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BONE MINERAL DENSITY, VEGETARIANISM, VITAMIN D, CALCIUM, AND ADIPOKINES: A CROSS-SECTIONAL INVESTIGATION

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ABSTRACT

Background and Objectives: Osteoporosis has widely been diagnosed and one reason could be the elimination of meat products from the diet. The aim of the study was to assess the possible impact of vegetarian diet to aBMD among Estonian vegetarians, considering other influencing factors such as age, gender, physical activity, adipokines, vitamin-D, calcium, body composition, smoking and alcohol consumption. *Materials and Methods:* Participants completed demographics and personal behaviour questionnaire. Whole body and regional aBMD was measured with dual-energy X-ray absorptiometry. In addition, calcium, vitamin D, leptin, and adiponectin were measured from the fasting blood samples. *Results:* Altogether 68 adult vegetarians (V; ♂: n=17; 20-48y; ♀: n=51; 20-53y); and 103 omnivores (O; ♂: n=22; 20-54y; ♀: n=81; 18-53y) were examined. V had significantly lower lumbar spine (L1L4; *p*=0.045) and femoral neck (FN; *p*=0.019) aBMD values in comparison with O group. Age and gender had an effect on BMD; FNBMD: V *vs* O coef-.045; *p*=0.028, female *vs* male coef-.08; *p*<0.001; whole body BMD: female *vs* male coef-.094, *p*<0.001; older *vs* younger coef.002, *p*=0.004; L1L4BMD older *vs* younger coef.002; *p*=0.038. Calcium levels were mostly in recommended level (2.02-2.60 mmol/l) in both groups, but significantly higher in O when compared with V (*p*=0.026). In addition, 43.5% of V and 35.1% of O had vitamin D level below the recommended level. Leptin levels were higher in omnivores (*p*=0.025) and adipokines playd important role for aBMD. *Conclusions:* V have lower FN aBMD, although vegetarianism was found to have a positive effect on calcium concentration.

KEYWORDS: Bone mineral density; vegetarian diet; vitamin D; calcium; physical activity; leptin; adiponectin.

INTRODUCTION

Osteoporosis significantly affects the quality of life of many people worldwide and increases health care costs.[1] Areal bone mineral density a(BMD) is considered the most consistent predictor of osteoporotic fracture.[2] Several factors have detrimental impact on bone health, namely age and gender.[3] lower body mass index (BMI),[4] lower intakes of vitamin B12,[5] calcium,[6] and vitamin D.[7,8] higher levels of circulating leptin,[9] and lower levels of adiponectin,[10] physical inactivity.[11] alcohol consumption.[12] and the use of tobacco.[13] However, the impact of vegetarianism on aBMD has not been thoroughly studied, although there are studies to demonstrate that compared with omnivores vegetarians have lower BMD.[14]

The proportion of self-reported vegetarians is approximately up to 5% of the general population in the European region.[15] and also in Canada,[16] and vegetarianism is audibly increasing in the Western world. The term “vegetarian” encompasses a variety of types of food intake in diets. The most restrictive diet is vegan, consuming no animal products at all. The association between vegetarian diets and bone mineral density is controversial because of conflicting findings from number of previous prospective and longitudinal,[17] studies. Anyway, the diet of vegetarians and vegans is often low in protein, calcium, vitamin D and B12 and other nutrients, which are necessary for the development and the maintenance of bone. On the other hand, vegetarians consume more potassium, and bear a much lower acid load, both being reported as positive for bone health.[18]

The aim of this cross-sectional study was to assess the possible impact of vegetarian diet to aBMD among Estonian vegetarians, considering other influencing factors such as age, gender, physical activity, adipokines, vitamin-D, calcium, body composition, smoking and alcohol consumption.

MATERIALS AND METHODS

Participants were recruited through online advertisements and local vegetarian societies. The inclusion criteria for vegetarians were as follows: age between 18-55y; adoption of a vegetarian diet for at least three years; white ethnicity; no history of pregnancy within the preceding 12 months. Exclusion criteria for all participants were pre-diagnosed bone problems and diseases affecting bone metabolism; menopause in females. We defined “vegans” who did not consume any animal products; and “lacto-ovo vegetarians” who consumed eggs and/or dairy products.[19]

Venous blood samples (16.5 mL) were drawn in the morning after an overnight fast using an antecubital vein with the participant sitting in the upright position. Haematological analyses were performed using the analyser MEK-6400J/K (Nihon Kohden, Tokyo, Japan). Plasma was separated and frozen at -20 °C for subsequent analysis. Clinical chemistry analysis was carried out in the laboratory of Tartu Health Care College using analysers BS-120 (Mindray BS 120, China, Nanshan), Cobas® c111 (Roche Diagnostics, Rotkreuz, Switzerland), and Immulite® 2000 (Siemens, Munich, Germany). Adiponectin was determined in duplicate via commercially available radioimmunoassay (RIA) kits (cat. No. HADP-61HK, Linco Research, St. Charles, MO, USA); the intra- and inter-assay CV values were <7%. Leptin was determined in duplicate by RIA (Mediagnost GmbH, Reutlingen, Germany), this assay has intra- and inter-assay CV values of less than 5%, and the lowest detection limit was 0.01 ng/ml. Vitamin D and calcium levels were measured using the ELISA and CPC methods.

All anthropometrical measurements were done according to the standard technique. Body height was measured with Martin metal anthropometer to the nearest 0.1 cm. Body mass was measured with minimal clothing to the nearest 0.05 kg with a medical electronic scale (A&D Instruments, Abingdon, UK). BMI was calculated as body mass (kg) divided by body height (m^2). Body fat %, and aBMD (g/cm^2) as whole-body (WB) aBMD, lumbar spine (LS; L1-L4) and femoral neck (FN) were detected by dual-energy X-ray absorptiometry (DXA, Hologic). Participants were scanned in light clothing while lying flat on their backs with arms on their sides. Coefficients of variation (CVs) for BMD were less than 2%.

On the same day as the blood sample collection and aBMD measures, participants completed demographics and personal behaviour questionnaires that comprised questions about age, gender, educational level, alcohol consumption, smoking, physical activity, vegetarian diet type and duration. Physical activity level was divided into three groups according to WHO recommendations [20]: inactive – physical activity (PA) <150 and <7X60 minutes a week; and very active – PA >300 and >7x60 minutes a week. The average group was defined as optimal or moderate PA group. Tobacco and alcohol use was defined as smoking any amount tobacco or none.

The software program Sigma Plot for Windows version 11.0 (GmbH Formation, Germany) and R 2.6.2 (A Language and Environment, http://www.r-project.org) were used for statistical analysis. Differences in proportions were compared with chi-square tests or Fisher’s exact tests, and differences in values were compared with t-tests or Mann-Whitney U tests as appropriate. To assess the influence of vegetarian diet on BMD in different locations and other parameters associated with aBMD univariate and multiple linear regression analysis adjusted for age and gender.[3,21] was applied. We did not consider physical activity in multiple linear analysis model as no influence of physical activity to aBMD in univariate logistic regression analysis was emerged. *P*-value of <0.05 was considered as significant.

RESULTS

Baseline characteristics

This prospective cross-sectional study comprised of 68 (27 of them vegans) vegetarians (♂: n=17; 20-48y; ♀: n=51; 20-53y) and 103 omnivorous (♂: n=22; 20-54y; ♀: n=81; 18-53y) as a control group. Vegetarians were firstly divided to lacto-ovo-vegetarian and vegan groups. Mean practice time of vegetarian diet was 8.1±5.1 years for lacto-ovo-vegetarians and 6.0±3.5 years for vegans and it did not differ between men and women. As there were no differences in measured parameters in vegans and lacto-ovo-vegetarians, all vegetarians were counted as one study group. Main characteristics of participants by diet group are presented in Table 1.

The educational and physical activity levels were similar between the study groups. Male vegetarians had significantly lower weight and BMI values compared with male omnivores (Table 1), while no differences in fat percentage were observed between the groups (fat%; Table 2). 44.1% of vegetarians consumed food additives (mostly vitamin D and B12) in daily bases, males more than females (p=0.024; Table 3). Male omnivores smoke more than male vegetarians. Compared to omnivores, female vegetarians had lower rates of alcohol consumption (p<0.025), but no differences in males.

Body composition parameters (results of DXA) and laboratory data in different diet groups

Bone mineral density at skeletal sites were lower in vegetarian group, but not WB aBMD where the lower numbers were shown in female group (Table 2; Figure 1). Male vegetarians had also lower WB BMD (p=0.02). *P*-value for all omnivores and vegetarians was 0.055. According to WHO,[22] 7 vegetarians (incl 5 females) and 6 omnivores (incl 4 females) had osteopenia (T-score -1 to -2.5 SD).

Leptin level was significantly higher (p=0.025) in omnivores than vegetarians. There were no differences in adiponectin and vitamin D level between the study groups. 43.5% of vegetarians and 35.1% of omnivores had vitamin D level under recommended level (50-250 nmol/l). Univariate linear regression analysis showed, that the use of additives enlarge the vitamin D level (coef 16.6; p=0.05). The multiple model, assessing the influence of vegetarian diet on BMD adjusted for gender, age and additionally for additive usage, showed the omnivorous diet negative influenced vitamin D level (coef -13.86; p=0.03). The average calcium level in vegetarians and omnivores was in accordance to reference values 2.02-2.60 mmol/l. Vegetarians had higher calcium level (Table 3), that also accompanied by the results of univariate linear regression analysis (coef 0.059; p=0.028).

Factors influencing measured aBMD values

According to the linear regression analysis the female gender and age were the most significant predictors of aBMD values (Table 4). Surprisingly, every year of life increased a BMD by 0.002 units. Current physical activity level had no impact on aBMD so we did not switch it in the multivariate linear regression model. The results of linear analysis did not coincide fully with previously given data (results of t-test). The negative influence of om-nivore diet to LS aBMD disappeared, but their lower FN BMD was fixated. It emerged more strongly when recon with age and gender.

Table 1: Baseline characteristics of participants.

	All participants		Females		Males	
	Vegetarians	Controls	Vegetarians	Controls	Vegetarians	Controls
n=	68	103	51	81	17	22
Age: year (mean; SD)	30.1; 8.9	30.8; 9.4	29.1; 8.2	30.1; 9.5	28.6; 7.4	33.3; 8.7
Body mass; kg (mean ±SD)	64.0; 11.2	70.2; 18.8	61.7; 10.1	65.7; 16.7	70.8; 12**	86.6; 17.4 **
BMI (mean; ±SD)	22.1; 2.9	23.8; 5	22.1; 3.1	22.9; 4.8	22.0; 2.1***	26.9; 4.8 ***
Height: cm (mean ±SD)	169.8; 9.2	170.5; 7.8	166.7; 6.5	168.0; 5.8	178.9; 10.2	179.4; 7.9
Inactive (%)	67.6	64.1	72.5	64.1	52.9	63.6
Optimally active (%)	29.5	24.3	23.6	24.2	47.1	22.7
Very active (%)	2.9	11.6	3.9	11.7	0	13.7
Food additives consumers (%)	44.1		35.3*		70.6*	
Smokers (%)	14.7	21.4	17.6	13.6	5.9**	50**
Alcohol consumers (%)	55.9**	76.7**	54.9*	75.3*	58·8	81.8

Table 2: The results of DXA and laboratory data in different diet groups.

	All participants		Females		Males	
Variables	Vegetarians	Omnivores	Vegetarians	Omnivores	Vegetarians	Omnivores
n=	68	103	51	81	17	22
FM: kg (mean ±SD)	18.3; 6.6	20.8; 8.6	19.7; 6.6	20.8; 8.3	13.9; 4.5*	20.7; 9.6*
FFM: kg (mean ±SD)	42.7; 7.9	45.4; 10.4	39.5; 4.7	41.0; 5.6	52.2; 8.1**	61.3; 8.2**
FM % (mean ±SD)	28.5; 7.7	29.8; 7.1	31.4; 6.2	31.5; 6.2	20.0; 4.9	23.3; 6.2
WB BMD: g/cm² (mean ±SD)	1.13; 0.09	1.16; 0.1	1.12; 0.08	1.13; 0.09	1.17; 0.13**	1.25; 0.07**
L1L4 BMD: g/cm² (mean ±SD)	1.02; 0.12*	1.05; 0.12*	1.02; 0.11	1.04; 0.12	1.02; 0.16*	1.11; 0.11*
FN BMD: g/cm² (mean ±SD)	0.84; 0.13*	0.88; 0.13*	0.82; 0.12	0.86; 0.12	0.89; 0.15	0.96; 0.13
Adiponectin: μg/ml (mean ±SD)	10.1; 2.89	10.37; 3.27	10.4; 2.99	10.93; 3.38	9.25; 2.45	8.47; 1.91
Leptin: ng/ml (mean ±SD)	10.8; 12.6*	14.8; 16.6*	12.9; 13.7	17.1; 18.0	4.2; 4.1	6.8; 5.7
Vitamin-D: nmol/l (mean ±SD)	60.1; 31.6	64.46; 30.0	61.64; 33.93	65.98; 29.16	56.03; 24.79	58.61; 33.15
Ca²+: nmol/l (mean ±SD)	2.22; 0.16*	2.16; 0.18*	2.22; 0.16*	2.15; 0.18*	2.21; 0.17	2.20; 0.20

BMI – body mass index; FM – fat mass; FFM – fat free mass; BMD – bone mineral density; WB – whole-body; L1L4 – lumbar spine (L1-L4); FN - femoral neck; *p<0.05; **p<0.01

Table 3. Influence of usage of food additives to blood parameters.

Dietary supplement	Parameter in the blood	Supplement users		Non-users	
		n=	Median ±SD		p=
Vitamin B12	Vitamine B12 (pmol/l)	10	359.8 ±242.8	228.9 ±87.4	0.213
B12+B complex	Vitamine B12 (pmol/l)	13	339.1 ±212.3	226.2 ±88.2	0.089
Vitamin D	Vitamine D (nmol/l)	19	72.2 ±33.0	55.2 ±30.0	**0.011**
Vitamin D	Ca (mmol/l)	19	2.2 ±0.1	2.2 ±0.2	0.548
Ca	Ca (mmol/l)	4	2.0 ±0.03	2.2 ±0.2	**0.006**

Table 4: Influence of vegetarian diet, gender and age to BMD and other associated parameters by the results of univariate (A) and influence of vegetarian diet adjusted for gender and age by the results of multiple linear regression analysis (B).

Marker	Vegetarians *vs* omnivores		Female *vs* male		Older *vs* younger	
	Coef	p	Coef	p	Coef	p
A - univariate						
WB BMD	NS	NS	**-0.094**	**<0.001**	**0.002**	**0.004**
L1L4 BMD	NS	NS	NS	NS	**0.002**	**0.038**
FN BMD	**-0.045**	**0.028**	**-0.08**	**<0.001**	NS	NS
BMI	**-1.657**	**0.014**	**-2.095**	**0.008**	**0.101**	**0.007**
FM %	NS	NS	**9.578**	**<0.001**	NS	NS
Adiponectin	NS	NS	**1.923**	**<0.001**	**-0.075**	**0.007**
Leptin	NS	NS	**9.837**	**<0.001**	NS	NS
Vitamin-D	NS	NS	NS	NS	NS	NS
Ca^{2+}	**0.059**	**0.028**	NS	NS	NS	NS
B - multiple adjusted for age and gender						
WB BMD	NS	NS	**-0.092**	**<0.001**	**0.002**	**0.012**
L1-L4 BMD	NS	NS	NS	NS	NS	NS
FN BMD	**-0.051**	**0.009**	**-0.086**	**<0.001**	NS	NS
BMI	**-1.573**	**0.017**	**-2.043**	**0.007**	**0.085**	**0.019**
FM %	**9.614**	**<0.001**	NS	NS	NS	NS
Adiponectin	NS	NS	**1.787**	**<0.002**	**-0.069**	**0.011**
Leptin	NS	NS	**9.420**	**0.002**	NS	NS
Vitamin-D	NS	NS	NS	NS	NS	NS
Ca^{2+}	**0.055**	**0.044**	NS	NS	NS	NS

BMD – bone mineral density; WB – whole-body; L1L4 – lumbar spine (L1-L4); FN - femoral neck; BMI – body mass index; FM – fat mass; NS – not significant

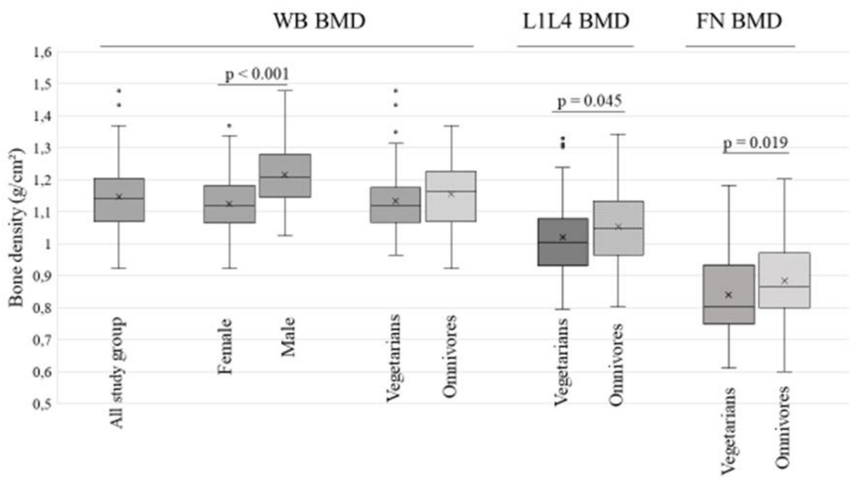

Figure 1: Bone mineral density in different diet groups (t-test).

DISCUSSION

To our best knowledge, this is the first study in vegetarians focusing on the associa-tion with several parameters possibly affecting aBMD. According to the study results, the strongest predictors for aBMD values were female gender and age. Surprisingly, older par-ticipants had higher BMD parameters, but it can explain by the age of our study group, that was early thirties. Previously have shown, that as there is solely decrease of BMD un-til 35-39 year in men and 40 years in women, in studied period this parameter is quite stable.[23] Women in menopausal period we excluded. Also, the fact that childhood and prepubertal years are crucial to increase aBMD through physical activity,[24,25] and children and youngster are more inactive than several decades ago, is a general knowledge. For example, in 2010 81% of adolescents (11-17y) were insufficiently physi-cally inactive globally,[26] and we can assume, that our participants might also been quite inactive in childhood.

Most of the participants in our study were females, which is explained by the fact that vegetarianism is more popular among them.[16,27] There are number of studies focused on female aBMD parameters only,[28-30] main reason for that might be higher prevalence of osteopenia and osteoporosis among females mostly after menopause.[3,31] For exam-ple, Alswat.[32] has shown four times higher rate of osteoporosis and tend to have frac-tures 5 - 10 years earlier in ≥ 50 years' women in comparing with men. Latter is the reason we excluded menopausal women in our investigation.

Nutrition is considered an important factor that affect aBMD,[33] but there have been conflicting results if vegetarian diet benefits or harms the bone health. Similarly to other investigations,[34-36] vegetarians in our study had lower aBMD parameters. Recent study has shown that compared with omnivores vegetarians have also higher risk for bone fractures.[37] It gives reason to presume that the time period not consuming meat prod-ucts plays an important role for bone health. For example, Xie et al.[38] suggested that short-term vegetarian diets (<1 year) probably do not have any adverse effects on aBMD at least in young adults (32.7±6.5y).

Calcium is the nutrient most commonly associated with bone health and the intake below 800 mg/day increases significantly the risk of hip fracture.[39] This mineral is ac-cumulated in dairy products, so calcium deficiency is not concern among lac-to-vegetarians.[40] and it is suggested, that only vegans may have a decreased aBMD and an increased fracture risk.[18] There is also a number of plant-based foods that contain well-absorbed calcium.[8] herewith thank to the choice of calcium-rich vegetables vegans might have almost normal aBMD.[18] According to the results of our study we could as-sume that our vegetarians prefer and choose their food products wisely and by intention so the lack of calcium in their menu is not something we should consider.

Only four of our study participants used calcium as food additive (three of them with vitamin D) and their serum level was higher than non-additive users. This result might be random, but the effect of calcium to aBMD could not be manifest when serum vitamin D is insufficient. Chon et al.[41] have shown that when daily calcium intake was sufficient, risks of osteoporosis significantly increased in the LS aBMD region of the skeleton if serum D vitamin was low (<20 ng/mL). However, there are studies showing that increasing cal-cium intake from dietary sources or calcium supplements increase aBMD by a similar amount, but the increases were small (1-2%) and non-progressive, and not meaningful in reduction in fractures.[42] Therefore it was concluded that for most individuals increas-ing calcium intake is unlikely to be beneficial for higher BMD.

Vitamin D plays a key role in bone health through its promotion of calcium absorp-tion and normal mineralization of bones.[8] Vitamin D concentrations in our study groups were relatively low. It is explained by the fact that vitamin D insufficiency is highly prevalent throughout the year among Estonians, because the country is situated at a high northern latitude,[43] where even with adequate sun exposure the dermal generation of vitamin D is low or missing in winter (the time of conducting our study was early spring) and thus increases demand for dietary intake.[44] The vitamin D level in our study groups were similar indicating that vegetarian were not in vitamin D deficiency. Our veg-etarians were more aware about the importance and need for the vitamin, so they con-sumed more vitamin D additives. Also dairy products are often fortified with vitamin D and it could be an important source for lacto-vegetarians.[8] Accordingly, we cannot completely confirm the fact that vegans.[7] and lactovegetarians have lower vitamin D in-takes than omnivores as previously suggested.[8]

There are several adipokines, leptin and adiponectin among them which are secreted by adipose tissue and considered to play direct or indirect role on bone resorption.[45,46] Leptin is highly correlated with body fat mass,[47] involved in fat metabolism,[48] and may modulate bone formation by enhancing differentiation of bone stromal cells into os-teoblasts, and by inhibiting osteoclasts` generation.[49] The effects of leptin to aBMD seems to be site-specific: different skeletal sites may be differentially associated with vari-ous components of bone, since bone from different skeletal sites may differ in composition e.g., different proportions of trabecular and cortical bones.[9] There are also several stud-ies.[10,50] that found no correlations between leptin and aBMD. Different results might be because of the fact that most studies are either based on small sample size or only the cor-relation between circulating leptin and aBMD has been studied.[9] Adiponectin levels are lower among obese individuals.[51] It is found that adiponectin is inversely correlated with aBMD, and association persist even after adjusting for confounders.[50,51] There were no differences in BM and fat% in vegetarians and omnivores in our study as we be-forehand expected, and the adipokines played important role for aBMD.

There were some limitations of the study that should be acknowledged. Firstly, rela-tively small number of participants. However, the sample size of studied vegetarians was similar to previous study in this area.[19] Secondly, our study did not provide detailed food information. Finally, cross-sectional study design does not allow say anything about any cause-and-effect inference about the relationship between vegetarian diet and bone health. Regardless prior statements, dual-energy X-ray absorptiometry technology for measuring BMD is considered a golden standard for assessing skeletal health; so far number of studies have observed vegetarians on short-term vegetarian diet (for example one year). Our participants had vegetarian diet at least three years before entering the study, which is necessary to have enough time to affect bone health.

CONCLUSIONS

The results of our study suggest that regardless of the fact that most important pre-dictors for aBMD are age and gender, vegetarian diet has also impact to aBMD. As there are differences between Estonian and some other populations regarding diet, and various risk factors associated with poor bone health comprehensive understanding in local and full scale situation is required. This is basic for accurate knowledge on diet (including di-etary supplements) that should begin from early childhood. In addition, bone strengthen-ing through exercises have the potential of being an effective strategy to prevent the loss of bone minerals.

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