ABSTRACT

Aim Adequate nutrition supports optimal bone development and prevents later metabolic bone diseases. The aim of this study was to assess calcium dietary intake and measure calcaneal bone mineral density and based on the obtained values to evaluate gender and age impact on the values itself as well as on their correlation.

Methods Subjects recruited for this study were 120 healthy adults. Calcaneal bone mineral density was measured by quantitative ultrasound and expressed as estimated bone mineral density (eBMD). Calcium intake was assessed using ten 24-hours recalls.

Results Values were compared between age and gender based sub-groups. The average value of eBMD of the study group was 0.543 g/cm². There was no difference between females and males in eBMD. The average daily dietary intake of calcium was low (661 mg/day), and males had statistically significantly higher daily dietary intake than females (805 mg/day and 599 mg/day, respectively). The younger group had higher average value for eBMD (0.560 g/cm²), and higher dietary intake of calcium (10.5 mg/kg of body weight) than the older group (eBMD=0.527 g/cm²; calcium intake=9.4 mg/kg of body weight), but the difference was not significant.

Conclusion Lack of statistically significant difference in eBMD values between two observed study subgroups based on the years of age, and low eBMD value itself, are obvious evidence of the compromised bone health status within the study population as well as the need for preventive actions.

Keywords: quantitative ultrasound, calcium, bone densitometry, osteoporosis, 24-hour recall
INTRODUCTION

The 21st century is the time of new epidemics, unknown and/or unrecognized until recently, which developed as a consequence of unhealthy lifestyle – unbalanced nutrition and lack of physical activity (1). Those epidemics, together with cardiovascular diseases and diabetes, have recently included osteoporosis, too, which in some countries counts as many hospital days and medical expenses as cardiovascular diseases. Yet, although osteoporosis has been increasing steadily worldwide in recent years, it is among the diseases that may be amenable to treatment through lifestyle modification or management across the lifespan (2).

In 1999 Croatia brought out the Croatian Food and Nutritional Policy, which emphasized that the content of some nutrients, such as calcium which counts for one of the most important nutrients for bone mineral density (BMD), was lower than recommended, and the strategy was to permanently monitor the nutritional status and dietary habits of all population groups, taking into consideration regional differences (3). Unfortunately, goals set in this Policy were neither achieved within the predicted period, nor the possibility of their achievements is predictable. Croatia, as many other countries round the Globe fight the battle with the growing incidence of chronic noncommunicable diseases (4), including osteoporosis (5), which are, among other factors, caused by unbalanced nutrition.

In Croatia about 200,000 females have osteoporosis, and additional 400 000 suffer from osteopenia (5). Considering its incidence, osteoporosis is a significant burden to health care systems in all countries, especially in the developing countries like Croatia. Consequently, there is an urgent need to develop effective strategies to prevent osteoporosis (like more optimal nutrients intake) and ultimately reduce the risk of osteoporosis-related fragility fractures among Croatians.

Genetics contributes up to 80% to the set point for peak bone mass in early life (6), but nutrition also has a unique role in the maintenance of bone status and may be a factor in optimizing bone health throughout life cycle (7,8). Results on the bone mineral density in Croatia are scarce and in most cases the same as in studies conducted round the world, related to older population of both sexes (9), especially for younger populations, who still have a possibility of taking some preventive measures (10-14).

A reliable assessment of nutrients intake is a hard task, and the choice of method depends on the aim of the study, accuracy of the dietary data required, and personnel and economic resources available. Variable nutritional habits, even in one country or the region, are problematic, which makes this assessment even harder (15). Available results on the calcium intake in Croatia are opposite, most often too low, sometimes too high (15,16). Beside genetic factors, geographic and socio-economic factors lifestyle can significantly affect bone mineral density as well (13,14).

Having in mind all the issues mentioned above, the primary aim of this study was to estimate dietary intake of calcium and to evaluate calcaneal bone mineral density. Also, we wanted to evaluate the correlation of dietary calcium intake and bone mineral density. It was expected that participants with higher dietary intake of calcium will also have higher calcaneal bone mineral density.

EXAMINEES AND METHODS

Study participants

One hundred and eighty healthy adults (age 18-55) were recruited for this study among working adults in eastern part of Croatia, and among university students. All subjects participated on a strictly voluntary basis. The study was approved by the Ethics Committee of the Faculty of Food Technology.

A total of 120 subjects for which complete data were collected were divided into two subgroups according to the age and gender. For the first criteria boundary value was at 30 years of age since, according to the different studies, before that age bone mass is accumulated and peak bone mass is achieved, and later on, after the menopause, bone mass starts to deteriorate (8,17). As a second criterion for the study participants sub grouping was gender (84 females and 36 males). More females than males were recruited for the study due to gender differences in the prevalence of osteoporosis.

Dietary assessment

All study participants filled out a personal data and lifestyle questionnaire, where they reported information on age, weight, height, health status, medication use, physical activity, smoking
habits, diagnosed bone diseases in close family members. Female participants also provided information on the number of pregnancies, menopause and hormone replacement therapy.

Upon recruitment, subjects underwent a dietary assessment by the 24-hour recall method. The 24-hour recall was collected ten times during a one-year period; 7 times for working days and 3 times for weekends or holidays. To introduce participants into the methodology, the first recall was conducted by personal interview, while the following recalls were collected either through personal or telephone interviews. Supplements were not considered in this study.

Quantitative ultrasound densitometry

Bone mineral density was measured by quantitative ultrasound densitometry (QUS). The QUS measurements of the calcaneus (heel bone) were performed using a Sahara sonometer (Hologic, USA). Results were expressed as estimated bone mineral density (eBMD) (g/cm²). Quality assurance was performed using a dedicated phantom (supplied by the manufacturer) before the first measurement of the day, and all measurements were conducted by the same person. The dominant foot was determined. Ultrasound gel was applied as a coupling medium to ensure good contact.

Data analysis

Dietary intake of calcium was calculated using a specially designed computer programme NutriPro 2001 based on national food composition tables (18). Dietary intakes were presented as the average daily intake (mg/day), and dietary intake per kg of body weight (mg/kg of body weight). Also, intakes were evaluated in comparison with Dietary Reference Intakes (DRIs): Estimated Average Requirements which represent average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. The DRI:EAR value for calcium is 800 mg/day for both females and males in the age span that corresponds to studied population (from 19 to 50 years, excluding pregnant or lactating females) (19).

Average daily dietary intake of calcium in gender and age based subgroups was compared on two levels, where recommended calcium dietary intake of 800 mg/day (19,20) was used as a boundary value. Results of calcium dietary intake were also expressed as mg/kg of body weight. For this purpose, as a boundary value, 9.9 mg/kg of body weight is set, based on the fact that this value represents an average dietary intake of the whole study group.

Comparison of the eBMD and dietary calcium intake between males and females, younger and older subjects, was performed using ANOVA. The normality of data distribution was checked by Kolmogorov-Smirnov test. Since there was a difference between the number of males and females for the comparison of those groups Mann-Whitney test was used.

RESULTS

The data analysis included 120 healthy adult volunteers of which 56 (46.7 %) were younger and 64 (53.3 %) older than 30 years of age. Females were more represented than males (70.0 % vs. 30.0 %, respectively) and both genders involved about the same number of younger and older subjects (40 younger and 44 older in females, and 16 younger and 20 older in males). Comparison of younger and older subgroup (p=0.108), as well as the comparison of females and males (p=0.448) showed the lack of statistically significant difference (Table 1). The average daily dietary intake of calcium was low, and males had statistically significantly higher intake than females. Yet, when average daily dietary calcium intake was expressed in mg/kg of body weight, there was no statistical difference between males and females.

The average age of younger and older age subgroups was 23.4 years and 42.9 years, respectively.

| Table 1. Age characteristics, bone mineral density and dietary calcium intake of total study population, and according to gender and age* |
|---|---|---|---|---|---|---|---|
| Parameters | Total (N=120) | Gender | Age (years) | <30 (n=56) | >30 (n=64) | p |
| Age (years) | 33.8±10.9 | 33.4±11.0 | 34.3±10.7 | NS | 23.4±2.6 | 42.9±6.3 | 0.000 |
| eBMD (g/cm²) | 0.543±0.112 | 0.539±0.118 | 0.552±0.098 | NS | 0.560±0.096 | 0.527±0.123 | NS |
| DCI (mg/day) | 661±244 | 599±186 | 805±301 | 0.000* | 650±242 | 671±248 | NS |
| DCI (mg/kg body weight) | 9.9±3.8 | 9.8±3.5 | 10.2±4.5 | NS | 10.5±3.8 | 9.4±3.8 | NS |

*Values are presented as mean±SD; eBMD, estimated bone mineral density; DCI, dietary calcium intake; NS, non-significant difference;
The younger group had a higher average value for eBMD than the older group, but the difference was not significant. Younger subgroup had lower absolute calcium daily dietary intake, but contrary, higher intake of calcium per kilogram of the body mass. In male subgroup with average daily dietary intake of calcium above 800 mg/day showed highest eBMD values. Females had lower values of eBMD than males, regardless of daily dietary intake of calcium (Figure 1).

Figure 1. Estimated calcaneal bone mineral density (eBMD) (mean and SD values) of males and females with average daily dietary intake of calcium ≤800 mg/day and >800 mg/day

Figure 2. Estimated calcaneal bone mineral density (eBMD) (mean and SD values) of males and females with average daily dietary intake of calcium ≤9.9 mg/kg body weight and >9.9 mg/kg body weight

An increase of eBMD values with the increase of dietary intake of calcium expressed as mg/kg of body weight for both genders, but with very weak correlation was found. The same as in case when daily dietary calcium intake was expressed in mg/day, females had lower eBMD than males regardless of dietary calcium intake. Yet, the differences were not statistically significant (Figure 2).

Figure 3. Estimated calcaneal bone mineral density (eBMD) (mean and SD values) of younger and older subgroup with average daily dietary intake of calcium ≤800 mg/day and >800 mg/day

Lower eBMD was found in older subgroup regardless of dietary calcium intake than in younger subgroup (Figure 4).

Figure 4. Estimated calcaneal bone mineral density (eBMD) (mean and SD values) of younger and older subgroup with average dietary intake of calcium ≤9.9 mg/kg body weight and >9.9 mg/kg body weight

DISCUSSION

Most often, BMD is measured by dual-energy X-ray absorptiometry (DEXA) (14,21). This method uses X-rays to obtain the results, and therefore, the ethics represents a great dilemma in cases when measurements should be conducted on a rather young and also healthy population. Quantitative
ultrasound therefore represents a method of choice for studies with younger population groups. A number of studies has shown the comparability of the results obtained by these two methods (14). This is especially useful in studies like ours, where relations among different study subgroups and comparison with other studies in different regions and countries are of a primary importance, while absolute value is not so important.

Our study has shown that males have 2.4% higher values of eBMD than females, but this difference is not statistically significant. Takeda et al. have reported the same results for their study (22).

In central Croatia, gender comparison revealed significant differences between ultrasound parameters in the three decades of life groups (60-69, 70-79, 80-89) (9). Significant differences in eBMD of males and females were obtained also for university students in Croatia and eBMD for some of their subgroups had similar values as the values obtained in our study (14).

Bone mineral density of our younger study subgroup was 6.3% higher than for the older subgroup, which is greater than the difference obtained for gender based subgroups. Yet, this difference is also not statistically significant, and that fact causes concerns, while it is expected that younger population should have a significantly higher eBMD.

Authors of a study conducted in one part of the Republic of Croatia reported considerably lower average value of eBMD than the values of our study (23). The average age of their study group was 25 years above our average age, and the difference in eBMD might be due to this age difference. The same fact on the age difference can explain a stronger negative correlation between the age and eBMD in the mentioned study (23), while the correlation in our study, though also negative, was very weak. Namely, eBMD values are more or less constant in the period between 30 years of age and menopause, and the decrease follows after that period.

Poniegjia et al. (24) studied bone mineral density of a group of young females (similar to our younger group) in central Italy and reported eBMD values slightly higher than ours for their study group. Differences can be explained by climatic conditions. Namely, the central part of Italy has more sunny days than eastern Croatia, which affects synthesis of vitamin D, another important contributor to bone mass.

Zoching et al. (25) studied characteristics of ultrasound based bones parameters, and reported that they give values of adequate precision for all age groups. On the other hand, Krieg et al. (26) in their Position Statement regarding the use of QUS in osteoporosis diagnostic, outlined that BMD cannot be applied with any other technology but DXA. Having in mind that our aim was not to diagnose osteoporosis, but to determine eBMD and to compare obtained values to those gathered on larger population by instrument producers, we can consider our obtained values of eBMD precisely enough. A small difference of eBMD between the two age groups is, unfortunately, most likely due to really bad bone health.

The values obtained in this study for both age groups are lower from those supplied by the manufacturer of the equipment used (27). Babaroutsi et al. (28) have also measured eBMD by quantitative ultrasound and one of their three groups, which corresponded to our younger group by age, also had similar values of eBMD. A study conducted on adults by Frost et al. (29) also gave results similar to those reported in our study.

Generally, in the Republic of Croatia dietary calcium intake is low (16,30,31). In our study average calcium intake, though substantially lower than recommended, is still higher than the intake reported in 1999 (32). That might be an indicator of the positive changes in consumer’s awareness on the importance of this mineral. Some developed countries have achieved a substantially positive change, and during the last decade calcium intake has increased for about one third (33). Three previous studies conducted in the Republic of Croatia using the FFQ for the dietary assessment, have reported higher dietary calcium intake: 965 mg/day (34), 1633 mg/day (15), and 1518 mg/day (35). Still, the last two of them represent an exception with such a high calcium intake in Croatia.

The analysis of the relationship between dietary intake and bone mineral density showed a very low correlation for dietary calcium intake expressed for daily intake (mg/day), calcium intake expressed per kg of body weight (mg Ca/kg of body weight) as well as the calcium intake in dependence of protein intake (mg Ca/g of proteins). Similar results were obtained by analysis...
of correlation between dietary intake of other nutrients important in bone metabolism (Na, K, P, Mg, dietary fibres and proteins) and bone mineral density. Recommendations for the calcium intake are almost never considered with regard to the body weight. A Japanese study implicated the fact that body size should be considered to estimate physiological need for calcium more precisely, and it reported that dietary intake of calcium should be 18.1 mg/kg of body weight for males, and 18.5 mg/kg of body weight for females (36). Dietary intake of calcium presented in our study as mg/kg of body mass revealed that all our study subgroups have only half of the intake reported in the aforementioned Japanese study.

Calcium intake does not vary only among the individuals, or groups, but also among different nations. Papers and reports from round the Globe show low calcium intake in the USA (37-39), Japan (36,40), Canada (41), China (42) and many other countries.

In one of our earlier studies (34) we found that only 61% of total calcium came from milk and milk product. That is the consequence of only about 45% of adult Croatians consuming milk on a daily basis (43). Therefore, it would be beneficial to initiate larger milk consumption as well as calcium-fortified milk consumption.

The results obtained in our study should be observed with having in mind a few facts which could lead to bias. Firstly, all measurements were performed on a heel bone whose structure might be in a bad condition due to specifics of subjects walking. Secondly, though results obtained in our study concerning the used methodology give precise values of bone mineral density and dietary calcium intake for the observed study population, wider studies should be conducted to be able to present results representative for the whole eastern Croatian population. Thirdly, our study population misses participants older than 55 when significant decline in bone mineral density could be expected due to menopause. It could have contributed to a lack of distinction in BMD between older and younger subgroups.

Having in mind the fact that this study involved rather young population sample, obtained results should be considered as an alarm for systematic education with the aim of osteoporosis prevention. Namely, even though bone mass deteriorates has not taken its rate yet, as it is visible from the lack of statistically significant difference in eBMD values between two observed study subgroups based on the years of age, low eBMD values themselves are obvious evidence of a bad bone health status within the study population. Intensive bone mass loss, which usually happens in the following life cycle period, if not prevented through education and changes in nutrition and lifestyle, might lead to high rates of osteoporosis.

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TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES


Mineralna gustoća petne kosti i prehrambeni unos kalcija zdravih odraslih osoba – utjecaj dobi i spola

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SAŽETAK

Cilj Adekvatna prehrana pozitivno utječe na razvoj kostiju i prevenira razvoj koštanih bolesti. Cilj ovog istraživanja bio je utvrditi mineralnu gustoću petne kosti i procijeniti prehrambeni unos kalcija zdravih odraslih osoba, te, temeljem dobivenih rezultata, procijeniti imaju li dob i spol utjecaja na iste, odnosno na njihovu povezanost.


Rezultati Prosječna vrijednost eBMD-a za cijelu ispitivanu skupinu iznosila je 0,543 g/cm², te nije bilo razlika između muških i ženskih ispitanika. Prosječan prehrambeni unos kalcija je bio nizak (661 mg/dan), s tim da su muškarci imali statistički značajno viši unos od žena (805 mg/dan u odnosu na 599 mg/dan). Mlađa podskupina ispitanika imala je višu prosječnu vrijednost eBMD-a (0,560 g/cm²) i viši prehrambeni unos kalcija (10,5 mg/g kg tjelesne mase) nego starija podskupina (eBMD=0,527 g/cm²; unos kalcija 9,4 mg/kg tjelesne mase), ali razlika nije bila statistički značajna.

Zaključak Nepostojanje statistički značajne razlike u vrijednostima eBMD-a između dvije promatrane dobne skupine, kao i same vrijednosti eBMD-a koje su bile relativno niske, dokaz su lošeg zdravstvenog statusa kostiju ispitivane populacije.

Ključne riječi: denzitometrija, osteoporozna, 24-satno prisjećanje