

Modifiable Unhealthy Behaviours Influencing Peak Bone Density of Turkish Young Women: Coffee and Smoking Affects Peak Bone Mass

Genç Türk Kadınlarında Zirve Kemik Yoğunluğunu
Etkileyen Değiştirilebilir Sağlıksız Davranışlar:
Kahve ve Sigara Tüketimi Zirve Kemik Kütlesini Etkiler

Aslı DOĞRUK ÜNAL, MD,^a
Sibel BAŞÇIL,^b
Neslihan B. TÜTÜNCÜ, MD^a

^aDepartment of Endocrinology and
Metabolism,
Başkent University Faculty of Medicine,
^bDepartment of Periodontology,
Başkent University Faculty of Dentistry,
Ankara

Geliş Tarihi/Received: 26.08.2010
Kabul Tarihi/Accepted: 02.03.2011

Yazışma Adresi/Correspondence:
Neslihan B. TÜTÜNCÜ, MD
Başkent University Faculty of Medicine,
Department of Endocrinology and
Metabolism, Ankara
TÜRKİYE/TURKEY
neslibascil@yahoo.com

ABSTRACT Objective: To determine the relationship between bone mineral density (BMD), periodontal diseases and modifiable unhealthy behaviors in healthy premenopausal young women. **Material and Methods:** A total of 50 healthy women from the hospital staff were included into study. A detailed questionnaire reporting the participants' daily tea, coffee, milk/ayran, and alcohol intakes, smoking status, sunlight exposure in a year, weekly physical activity were reviewed. Their periodontal status' were identified by the same periodontologist. Bone masses were assessed by the measurement of BMD. **Results:** Total of 23 participants were smokers. There was negative correlation between BMD of lumbar and femur Ward's with the number of cigarette smoked ($p=0.035$ and $p=0.012$, respectively). A total of nine participants were heavy coffee consumers. They have significantly lower BMD at lumbar vertebrae and femur trochanter ($p=0.008$, $p=0.050$, respectively). There were nine participants with osteopenia in all three sites. Heavy coffee consumers were significantly more in this subgroup ($p=0.024$) and body mass indexes (BMI) of these participants were significantly lower ($p=0.044$). **Conclusion:** Heavy caffeine consumption and smoking were found to be two important habitual factors negatively influencing the final bone mass that a young women can achieve.

Key Words: Bone diseases, metabolic; caffeine; smoking

ÖZET Amaç: Sağlıklı premenopozal genç kadınlarda değiştirilebilir davranış biçimleri ile kemik mineral dansitesi (KMD) ve periodontal hastalıklar arasındaki ilişkiyi belirlemek. **Gereç ve Yöntemler:** Toplam 50 sağlıklı kadın hastane personeli çalışmaya dâhil edildi. Katılımcılardan günlük tükettikleri çay, kahve, alkol, süt/ayran, sigara miktarlarını ve güneşe maruziyet sürelerini, fizik aktivite durumlarını belirten anket formu doldurmaları istendi. Periodontolojist tarafından periodontal hastalıklar yönünden sorgulanıp muayeneleri yapıldı. KMD ölçümleri yapıldı. **Bulgular:** Katılımcıların 23'ü sigara içicisi idi. Günlük tüketilen sigara sayısı ile lumbar ve femur Ward's KMD ölçümü arasında negatif korelasyon tespit edildi (sırası ile $p=0.035$ ve $p=0.012$). Yoğun kahve tüketenlerin sayısı 9 idi. Yoğun kahve tüketicilerinin lumbar vertebra ve femur trokanter KMD değerleri belirgin olarak düşük bulundu (sırası ile $p=0.008$, $p=0.050$). Her 3 bölgede de osteopeni tespit edilen katılımcı sayısı 9 idi ve yoğun kahve içicileri bu grupta fazla idi ($p=0.024$). Bu 9 katılımcının aynı zamanda beden kitle indeksi de düşük olarak bulundu ($p=0.044$). **Sonuç:** Yoğun kahve tüketimi ve sigara içimi genç kadınlarda KMD ölçümünü olumsuz etkileyen değiştirilebilir önemli sağlıksız davranışlardır.

Anahtar Kelimeler: Kemik hastalıkları, metabolik; kafein; sigara içme

Türkiye Klinikleri J Endocrin 2011;6(2):47-54

Bone density of an elderly at any age is determined by the sum of peak bone density achieved at the end of adolescence and that lost till that age. Thus, low bone mineral density (BMD) as an older adult may

partially reflect the failure to achieve peak BMD, most of which is obtained before the age of 30. The exact age at which values for bone mass reach their peak has received considerable attention. It is likely that the timing of peak values differs between the axial and appendicular skeletons. Peak bone mass in the axial skeleton in women is achieved near the end of their second decade of life.¹ The studies using computed tomography have demonstrated that the density and the size of vertebral bone reach their peak soon after the sexual and skeletal maturity.² In the appendicular skeleton, the range of ages for the timing of peak bone mass has varied significantly, from 17 to 18 years of age to as late as 35 years of age.³⁻⁷

Hereditary factors influence both the timing and the quantity of peak bone mass. That is, three fourths of the variance in peak bone mass is attributable to hereditary factors.^{8,9} The remaining fraction of the variance in peak bone mass is assumed to be caused by modifiable lifestyle determinants especially exercise, calcium intake, coffee and tea consumption, alcohol use, sunlight exposure, and smoking. The role of these modifiable risk factors for the achievement of peak bone mass is not fully studied as those for their influence on bone in elderly.⁹⁻¹⁸ Determining the factors that influence the BMD of young women could help to identify modifiable unhealthy behavior patterns which influences future fracture risk.

It is known that dietary factors optimize bone health from childhood through late adulthood. The influence of calcium intake on achievement of peak bone mass during adolescence has been examined in retrospective studies.^{13,14} Studies demonstrate that augmented calcium intakes increase bone acquisition during growth, slow the age-related bone loss and reduce fractures in the elderly.⁷ The influence of habitual coffee consumption on bone metabolism are still controversial, although several studies have suggested that heavy coffee consumption is associated with a significant increase in risk of fracture, osteoporosis and periodontal disease.¹⁹⁻²³ Tobacco is invariably associated with reduced BMD in most studies.²⁴⁻²⁷ Little is known about the effects of alcohol in adolescents. Excess alcohol in-

take seems to have an adverse effect on the preservation of bone mass, mainly by suppressing bone formation.²⁸

Osteopenia increases susceptibility to infectious destruction of periodontal tissue. It is an important risk factor for chronic periodontitis. Although the etiopathogenesis is not well known, loss of matrix and mineral of the alveolar bone may lead to an unhealthy environment for the periodontal soft tissue attachment. Women with osteopenia are at increased risk for periodontal attachment loss and tooth loss.^{29,30}

In the present study we aimed to determine the relationship between BMD and habitual coffee, tea and cigarette consumption and periodontal status in healthy women in their early thirties.

MATERIAL AND METHODS

The 50 participants in our study were recruited from a random sample (n= 240) of female hospital staff, age 19-35 years, at Başkent University Hospital. Of the 240 recruited, 204 responded to a short questionnaire to determine eligibility for the study. To be eligible, women had to be nulligravid, between 80-150% of their ideal body weight, and have reached menarche before age 19 years. They also had to be free of the following: Amenorrhoea (experiencing no menstrual periods during the previous 12 months); a history of chronic medical conditions that could affect BMD including type 1 or type 2 diabetes mellitus, thyroid or kidney problems, and rheumatoid arthritis; a history of conditions that could alter nutrient absorption, including eating disorders and disorders of digestive tract; a history of using drugs that could affect BMD, including medications of seizure, use of glucocorticoids; and significant limitations in their physical activity during the previous six months because of injury or illness. Of those contacted 106 (51%) were eligible for the study. Among this group 58 (55%) agreed to participate. Three women who participated in the study were dropped because of the insufficient/confusing data about the physical exercise status and information documented in the food consumption diaries and five participants were dropped from the study due to refusal to con-

duct BMD. There were no significant differences between eligible persons participating and eligible persons not participating in the study with regard to age, body mass index or age at menarche. Informed consent was obtained from all participants in the study in accordance with Helsinki Declaration. This study was approved by the local ethical committee.

To characterize milk/ayran, alcohol, coffee, and tea consumption, all the participants were asked to report how frequently (times per day, week, month or year) they consumed these items including their serving sizes. Participants were also asked to fill diaries for 14 days documenting their daily food intake, number of cigarettes smoked per day. They also noted their average sunlight exposure in a year (hours per day, week and year), physical activity performed per week. Radiographic and clinical parameters of periodontal status including loss of alveolar crestal height, clinical attachment level, probing attachment level, probing depth, and percentage of sites with bleeding on probing, and measurement of plaque and gingival index, were evaluated after controlling for known confounders by the same periodontologist.

DIETARY INTAKE AND LIFESTYLE FACTORS

Subjects were asked to complete a 14-day food diary and nutrient supplement record. Their mean daily consumption of coffee, tea, milk/ayran, and alcohol were estimated using their diaries consisting of food frequency questionnaire. Alcohol, tea, milk/ayran, and coffee consumption were assessed by questionnaires determining frequency, amount and source of each. Coffee drinkers were categorized as drinking 2 or less cups (≤ 400 mg coffee/day; $\sim \leq 300$ mg caffeine /day; low caffeine consumers), and >2 cups/day (>400 mg coffee /day; $\sim >300$ mg caffeine /day; heavy caffeine consumers). Each cup (about 172 g or 6 oz) of brewed coffee contains about 103 mg caffeine. Tea drinkers were categorized as drinking 1-4 tea-glasses (≤ 80 mg; low tea consumers), and >4 tea-glasses/day (>80 mg; heavy tea consumers). Milk and ayran (yogurt diluted with water) intake were also categorized as drinking one or more cups/day. Those

drinking one or more cups of milk or ayran were recorded as milk or ayran consumers. Current smokers were classified as smokers, and those who had never smoked and past smokers (who didn't smoke during the last five years) were classified as nonsmokers. For smokers, number of years and packages smoked per day was recorded to calculate packs-year for smoking. Those with history of more than 5 pack-years smoking were classified as heavy smokers. Physical activity levels from both lifelong and current perspective were obtained by questionnaire. Past physical activity was named as highly active (≥ 5 hours of exercise per week during the last year), moderately active (3-5 hours of exercise per week during the last year) and mildly active (<3 hours of exercise per week during the last year). Those mildly active or stopped exercising in the last 3 months were reported as sedentary. The other participants were reported as physically active. Alcohol use was stratified as drinkers (more than 3 oz per week) and nondrinkers.

BONE MASS AND ANTHROPOMETRIC MEASUREMENTS

Weight was measured with a calibrated electronic scale and height was measured with a wall-mounted stadiometer by the same trained observer in the same scale and stadiometer with subjects wearing light clothing and no shoes and body mass index (BMI; in kg/m^2) was calculated.

BMD (in g/cm^2) was measured by dual-energy X-ray absorptiometry (model Hologic QDR-4500A dual-energy X-ray absorptiometer) (DEXA). BMD values were used throughout this study. T-score and aBMD (g/cm^2) of anteroposterior (L1-4), femur neck, trochanter, and Ward's triangle were assessed. The origin of the T score was determined. Results were compared with the locally determined BMD values of healthy Turkish population ($n=323$; 171 women, 152 men) and expressed as T-score.²⁹

STATISTICAL ANALYSIS

All continuous data were expressed as mean \pm SD. Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS for Windows version 11.0; SPSS Inc., Chicago, IL, USA). *P* values

< 0.05 were considered statistically significant. Continuous, normally distributed variables were compared between subgroups of participants with the Student *t* test. Mann-Whitney U test and chi-square test were performed accordingly.

RESULTS

A total of 50 healthy females (mean age 27.1±3.3 years, 23-38 years of age) were analysed for the study. Clinical characteristics of the participants including their BMI, daily milk/ayran, alcohol, tea, coffee consumption and cigarette smoking, physical activity levels, and BMD and T-scores of lumbar vertebrae and femur are given in Table 1.

Analysis of the medical history questionnaire revealed that 18 participants had history of euthyroid goiter and 14 women who received supplemental vitamins and calcium preparations irregularly (equal to or less than two tablets in a week), three women had history of fractures (one had fracture in distal fibula, two had fracture of metatars after trauma) and four participants had chronic periodontitis. These participants with chronic periodontitis revealed normal bone mineral densities in all the examined anatomic sites.

A total of 23 participants were smokers. There were no differences in bone mineral densities in those with smokers and nonsmokers. Correlation analysis revealed negative correlation between BMD of lumbar and femur Ward's with the number of cigarette smoked (pearson= -0.298, *p*= 0.035 for lumbar vertebrae; pearson= -0.351, *p*= 0.012 for femur Ward's). Subgroup analysis for the heavy smokers (*n*=14), who smoked more than 5 pack-years, revealed lower lumbar BMDs than those smoked less and lower than those never smoked (BMD=0.73± 0.1 vs 0.78 ± 0.1, *p*=0.042, respectively).

A total of 9 participants were heavy coffee consumers (~>300 mg/caffeine/day). Correlation analysis revealed negative correlation between BMD of lumbar vertebrae and femur trochanter with the daily coffee consumption (pearson= -0.373, *p*= 0.008 for lumbar vertebrae; pearson= -0.278, *p*= 0.050 for femur trochanter). Heavy coffee consumers revealed lower BMD at lumbar verte-

brae and femur trochanter than the low coffee consumers (*p*= 0.008, *p*= 0.050, respectively). Many of the heavy coffee consumers were also drinking milk (44% vs 12%, *p*=0.024) (Table 2).

TABLE 1: Clinical characteristics of the participants.

n	50
Mean age (year)	27.68±3.31
Body mass index (kg/m ²)	21.74±3.51
Milk consumers (n)	9
Ayran consumers (n)	6
Heavy coffee consumers (n)	9
Heavy tea consumers (n)	28
Alcohol consumers (n)	6
Smokers (n)	23
Highly active (n)	2
Moderately active (n)	8
Sedantary life (n)	40
Mean Cigarette per day for smokers (n)	9.29±5.3
BMD (g/ cm ²)- Lumbar vertebrae	0.76±0.02
BMD (g/cm ²)- Femur Trochanter	0.66±0.05
BMD (g/cm ²)- Femur Ward's	0.69±0.11
T-score Lumbar vertebrae	-0.63±0.92
T-score Femur Trochanter	-0.47±0.81
T-score Femur Ward's	-0.34±1.03

TABLE 2: Comparison of risk factors with respect to daily caffeine consumption.

	Low coffee consumers	Heavy coffee consumers	P
n	41	9	
Mean age (years)	27.3±2.9	29.4±4.2	-
Body Mass Index (kg/m ²)	21.4±3.0	23.1±5.2	-
Daily milk consumers (n)	5	4	0.024
Daily ayran consumers (n)	5	1	-
Active women (n) (moderate-high physical activity)	8	2	-
Daily tea consumers (n)	22	6	-
Smokers (n)	18	5	-
Alcohol consumers (n)	4	2	-
BMD (g/ cm ²) Lumbar vertebrae	0.8 ± 0.1	0.7 ± 0.1	0.008
BMD (g/cm ²) Femur Trochanter	0.7 ± 0.1	0.6 ± 0.6	0.050
BMD (g/cm ²) Femur Ward's	0.7 ± 0.1	0.6 ± 0.1	-
T-score Lumbar vertebrae	-0.5 ± 0.8	-1.2 ± 1.0	0.021
T-score Femur Trochanter	-0.4 ± 0.8	-0.9 ± 0.6	0.039
T-score Femur Ward's	-0.2 ± 1.0	-1.0 ± 0.7	0.022

Participants with osteopenia in all three sites (n=9, 18%) were re-evaluated with respect to the risk factors (Table 3). Heavy coffee consumers were significantly more in this osteopenic group (44 % vs 12%, p=0.024). BMI of these osteopenic participants were significantly lower (19.6 ± 1.3 vs 22.2 ± 3.7 ; p=0.044).

There were more heavy coffee consumers in the subgroup with femur osteopenia than the subgroup of participants with normal femur BMD (42% vs 10% respectively, p=0.015). On the other hand, subgroup of participants with lumbar osteopenia (n=16) revealed similar characteristics of modifiable risk factors with those with normal lumbar BMD.

Data reported from the life style questionnaire revealed that daily calcium intake (milk, yogurt and ayran consumption), physical activity (moderate and high) and alcohol consumption were similar in those smoking and nonsmoking participants and also in heavy coffee consumers and non-coffee consumers. There were only 6 alcohol drinkers in our study and thus we could not drive any conclusions regarding the influence of alcohol on BMD.

DISCUSSION

Although genetics is the most important contributor (up to 80%) to achieve peak bone mass early in the third decade, a substantial proportion of the variance in peak bone mass found in the general population can not be explained by the known genetic factors. That is both genetic and lifestyle factors are considered important for the achievement of peak bone mass and thus the risk of osteoporosis.^{7-9,11,12,31,32}

In the present study about half of our participants were smokers and more than half of the smokers were heavy smokers (about 30% of the participants). Not surprisingly, number of cigarettes smoked per day and thus total number of cigarettes smoked in life was found to be negatively correlated with BMD in our study. BMD of lumbar vertebrae was significantly lower in heavy smokers. Unlike the contradictory results about the effects of caffeine on bone, cigarette smoking is almost always cited as a risk factor for osteoporosis and associated fractures.²³⁻²⁷ Although the exact mechanism by which smoking exerts its negative effect on bone is not yet fully known, it decreases circulating levels of estrogen by increasing its degrada-

TABLE 3: Modifiable risk factors in participants with lumbar and femur osteopenia.

	Those with normal BMD in femur and/or lumbar vertebrae	Those with lumbar and femur osteopenia	P
n	41	9	
Mean age (year)	27.8 ± 3.4	27.0 ± 2.9	-
Body Mass Index (kg/m ²)	22.2 ± 3.7	19.6 ± 1.3	0.044
Daily milk consumers (n)	7	2	-
Daily ayran consumers (n)	5	1	-
Active women (n) (moderate-high physical activity)	9	1	-
Daily tea consumers (n)	22	6	-
Coffee consumers (n)	5	4	0.024
Smokers (n)	17	6	-
Alcohol consumers (n)	4	2	-
BMD (g/ cm ²) Lumbar vertebrae	0.8 ± 0.1	0.7 ± 0.0	0.000
BMD (g/cm ²) Femur Trochanter	0.7 ± 0.1	0.6 ± 0.0	0.000
BMD (g/cm ²) Femur Ward's	0.7 ± 0.1	0.6 ± 0.1	0.000
T-score Lumbar vertebrae	-0.4 ± 0.8	-1.8 ± 0.4	0.000
T-score Femur Trochanter	-0.3 ± 0.7	-1.4 ± 0.4	0.000
T-score Femur Ward's	-0.1 ± 0.1	-1.4 ± 0.6	0.000

tion and decreasing its production. It also has direct toxic effect on bone cells and causes calcitonin resistance.³² Smoking is a very important health problem especially in the developing countries like ours where first age of smoking can be as low as the prepubertal ages in some regions of the country. Thus smoking not only has impact on bone mass in elderly but also in children and adults before achieving peak bone mass.

In the present study heavy coffee consumption is found to be an important negative contributor to the peak bone mass achieved in early thirties. Coffee is one of the most popular beverages in the world and it is also a very popular drink in our country. One of the most important constituents of coffee is the caffeine (~50 mg caffeine in 3 oz brewed coffee). Studies of caffeine as a probable risk factor for osteoporosis have yielded conflicting results in elderly. In several cross sectional studies, caffeine consumption has been reported to decrease BMD, increase the risk of hip fracture and negatively influence calcium retention in postmenopausal women.^{19,21,23,33} In a recent study caffeine was found to decrease bone mass by downregulating some important events in osteogenesis in rat bone marrow-derived mesenchymal stromal cells.³⁴ In a prospective study of Sakamoto, it was found that high caffeine intake (> 300 mg/day) increased the rate of bone loss significantly in the spine in elderly postmenopausal women studied longitudinally. This negative effect of caffeine on bone mass was found to be further accentuated by low calcium intakes. It was interesting to find in the same study that, postmenopausal elderly women with the tt genetic variant of vitamin D receptor appeared to be more susceptible to the negative effect of caffeine as evidenced by higher rates of bone loss.³⁵ In the study of Hallström et al., it was found that those having CYP1A2 genotype are rapid caffeine metabolizers and have increased risk of losing BMD of proximal femur in elderly.³⁵ However, most of the other studies reported no overall association between caffeine intake and BMD, fracture rate or calcium metabolism.^{22,31-40} In our study we found a negative correlation between BMD and frequency of coffee consumption. Heavy

coffee consumers revealed significantly lower BMD's. In the present study we also found that daily milk consumption was significantly higher in heavy coffee consumers. This finding is probably the result of habitual drinking of coffee with milk. Nevertheless, milk being one of the most important source of calcium doesn't seem to be enough to protect bone from deleterious effects of caffeine in heavy coffee consumers.^{19,23,40}

As it is well known that increasing body weight has positive impact on bone metabolism it was not surprising to find that thinner participants in our study revealed the lowest BMD's of all the anatomic sites studied.

Tea is one of the two most popular drinks in our country and coffee is the other one as mentioned above. In the present study daily tea consumption was found to be very high as expected but didn't influence the bone mass. Studies conducted in elderly women, tea was reported to protect against hip fractures.^{15,16,31} Possible protective role of tea on bone was explained by the phytoestrogens or fluoride components of the tea in those studies.^{31,40-47}

Osteopenia is an important risk factor for periodontal diseases and thus tooth loss in elderly.^{29-30,48} Its effect in young is not known. In the present study we also tried to determine the relationship between metabolic bone disease and periodontal disease in early ages. We found no active or chronic periodontitis in our young women with osteopenia. This finding may be confounded by the small number of participants examined for this study and absence of severe metabolic bone disease in our participants. Large groups of young women should be examined to find out the influence of different stages of metabolic bone disease from osteopenia to severe osteoporosis on periodontal attachment in early ages.

In the present study which was conducted to specify the roles of modifiable behavioural risk factors on bone mass in women at their thirty's, heavy caffeine consumption and heavy smoking were found to be two important habitual factors influencing the final peak bone mass that young woman can reach. Although our study would have been

more powerful if larger group of young women were examined to find out the influence of unhealthy behaviours on bone metabolism, this study is amongst the very few studies if any, documenting the deleterious effects of high coffee intake and

smoking on peak bone mass. Our study forms basis for further prospective and controlled studies in large groups of participants to identify the role of these modifiable risk factors on bone in early decades of life.

REFERENCES

1. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab* 1992;75(4): 1060-5.
2. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE. Peak trabecular vertebral density: a comparison of adolescent and adult females. *Calcif Tissue Int* 1988;43(4):260-2.
3. Gordon CL, Halton JM, Atkinson SA, Webber CE. The contributions of growth and puberty to peak bone mass. *Growth Dev Aging* 1992;55(4):257-62.
4. Matkovic V, Jelic T, Wardlaw GM. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. *J Clin Invest* 1994;93(2):799-808.
5. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. *JAMA* 1992;268(17): 2403-8.
6. Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. *J Clin Endocrinol Metab* 1991;73(3):555-63.
7. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, et al. Peak bone mass. *Osteoporos Int* 2000;11(12):985-1009.
8. Christian JC, Yu PL, Slemenda CW, Johnston CC Jr. Heritability of bone mass: a longitudinal study in aging male twins. *Am J Hum Genet* 1989;44(3):429-33.
9. Kelly PS, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA. Genetic factors in bone turnover *J Clin Endocrinol Metab* 1991;72(4):808-13.
10. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Foulkner RA. A six year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *J Bone Miner Res* 1999;14(10): 1672-9.
11. Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A, et al. Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. *J Bone Miner Res* 1998;13(3):500-7.
12. Slemenda CW, Peacock M, Hui S, Zhou L, Johnston CC. Reduced rates of skeletal remodeling are associated with increased bone mineral developments during the development of peak skeletal mass. *J Bone Miner Res* 1997;12(4):676-82.
13. Bonjour JP, Rizzoli R. [The property of calcium in the child and the adolescent: importance in the acquisition of bone mineral density]. *Arch Pediatr* 1999;6(Suppl 2):155s-157s.
14. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: Randomized, controlled intervention trial. *Br Med J* 1997;315(7118): 1255-60.
15. Oden A, Dawson A, Dere W, Johnell O, Jonsson B, Kanis JA. Lifetime risk of hip fractures is underestimated. *Osteoporos Int* 1998;8(6): 599-603.
16. Johnell O, Gullberg B, Kanis JA, Allender E, Ellfors L, Dequeker J, et al. Risk factors for hip fracture in European women: the MEDOS Study. *Mediterranean Osteoporosis Study. J Bone Miner Res* 1995;10(11):1802-15.
17. Rico H, Canal ML, Manas P, Lavado JM, Costa C, Pedrera JD. Effects of caffeine, vitamin D, and other nutrients on quantitative phalangeal bone ultrasound in postmenopausal women. *Nutrition* 2002;18(2):189-93.
18. Albrand G, Munoz F, Sornay-Rendu E, DuBoeuf F, Delmas PD. Independent predictors of all osteoporosis-related fractures in healthy postmenopausal women: the OFELY study. *Bone* 2003;32(1):78-85.
19. Kiel DP, Felson DT, Hannan MT, Anderson JJ, Wilson PW. Caffeine and the risk fracture. *Am J Epidemiol* 1990;132(4):675-84.
20. Yano K, Heilbrun LK, Wasnich RD, Hankin JH, Vogel JM. The relationship between diet and bone mineral content of multipl skeletal sites in elderly Japanese-American men and women living in Hawaii. *Am J Clin Nutr* 1985;42(5): 877-8.
21. Harris SS, Dawson-Hughes B. Caffeine and bone loss in healthy postmenopausal women. *Am J Clin Nutr* 1994;60(4):573-8.
22. Cooper C, Atkinson EJ, Wahner HW, O'Fallon WM, Riggs BL, Judd HL, et al. Is caffeine consumption a risk factor for osteoporosis? *J Bone Miner Res* 1992;7(4):465-71.
23. Barret-Connor E, Chang JC, Edelstein SL. Coffee-associated osteoporosis offset by daily milk consumption. The Rancho Bernardo Study. *JAMA* 1994;271(4):280-3.
24. Ortego-Centeno N, Munoz-Torres M, Jodor E, Hernandez-Quero J, Jurado-Duce A, de la Higuera Torres-Puchol J. Effect of tobacco consumption on bone mineral density in healthy young males. *Calcif Tissue Int* 1997;60(6):496-500.
25. Turner JG, Gilchrist NL, Ayling EM, Hassal AJ, Hooke EA, Sadler WA. Factors affecting bone mineral density in high school girls. *N Z Med J* 1992;105(930):95-6.
26. Välimäki MJ, Kärkkäinen M, Lamberg-Allardt C, Laitinen K, Alhava E, Heikkinen J, et al. Exercise, smoking and calcium intake during adolescence and early adulthood as determinants of peak bone mass. *Cardiovascular Risk in Young Finns Study Group. Br Med J* 1994;309(6949):230-5.
27. Hopper JL, Seeman E. The bone density of female twins discordant for tobacco use. *N Engl J Med* 1994;330(6):387-92.
28. Turner RT. Skeletal response to alcohol. *Alcohol Clin Exp Res* 2000;24(11):1693-701.
29. Al Habashneh R, Alchalabi H, Khader YS, Hazza'a AM, Odat Z, Johnson GK. Association between periodontal disease and osteoporosis in postmenopausal women in Jordan. *J Periodontol* 2010;81(11):1613-21.
30. Koduganti RR, Gorthi C, Reddy PV, Sandeep N. Osteoporosis: A risk factor for periodontitis. *J Indian Soc Periodontol* 2009;13(2):90-6
31. Gurlek A, Bayraktar M, Ariyurek M. Inappropriate reference range for peak bone mineral density in dual-energy X-ray absorptiometry: Implications for the interpretation of T-scores. *Osteoporos Int* 2000;11(9):809-13.
32. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mass density in older women. *Am J Clin Nutr* 2000;71(4):1003-7.

33. Rapuri PB, Gallagher JC, Balhorn KE, Ryschon KL. Smoking and bone metabolism in elderly women. *Bone* 2000;27(3):429-36.
34. Zhou Y, Guan X, Zhu Z, Guo J, Huang Y, Hou Y, et al. Caffeine inhibits the viability and osteogenic differentiation of rat bone marrow-derived mesenchymal stromal cells. *Br J Pharmacol* 2010;161(7):1542-52.
35. Hallström H, Melhus H, Glynn A, Lind L, Syvänen AC, Michaëlsson K. Coffee consumption and CYP1A2 genotype in relation to bone mineral density of the proximal femur in elderly men and women: a cohort study. *Nutr Metab (Lond)* 2010;7:12.
36. Rapuri PB, Gallagher JC, Kinyamu HK, Ryschon KL. Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. *Am J Clin Nutr* 2001;74(5):694-700.
37. Massey LK. Is caffeine a risk factor for bone loss in the elderly? *Am J Clin Nutr* 2001;74(5):569-70.
38. Conlisk AJ, Galuska DA. Is caffeine associated with bone mineral density in young adult women? *Prev Med* 2000;31(5):562-8.
39. Heaney RP. Effects of caffeine on bone and the calcium economy. *Food Chem Toxicol* 2002;40(9):1263-70.
40. Huang TH, Yang RS, Hsieh SS, Liu SH. Effects of caffeine and exercise on the development of bone: A densitometric and histomorphometric study in young Wistar rats. *Bone* 2002;30(1):293-9.
41. Lloyd T, Rollings NJ, Kieselhorst K, Eggle DF, Mauger E. Dietary caffeine intake is not correlated with adolescent bone gain. *J Am Coll Nutr* 1998;17(5):454-7.
42. Sakamoto W, Nishihira J, Fujie K, Iizuka T, Handa H, Ozaki M, et al. Effect of coffee consumption on bone metabolism. *Bone* 2001;28(3):332-6.
43. Hertog MG, Hollman PC, Kardon MB, Kramhau D. Estimation of daily intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr Cancer* 1993;20(1):21-9.
44. Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol* 1993;44(1):37-43.
45. Adlercreutz H, Hämäläinen E, Gorbach S, Goldin B. Dietary phyto-oestrogens and the menopause in Japan. *Lancet* 1992;339(8803):1233.
46. Medrele Kuder E. [Unhealthy behaviour patterns encouraging the development of osteoporosis]. *Rocz Pantstw Zacl Hig* 2009;60(2):181-4.
47. Kara HI, Aydın S, Gemalmaz A, Akturk Z, Yaman H, Bozdemir N, et al. Habitual tea drinking and bone mineral density in postmenopausal Turkish women: Investigation of prevalence of postmenopausal osteoporosis (IPPOTStudy). *Int J Vitam Nutr Res* 2007;77(6):389-97.
48. Martínez-Maestre MÁ, González-Cejudo C, Machuca G, Torrejón R, Castelo-Branco C. Periodontitis and osteoporosis: a systematic review. *Climacteric* 2010;13(6):523-9.