	0.04	-0.25*
Age when irregular menstruation began	119	14.1 (± 2.1)	0.12	0.03
Time spent outdoors during summer (mins)	187	324 (± 184)	0.008	0.25***
Time spent outdoors during winter (mins)	196	102 (± 68)	0.045	0.18*

\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001

79% admitting some alcohol consumption. The majority chose the medium/moderate alternatives for the questions on dietary habits.

BMD correlated positively with body mass index, body weight (Table 1) and physical activity (Table 2). BMD also correlated positively with chronological age and hormonal age but not with age at menarche. There was a negative association between BMD and DPD. There were no significant correlations between BMD and height, current calcium intake, alcohol or tobacco consumption. Students with a high physical activity level had significantly higher BMD than students with lower physical activity levels. They also had their menarche later (median 13 years) than girls who were less active (12 years) ( $p = 0.049$ ). A high fiber intake was associated with a high physical activity level and with a low sugar intake.

Multiple regression analyses showed that hormonal age was a stronger predictor of BMD than chronological age (Table 3). Physical activity, weight and regular menses seemed to be important predictors of BMD, but other potential predictors were not. The final model accounted for 24% (adjusted  $r^2 = 0.24$ ) of the variance of BMD in the population.

Correlations between DPD and chronological age as well as hormonal age were negative. The most marked correlation was with hormonal age. Significant positive correlations were found between DPD and with time spent outdoors during summer, as well as with sugar intake, while correlation to fiber intake was negative.

In the multiple regression analysis, hormonal age was more strongly associated with a decreasing DPD than chronological age. In the final model

**Table 2** Correlation between high and low physical activity (%), dietary intake ( $n = 218$ ) and correlation coefficient ( $r$ -value) and  $p$  value between bone mineral density (BMD), deoxypyridinoline (DPD), and anthropometric characteristics with linear regression

		Level (%)		BMD	DPD
		Low	High	$r$ -value	
Level of physical activity	215	87	13	0.30***	-0.07
Food intake	218	88	12		
Fiber intake	214	82	18	0.10	-0.15*
Fat intake	214	91	9		
Sugar intake	215	82	18	-0.04	0.18**

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

**Table 3** Multiple regression with bone mineral density (BMD) as dependent variable

Predictors	Unstandardized coefficient (b)	SE	$p$ value
Constant <sup>a</sup>	0.361	0.077	0.000***
Hormonal age	0.0082	0.004	0.047*
Chronological age	-0.0033	0.005	0.499***
Weight (kg)	0.0031	0.001	0.000***
High physical activity	0.085	0.016	0.000
Regular menses	0.026	0.012	0.025*
Alcohol <sup>b</sup>	-0.00009	0.000	0.838
Smoking <sup>b</sup>	-0.0092	0.013	0.477
Current calcium intake <sup>b</sup>	-0.00001	0.000	0.451
Contraceptive pills <sup>b</sup>	-0.0015	0.013	0.909
DPD <sup>b</sup>	-0.0063	0.003	0.050

<sup>a</sup>Dependent variable, BMD; <sup>b</sup>potential predictors included separately into the model. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

**Table 4** Multiple regression with deoxyipyridinoline (DPD) as dependent variable

Predictors	Unstandardized coefficients (b)	SE	p value
Constant <sup>a</sup>	9.082	1.622	0.000***
Hormonal age	-0.376	0.092	0.000***
Chronological age	0.006	0.108	0.955
High sugar intake	0.813	0.324	0.013*
Weight loss	0.669	0.282	0.019*

<sup>a</sup>Dependent variable, DPD. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

the best predictors describing DPD were hormonal age, sugar intake and weight loss (Table 4) and the final model accounted for 28.5% (adjusted  $r^2 = 0.285$ ) of the variance of DPD.

## DISCUSSION

The results revealed a lower mean BMD (0.568 g/cm<sup>2</sup>) than has been obtained in other studies which used other measuring sites and had a wider age span. There are no data available for a direct comparison in young women of the same age range and measuring at the calcaneus. The lower BMD mean might be explained by the high content of trabecular bone in the calcaneus compared with other bones, e.g. spine, radius and hip. Several other studies show a lower BMD in bones with a high trabecular content compared with bones with less trabecular content<sup>12-15</sup>.

Students with a high physical activity level had a later menarche than less physically active students, but this had no negative impact on BMD. Irregular menses were negatively associated with BMD, but a late menarche together with a high level of physical activity was not correlated with a low BMD. Students with late menarche might constitute a risk group for a low BMD<sup>7</sup>. Hence, it seems important to outline the reason for a late menarche and if dieting or 'healthy' high physical activity are involved. It has been suggested on the basis of family and twin studies, that 70-85% of the variance of bone mass is genetically determined<sup>2</sup>. Thus 24% of the variance in BMD in this group could be considered acceptable. However, irregular menstruation and bodyweight might both be influenced by lifestyle and genetic factors. Physical activity, regular menstruation and body weight

interact in the accrual of BMD and it is complicated to assess possible interactions between these variables. Young women with irregular menses have a low BMD, be it anorectic females with a low body weight or female elite athletes. This suggests that physical activity and body weight have a positive influence on BMD as long as the hormonal balance is not disturbed. The results from the study of Prior and colleagues<sup>8</sup> confirm this assumption since neither physical activity nor calcium supplementation could prevent bone loss if a normal hormonal pattern was not simulated. To delineate further the importance of lifestyle factors, such as calcium intake, physical activity and body weight, it seems reasonable to consider the young women's hormonal balance and to investigate the impact of whether they have regular or irregular menstruation.

Results from Valimaki and colleagues' study<sup>16</sup> suggests that the impact of lifestyle factors, such as smoking, influence BMD negatively among males but not among females. These results were found after 11 years' follow-up in subjects aged 20 to 29 years at the follow-up. The study by Fehily *et al.*<sup>17</sup> found alcohol consumption to have a negative effect on BMD among young men but not among young women. These results regarding women were confirmed by Fujita *et al.*<sup>18</sup>. Since neither alcohol consumption nor smoking correlated significantly with BMD in the current study it might be considered that hormonal age and regular menstruation could have a large impact on accrual of bone mass, which might explain the different results between men and women, as well as between different women. The impact of smoking and alcohol consumption might vary between different age groups of women and become more important with increasing age. Also the effect of alcohol consumption and smoking may not show in a short perspective or perhaps the positive influence of hormones among young women is stronger than the negative effect of smoking and alcohol. However, results from a population-based cohort study with 40 years of prospectively collected data on smoking, showed that neither current smoking, last 10 years of smoking, nor early adulthood smoking resulted in significantly lower BMD among women who had not taken estrogen<sup>19</sup>. Hence, it seems likely that women are protected from the negative impact of smoking by their natural hormone status.

Bone resorption markers such as DPD seem to be increased by weight reduction among otherwise healthy young women. The Western lifestyle with its slim ideal for young women and its encouragement of dieting on and off even at an early age, could elicit the natural attenuation of bone turnover after menarche which in turn might lead to a lower peak bone mass. The suggested peak at menarche in bone turnover and consequently in DPD values and the decreasing levels towards adult values, in late puberty<sup>20-22</sup>, might be disturbed by weight reduction. A cross-sectional study reported that reduced rates of skeletal remodelling were associated with increased BMD during the development of peak skeletal mass<sup>23</sup>. The negative association in this study could be explained by the faster breakdown of persisting bone compared with the mineralization process of newly synthesised bone, i.e. time-dependent reaction. However, this needs to be confirmed by a longitudinal study. The physiological variation of sex hormone levels during the menstrual cycle seem to be associated with monthly fluctuations in bone turnover<sup>24</sup>. These results might raise the question of whether the physiological variation of sex hormones is also disturbed among young women who are dieting, and if this is of any significant importance for future bone health. Young women are more likely to attempt to lose weight and to perceive themselves as overweight than men<sup>25</sup>. They are also more likely to diet in order to reduce weight while men are more likely to exercise<sup>25</sup>.

The positive correlation between carbohydrate intake and DPD could be a coincidence, or it could indicate different lifestyles with a high sugar consumption, a low fiber intake and a low level of physical activity. Dieting and weight loss might explain poor dietary habits that could result in lack of proteins essential for collagen formation, which might contribute to the increased negative association between high DPD and low BMD. Girls aged 7 to 14 years with a consumption of carbonated beverages above 350 ml/day have significantly lower total body bone calcium as soft drinks might substitute consumption of calcium-rich foods<sup>26</sup>. Also, too high a consumption of dairy products might replace a balanced diet. The impact of dieting on BMD in a time perspective remains to be clarified.

To the best of our knowledge, no study has focused exclusively on healthy females in this age

range and with a broad perspective of lifestyle factors and physiological factors, such as bone turnover and both chronological and hormonal age. It has been an implicit assumption in the literature that changes in BMD are positively related to chronological age. Because of this, other studies have used chronological age as a predictor for BMD, and in female populations with a wider age range, and also in men, BMD correlates with chronological age. Studies have used age at menarche instead of chronological age but from a statistical point of view these two variables are related and act in the same way in a statistical analysis based on linear associations. Since menarche is considered to have a high impact on bone development, the slope of the line when using hormonal age illustrates this better than chronological age and age at menarche. For the same reason, chronological age might also be inadequate when predicting BMD in menopausal women and hormonal age, counted from menopause, might be more appropriate. Results in a young female population showed that bone mineral content increased independently with age for each pubertal stage<sup>27</sup> indicating that hormonal age is a stronger predictor during the years following menarche. Our results confirm the assumption that hormonal age is the stronger and more significant predictor during a period after menarche. Hence, when designing studies in connection with menarche or menopause, it seems important to select a population using their hormonal age rather than their chronological age.

In this age range of women, hormonal and lifestyle factors seem to be the most important factors in accrual of peak bone mass. This suggests that hormonal balance and lifestyle are of greater importance than chronological years.

### Comments on method

Students taking part in the study were recruited from educational programs containing health information. It can be assumed that they have a greater health consciousness than most girls of the same age and that they are likely to underestimate their consumption of cigarettes and alcohol; although regarding smoking prevalence they do not differ from mean values in Sweden.

Since there were outliers regarding hormonal age we wanted to control the stability of the

regression coefficients in the final model (multiple regression) by making an analysis without these individuals (hormonal age: one,  $n = 4$ ; two,  $n = 4$ ; 12,  $n = 8$ ; 13,  $n = 2$ ; and 15,  $n = 1$ ). The effect of hormonal age on BMD is stable but with a non-significant  $p$  value of 0.074. The impact of physical activity on BMD seems to vary slightly with hormonal age. The effect of weight and also regular menses is stable. This indicates that weight and regular menses seem to be stable predictors among women of this age. Excluding outliers, the effect of hormonal age on bone turnover decreases slightly (or stable) from  $-0.39$  to  $-0.36$ , the effect of high sugar intake remains stable at 0.81 to 0.80 and the effect of weight loss increases slightly from 0.65 to 0.71. This indicates that hormonal age, sugar intake and weight loss are stable predictors.

## Conclusions

A healthy lifestyle including physical activity and maintenance of healthy body weight contributes to a high bone mineral density. Hormonal age was a more stable predictor of BMD than chronological age. Irregular menstruation could be considered an indication for measuring BMD among young women but it needs further investigation.

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