Scientific Paper

DIFFERENCES IN PEAK BONE DENSITY BETWEEN MALE AND FEMALE STUDENTS

Selma CVIJETIĆ AVDAGIĆ[1], Irena COLIĆ BARIĆ[2], Irena KESER[2], Ivana CECIĆ[2]
Zvonimir ŠATALIĆ[2], Jasminka BOBIĆ[1], and Milica GOMZI[1]

*Institute for Medical Research and Occupational Health[1], Laboratory for Food Chemistry and Nutrition,
Faculty of Food Technology and Biotechnology, University of Zagreb[2], Zagreb, Croatia*

As an important determinant of osteoporotic fracture risk, peak bone density tends to be higher in men than in women. The aim of this study was to see whether young men and women differed in the time and skeletal region of peak bone density. We also investigated the influence of diet and physical activity on bone mass. The study group included 51 male and 75 female students aged 19 to 25 years. Bone mineral density was measured for the spine, total femur, and the distal third of the radius using dual energy x-ray absorptiometry. Dietary data were obtained using a specially designed semiquantitative food frequency questionnaire. Bone mineral density (BMD; g cm^{-2}) was higher in boys than in girls at all measured sites, while bone mineral apparent density (BMAD; g cm^{-3}) was higher in girls. Age negatively correlated with bone mineral density in all measured sites except in the boys' spine. Sodium, protein, and fibres were nutrients that significantly correlated with bone mineral density. The study suggests that boys achieve peak bone density later than girls, and that this delay is the most prominent in the spine. In our study group, this difference could not be explained by different nutrition or the level of physical activity.

KEY WORDS: *bone mineral density, diet, osteoporosis, physical activity, young people*

The risk of developing symptomatic osteoporosis later in life is influenced to a large extent by the levels of peak bone mass attained in early adulthood. Peak bone mass is thought to be determined by an interaction between lifestyle and genetic factors (1-3), but the ways in which they interact with each other remain incompletely understood. Young people's lifestyle and dietary habits have changed over recent decades toward non-physical activities and fast food. Many cross-sectional and follow-up studies indicate that physical activity and calcium supplementation are beneficial through childhood and adolescence. Bone mass is higher in children and young people who are physically active (4-6). The majority of studies on children and adolescents support a positive relation between calcium intake and bone mineral density (BMD) (7-9), but there are also studies where no such association has been observed (10, 11).

Although the determinants of peak bone mass are well recognised, its exact timing is still controversial. It is widely accepted that most of the skeletal mass is acquired by the age of 20, and it continues to grow slowly until the end of the third decade of life or even later. More recent studies have suggested that peak bone density is achieved by the end of adolescence (12, 13). Cross-sectional studies also observed that the age of attainment of peak bone mass varies with the site of bone measurement (14-15). Lin (16) proposed that peak bone mass is attained earlier at the hip than at the spine.

However, there is little data about the differences in obtaining peak density between young men and women in different skeletal regions. This study investigated whether bone mineral density in the cortical and trabecular bone of healthy student population aged 19 to 25 years had already attained

peak bone mass, and whether it is related to lifestyle (dietary intake and physical activity).

MATERIALS AND METHODS

Subjects

The study included 51 male and 75 female students from the University of Zagreb. They were generally healthy young people, without a history of disease or therapy which could interfere with bone metabolism. They were all informed about the study protocol, which has been approved by the Ethical Committee of the Institute for Medical Research and Occupational Health. Informed consent was signed by all participants.

Anthropometry and bone density measurement

Height and weight were measured using a portable stadiometer and an electronic scale. Body mass index (BMI) was calculated as weight (kg) divided by square height (m^2).

Areal bone mineral density (BMD; $g\ cm^{-2}$) was measured using dual energy X-ray absorptiometry (Lunar – Prodigy, Madison, WI). Measurements were made in the lumbar spine (L2-L4), proximal femur, and the distal third of the radius. To minimise the effect of the skeleton size, we made a volumetric, three-dimensional approximation of bone density, called bone mineral apparent density (BMAD; $g\ cm^{-3}$) using the Katzman/Carter formula: $BMAD=BMC/area^{3/2}$, where BMC stands for bone mineral content (17). BMD was also expressed as a T-score, that is, the number of standard deviations from the mean BMD of a control population aged between 20 and 40 years given in the manufacturer's reference values.

Diet assessment

To assess the diet, the subjects were interviewed by trained personnel using a Quantified Food Frequency Questionnaire (FFQ). The FFQ included 173 food items arranged in groups. The reference period was the previous year, and each subject was asked to recall consumption frequency ranging from once a month to once or more a day. The portion size was estimated showing photographs of food in actual size (18).

The frequency of specific nutrients was established using the U.S. Department of Agriculture (USDA) food composition tables (19). For nutrient composition of Croatian brands, we used manufacturer labels we contacted the manufacturer.

Energy and nutrient intakes were compared with Croatian reference values for vitamins and minerals (valid since 2004) (20) with Croatian Recommended Dietary Allowances (RDA) for energy and protein intake (valid since 1994) (21, 22), and with Dietary Reference Intakes (DRI) for dietary fibre, fats, carbohydrates, sodium, and potassium (23).

Physical activity

Physical activity was measured by quantifying sport and work activities: duration (months/years) and frequency (hours per week) of sport activity and intensity (moderate or hard) and frequency (hours per week) of other physical activities. Moderate physical activities included bicycle riding and, walking, and such, while hard physical activities included heavy object lifting, fitness, and such. The frequency of sport activities and the frequency of other moderate and/or hard physical activities were categorised and scored as: 1) never; 2) (0,5 to 1) hours per week; 3) (2 to 3) hours per week; 4) (4 to 6) hours per week; 5) (7 to 10) hours per week; 6) (11 to 20) hours per week; 7) (21 to 30) hours per week; 8) >31 hours per week. The final score of physical activity was calculated for each subject by summing up years of sport activities with the frequency of moderate and hard work activity score (1 to 8). Subjects with higher final score were considered more physically active.

Table 1 *Subject profile and physical activity*

	Mean±SD	
	Male students (N=51)	**Female students (N=75)**
Age / year	22.2±1.3	22.4±0.6
Time after menarche / year	/	5.5±1.6
Height / cm	179.4±5.6	167.5±5.8[1]
Weight / kg	78.4±7.1	59.9±6.8[1]
Body mass index / kg m^{-2}	24.4±2.2	21.3±1.9[2]
Fat tissue / %	24.1±6.8	30.8±7.2[1]
Smoking	(N=10)	(N=12)
Number of cigarettes	12.7±5.8	12.5±10.6
Years	5.0±1.7	4.0±1.4[2]
Physical activity score	12.4±5.8	7.0±3.8[1]

[1]*p<0.0001 t-test (male vs. female students)*
[2]*p<0.01*

Statistics

The data were analysed using statistical software Statistica, version 5.0 (StatSoft Inc., Tulsa, OK). The results are shown as means ± standard deviation (SD). The distribution of variables was tested with the Kolmogorov-Smirnov test. If the test was significant, then the hypothesis that the respective distribution was normal was rejected. Variables which were not distributed normally were log-transformed. As only five students were several months younger than 20 years (the lower limit of age range was 19.4 years), we calculated their T-scores as if they had been 20 years old. The differences between two mean values were analysed using the *t*-test. Correlation matrices were used to measure the relation between two or more variables. The association between bone mass and different predictor variables was tested with multiple regression. P value lower than 0.05 was considered significant.

RESULTS

The mean age of students was 22.3 years (22.2 years for boys and 22.4 years for girls), and it ranged from 19.4 years to 25.8 years (Table 1). Male students were significantly taller, heavier, and with greater BMI (p<0.0001). However, they had a significantly lower percent of fat tissue (p<0.0001) and were involved in sport activities for longer (p<0.001) than the girls. Twelve girls and ten boys were smokers.

The boys had a higher BMD and also a significantly higher BMC and bone area than the girls (Table 3). BMAD was higher in the girls than in the boys; the difference was particularly significant in the distal radius (p<0.0001). In all students, mean T score in the distal radius (-0.89±0.7) was significantly lower than the T scores in other regions [(0.66±1.07) spine; (0.49±1.03) total femur] (p<0.0001). BMD and age showed negative correlation in all measured sites in girls, which was significant for the spine (r=-0.21; p<0.05) and total femur (r=-0.39; p<0.0001). In boys, age positively correlated with spine BMD and negatively with femur and radius BMD. These correlations, however, were not significant.

Twenty percent of male and 14.1 % of female students had had fractures caused by trauma, but there was no difference in BMD between students with and without fractures. There were also no significant differences in BMD between smokers and non-smokers.

Save for vitamin E in girls (Table 2) all subject were taking nutrients within the RDA.

Multiple regression was performed to see whether any nutrient variable and physical activity predicted bone mass (Table 4). The analysis was controlled for age, height, and weight. The most significant positive predictor of bone density in girls was body weight. Dietary fibre intake negatively correlated with BMD in girls, and this correlation was significant for the spine and femur (p<0.01). Protein and sodium intake significantly negatively correlated with spine BMD in boys (p<0.05).

Table 2 *Average daily energy and nutrient intake in male and female students and age-adjusted recommended levels*

Nutrition	Mean ± SD		Recommended level of daily intake (RDA)
	Male students	**Female students**	
Energy / kcal d^{-1}	3207.4±1515.2	2879.4±1449.4	2200
Protein / g d^{-1}	148.2±110.8s	109.2±43.7	46
Fat / kcal d^{-1}	125.2±67.1	98.2±43.1	10 to 35
Carbohydrates / g d^{-1}	369.2±152.8	339.6±144.8	130
Dietary fiber / g d^{-1}	25.3±13.2	26.1±12.2	25
Calcium / mg d^{-1}	1436.9±607.1	1377.1±630.2	800
Sodium / mg d^{-1}	5903.8±3097.2	4713.72±2203.2	1500 to 2300
Iron / mg d^{-1}	17.4±7.9	15.8±6.8	14
Vitamin A / µg d^{-1}	970.9±7138.7	801.9±6391.8	800
Vitamin C / mg d^{-1}	222.1±237.9	181.6±120.9	60
Vitamin E / mg d^{-1}	17.6±29.1	9.2±5.6	10
Vitamin K / µg d^{-1}	181.2±148.6	236.2±167.6	65
Vitamin B$_{12}$ / µg d^{-1}	9.1±7.7	6.3±3.0	1.0
Folic acid / µg d^{-1}	626.6±286.7	540.0±259.7	200

DISCUSSION

The results of this study suggest that there are differences in peak bone density at different skeletal regions between young male and female adults. Our male participants had a higher areal bone density in all measured sites. However, with respect to estimated volumetric bone mineral density, the difference in BMD between boys and girls could be explained by a greater increase in bone size in boys during growth. In a longitudinal study of 116 men and women, Walsh found that peak BMC velocity occurs at an age of 12.5 years in girls and 14 years in boys (24). In our study, the increase in BMC was greater than the increase in BMAD, which also points to BMAD as a more accurate parameter for the assessment of bone density in the growing skeleton. A significant negative correlation between age and bone density suggests that our students in their early twenties had already acquired peak bone density and started to lose bone. The exception was the spine in boys, since bone density at that site did not negatively correlate with age. Since it is expected that men will gain a higher peak bone density than women, we can conclude that male students had not yet achieved their peak bone density in the spine. Due to insignificant negative correlation between age and the BMD of the femur and radius, we can also conclude that boys gain peak bone density at later age than girls.

It is known that the age at which peak BMD is achieved depends on the size of the skeleton. Several authors showed that BMD at the proximal femur peaked earlier than in the spine (13, 16, 25), which is consistent with our results. According to T scores, our students had a higher BMD of the spine and proximal femur and significantly lower BMD of the radial shaft than the reference NHANES values. Our previous results for female students suggest that young Croatian women have a lower cortical peak bone density than their American counterparts (26). These differences may be related to geographical variations in BMD worldwide, as shown in many cross-sectional, population-based studies (27, 28). Discrepancies in age at which peak bone density is achieved could partly be attributed to the so called "consolidation", which is a continued increase in bone mass after bone linear growth has stopped at the end of puberty (29). This process may be the result of continued increase in bone size, but also the result of a gain in volumetric bone density.

We also analysed whether the reason for the difference in peak bone density between our male

Table 3 *BMD, BMC, area, and BMAD (mean ± SD) in the spine, neck and distal radius in male and female students*

	Spine		Neck		Distal radius	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
BMD / g cm^{-2}	1.313±0.138	1.305±0.139	1.183±0.166	1.135±0.159	0.828±0.161	0.783±0.109
BMC / g	79.60±12.41[1]	69.61±13.54	6.73±1.20[2]	5.24±1.05	2.13±0.18[1]	2.03±0.25
Area / cm^2	62.16±5.58[2]	53.50±7.59	5.61±0.68	5.13±4.61	2.87±0.25[3]	2.53±0.26
BMAD / g cm^{-3}	0.166±0.018[3]	0.184±0.028	0.505±0.082	0.524±0.082	0.189±0.037[3]	0.496±0.075

[1] *p<0.01 men vs. women (t-test)*
[2] *p<0.001 men vs. women (t-test)*
[3] *p<0.0001 men vs. women (t-test)*

Table 4 *Significant predictors of BMD at different skeletal sites in male and female students*

Dependent variable	Predictor variables	p-level	
		Male students	**Female students**
BMD Spine	Body weight	/	<0.001
	Proteins	<0.05	/
	Sodium	<0.05	/
	Dietary fibre	/	<0.01
BMD Total femur	Body weight	/	<0.0001
	Dietary fibre	/	<0.01
BMD Radius	Body weight	/	<0.01
		/	

and female students could be any of the lifestyle factors investigated (diet and physical activity) which might vary between them. Furthermore, diet is a key determinant for the balance between bone turnover and bone mass. This study included a wide spectrum of nutrients and their possible relation to bone mass. A number studies showed a positive correlation between calcium intake and bone mass (30-32), but our results are closer to studies that do not support the theory that larger dietary calcium intake is associated with greater bone density (10, 16, 33).

A significant negative association between dietary fibre intake and bone density in girls could be the consequence of increased urinary calcium excretion associated with increased dietary fibre intake and lower oestradiol levels in pre- and post-menopausal women (34, 35). This may imply that increased dietary fibre intake may adversely affect bone mass. However, the effect remains controversial, because dietary fibre may affect on calcium absorption through phytate, which is present in substantial amounts in cereals (36). Therefore, high fibre intake from a mixed diet need not adversely affect bone health , as shown by Yano et al. who found no association between dietary fibre intake and bone health (37).

The subjects in our study reported a very high intake of sodium and proteins. Over the past several decades dietary habits have shifted toward consumption of processed foods that are high in sodium. It is known that high sodium intake can accelerate calcium excretion and that hypercalciuria is a risk factor for osteoporosis (38-40). Dietary protein has also been shown to increase urinary calcium resulting from an increase in calcium bone resorption (41, 42). Although there is evidence of the beneficial effect of protein intake on bone health (43, 44), its negative correlation in our study could be the consequence of excessive protein intake in male students, which was three times the normal.

We can not explain the differences in the timing of peak bone density between the sexes with dietary factors, since all students had a satisfactory DRI, and there were no significant differences in nutrient intake between them.

A shortcoming of this study is a relatively small number of subjects, which might have led to a type I error and a false positive result. However, the aim was to establish the difference in peak bone density between men and women, not to determine the prevalence. In addition, there are several other studies on peak bone mass which have been based on a relatively small number of participants (15, 24, 28, 45, 46).

We can conclude that peak bone density in our study population occurred before the age of twenty in most of skeletal regions. The analysis of correlation between age and bone density has shown that peak bone density occurs later in boys than girls, especially in the spine. In our study, this difference can not be explained by different diet or level of physical activity, but rather by hormonal and genetic differences. Bearing in mind that the pathogenesis of osteoporosis could be traced back to childhood or earlier, identification of the genes responsible for these effects would have a major impact on the prediction of peak bone mass and prognosis of the disease.

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Sažetak

RAZLIKE U VRŠNOJ KOŠTANOJ MASI IZMEĐU STUDENTICA I STUDENATA

Vršna koštana masa važna je odrednica nastanka osteoporotskih prijeloma i poznato je da je veća u muškaraca nego u žena. Cilj istraživanja bio je analizirati razlike u postizanju vršne koštane mase na različitim regijama skeleta između mladih muškaraca i žena. Također je procijenjen utjecaj prehrane i tjelesne aktivnosti na mineralnu gustoću kostiju. Ispitanici su studenti u dobi od 19 do 25 godina, 51 muškog i 75 ženskog spola. Mineralna gustoća kostiju određena je metodom dvoenergetske apsorpciometrije X-zraka na kralježnici, ukupnom femuru i distalnoj trećini radijusa. Podaci o prehrani dobiveni su semikvantitativnim upitnikom o prehrani. Mineralna gustoća kostiju (BMD; g cm^{-2}) bila je veća u studenata na svim mjerenim regijama, dok je procijenjena volumetrijska koštana gustoća (BMAD; g cm^{-3}) bila veća u studentica. U studenata obaju spolova vrijednosti mineralne gustoće kostiju smanjuju se s porastom dobi, jedino se s dobi povećava mineralna gustoća kralježnice u muških studenata. Unos proteina, natrija i vlakana značajno je povezan s koštanom masom. Dobiveni rezultati upućuju na zaključak da muškarci kasnije postižu vršnu koštanu masu, što je najizraženije na kralježnici. U ispitivanoj grupi te se razlike ne mogu objasniti mogućim razlikama u prehrani i/ili tjelesnoj aktivnosti između muških i ženskih ispitanika.

KLJUČNE RIJEČI: *mineralna gustoća kosti, mladi ljudi, osteoporoza, prehrana, tjelesna aktivnost*

CORRESPONDING AUTHOR:

Selma Cvijetić Avdagić
Institute for Medical Research and Occupational Health
Ksaverska cesta 2, 10001 Zagreb, Croatia
E-mail: *scvijetic@imi.hr*