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THE FREQUENCY OF MEAT CONSUMPTION AND BONE MINERAL DENSITY IN FEMALE POPULATION

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Abstract
The objective was to investigate the impact of the frequency of meat consumption on bone mineral density (BMD) in females. BMD was measured in 210 females by DEXA denzitometar. For the manner of nutrition a Questionnaire was used. The females were divided into 4 age groups, and 4 subgroups: those consuming meat on daily bases; 3-5 times/week; 1-2 times/week; and no consumers. Data analysis was performed by statistical program Statistica 7.1 for Windows and SPSS Statistics 17.0. The significance was determined by p <0.05. 40-49 years old females, did not have significant differences in BMD no matter of the meat consumption frequency. 50-59 years old females who consumed meat 3-5 times weekly had significantly lower BMD compared to those with 1-2 weekly meat consumption (p<0.001) and to no consumers (p<0.01), while BMD was not significantly different between 1-2 weekly consumers and no consumers (p>0.05). 60-69 years old females on 3-5 times consumption had significantly lower BMD compared to no consumers (p<0.001) and 0.33 g/cm2 lower BMD compared to no consumers, which was significant difference (p<0,001). However, 1-2 weekly consumers had significantly (0.18 g/cm2) lower BMD compared to no consumers (p<0.01). Every day meat consumers > 69 had significantly lower BMD compared to 1-2 weekly consumers (p<0,001). Despite this, there was no significant difference in BMD between 1-2 and 3-5 weekly consumers and no consumers (p>0.05).The results suggest that frequency of consumption in age of 40-49, does not have negative impact on the BMD of females. In age of 50-59 and 60-69 frequent meat consumption significantly correlated with lower BMD. However, in age over 69, despite the findings that BMD of females on 1-2 weekly meat consumption was significantly higher than of those on everyday consumption, the correlation between BMD and meat consumption frequency was insignificant.

Keywords: Densitometry, questionnaire, age groups, meat consumption.

Introduction

Meat and its numerous species and products are important part of human diet and culture worldwide as a source of lipids, proteins with high biological capacity, trace elements and vitamins (Zang et al. 2010,Wyness et al. 2013). High levels of SFA were linked with cardiovascular diseases in the past, but resent data of Siri-Tarino et al. (2010), found no significant evidence for SFA and cardiovascular diseases association. Losses in high quality protein, especially in older adults, cause sarcopenia and sarcopenic obesity by replacing lost skeletal muscle into fat (Paddon-Jones and Leidy 2014). It has been demonstrated that collagen has a positive influence on the delivery and bioactivity of bone morphogenic protein-2 and ectopic bone formation, enhancing bone healing (Bhakta et al. 2013). Other beneficial effects on health by meat peptides include antihypertensive, antioxidant, antithrombotic, immunomodulatory, anticancer, and antimicrobial activities (Di Bernardini et al. 2011). However, what is the potential role of proteins, amino acids and peptides from meat and other type of food in bone health and bone mineral density (BMD) maintenance through aging; whether recommended protein intakes should be increased in order to prevent or reduce osteoporosis and sarcopenia is the issue for scientists, clinitians, nutritionists and public health professionals. Bone health is multifactorial issue, it depends on genetics, BMD peak, sun exposure, lifestyle and exercise (Kanis, 2013). Amount and type of protein influences bone health. Protein has been identified as being both detrimental and beneficial to bone health, depending on a variety of factors, including the level of protein in the diet, the protein source, calcium intake, weight loss, and the acid/base balance of the diet. Loss of bone mass (osteopenia) or its heavier form (osteoporosis) and loss of muscle mass (sarcopenia) that occur with age are closely related and track together over the life span. Calcium and protein intake interact constructively to affect bone health. Intakes of both calcium and protein must be adequate to fully realize the benefit of each nutrient on bone. Concerns about dietary protein increasing urinary calcium appear to be offset by increases in absorption. Likewise, concerns about the impact of protein on acid production appear to be minor compared with the alkalinizing effects of fruits and vegetables. Perhaps more concern should be focused on increasing fruit and vegetable intake rather than reducing protein sources (Heaney and Layman 2008). Clinical studies do not support the idea that animal protein has a detrimental effect on bone health or that vegetable-based proteins are better for bone health (Kerstetter et al. 2003). There is increasing evidence that a higher protein intake may have beneficial effects on BMD (Heaney, 2001, Rapuri, 2003), and may reduce bone loss in patients with recent hip fracture (Schurch et al. 1998), particularly in the elderly (Hannan, 2000), and in those consuming adequate calcium levels. The researchers identified a positive association between dietary protein intake and change in bone mass density in those with the highest intake of protein who were supplemented with calcium and vitamin D. There was no benefit from supplementation among those with lower intakes of protein. (Dawson-Huges and Harris 2002, Whiting et al. 2002, Dawson-Hughes, 2003). The higher protein content in the calcium-rich DASH diet and sodium reduction improve the markers of bone turnover and calcium metabolism in adults (Lin et al. 2003). While protein seems to have a direct anabolic effect on bone, the relation between protein intake and bone is further complicated by the potential negative effect of overall dietary acid-base balance. A Western-type diet has been reported to be associated with osteoporosis and urinary calcium loss (Mauer et al. 2003). Urinary calcium has been found to be increased with acid-forming foods, such as meat, fish, eggs, and cereal, and negatively associated with plant foods and is likely determined by the acid-base status of the total diet. Bone loss may be attributable, in part, to the mobilization of skeletal salts to balance the endogenous acid generated from acid-forming foods (Spence and Weaver 2003). Moreover, it has been suggested that animal protein–based diets might have a greater negative effect on skeletal health than do vegetable-based diets (Sellimeyer et al. 2001) because dietary animal protein induces a greater increase in urinary calcium excretion than vegetable protein. In a large group of middle-aged and elderly women in China, urinary excretion of calcium was correlated positively with intake of animal protein (Zhao et al. 1993). Several studies examining the effect of meat have found no effect on either bone mineral density (BMD) or BMD markers. A 16-wk randomized crossover study of healthy postmenopausal women found that consuming a high-meat diet (297 g/d of meat), providing 117 g of protein, did not adversely affect urinary calcium excretion, calcium retention, or clinical indicators of bone formation and resorption compared with a low-meat diet (45 g/d of meat and 68 g of protein) (Roughead et al. 2003). Considering the contradictory findings in literature about the effect of animal proteins on BMD of female and male population; no data about the impact of nutrition on BMD; nor specific clinical trials conducted about the association of meat consumption and BMD of Macedonian population, the focus of this study was to investigate the link between the frequency of meat consumption and BMD in female population of Republic of Macedonia, and possibly answer to the question whether frequent meat consumption, might be recommended or denied for prevention of osteoporosis in females or in some of the female age subgroups.

Material and methods

BMD (in g/cm^2 and t-score) at lumbar spine was measured by Dual Energy X-ray Absorptiometry (DEXA) in 210 females. For the manner of nutrition a Questionnaire was used. The females were divided into 4 age groups (40-49; 50-59; 60-69; and > 69 years old), and 4 subgroups according to meat intake: those consuming meat on daily bases; 3-5times/week (t/w); 1-2 t/w; and no consumers (used as reference data). Data analysis for the whole female group no matter of age, and for every age subgroup separately was performed by statistical program Statistic 7.1 for Windows and SPSS Statistics 17.0. Numerical data (age), were analyzed by descriptive statistics (Mean; Std. Deviation; ±95. 00% CI; Minimum; Maximum). Data distribution was tested with Kolmogorov-Smirnov test; Lilliefors test; and Shapiro-Wilks test (p); BMD as dependent phenomenon; and age and frequency of meat consumption as independent phenomena ratio was tested by Multiple Regression analysis (R). The significance was determined by $p < 0.05$.

Results and discussion

Females categorized in 4 subgroups according to the meat consumption frequency (MCF) were analyzed in context of number/percentage (n/%) of females within a frequency subgroup who had normal level (NL) of BMD according to t-score values which are compatible with their age (t-score of 1 to -1); osteopenia (t-score of -1 to -2.5); osteoporosis (t-score bellow -2.5 to -3.5): and a heavy form (HF) of osteoporosis prone to spontaneous fractures (lower than -3.5 to -5) -Table 1 below.

Table 1. BMD level related to meat consumption frequency

MCF	NL		Osteopenia		Osteopor.		HF		Total	
	n	%	n	%	n	%	n	%		
Everyday	3	25	3	25	3	25	3	25	12	100
3-5 t/ w	14	16.9	25	30.1	28	33.7	16	19.3	83	100
1-2 t/w	22	22	47	47	12	12	19	19	100	100
No consum	6	40	3	20	6	40		/	15	100

Results showed that no meat consumers had the highest percentage of normal BMD level compared to females on everyday consumption, 3-5 t/w and 1-2 t/w consumption (40 % v. 25% v. 16.9% v. 22%); as well as they had the lowest percentage of osteopenia compared to other consumers subgroups (20% v. 25% v. 30.1% v. 47%). However, the percentage of osteoporosis occurrence was highest in no consumers subgroup but there was no evidence of heavy form of osteoporosis. The highest percentage of heavy form of osteoporosis was found in everyday meat consumes (25%). When we summarized the percentage of osteoporosis occurrence and its heavy form, the highest percentage was found in 3-5 t/w consumers. Therefore, these results suggest that frequent meat consumption on everyday basis and 3-5t/w does not prevent occurrence of osteoporosis and its heavy forms, thus the fracture risk. However, it should be noted that the level of calcium intake was not investigated in those females. Feskanich et al (1996) reported that the risk of forearm fractures with a high intake of protein (> 90 g/d) is exacerbated by a low calcium intake (< 541 mg/d). Similar observations were made by Meyer et al (1997) who reported an elevated risk of fracture in elderly men and women with a high intake of protein from non dairy sources and calcium intakes < 400 mg/d. However, both authors did not find a positive effect of high calcium and high protein intakes on fracture risk. The results (Table 2) are related to investigated ratio between BMD in g/cm^2 as dependent phenomenon; and meat consumption frequency (every day, 3-5 t/w, 1-2 t/w and no consumers) like independent phenomena.

Table 2. BMD & Meat Consumption Frequency

MCF	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			1.14	0.03	32.87	0.000
Everyday	-0.43	0.07	-0.38	0.06	-6.17	0.000
3-5 t/w	-0.82	0.11	-0.29	0.04	-7.55	0.000
1-2 t/w	-0.49	0.11	-0.17	0.04	-4.45	0.000

Moderately strong correlation was determined for the ratio investigated for R=0.53 and p<0.001(p=0.000). No consumer female subgroup was used as referent category. 3-5 t/w meat consumption had the highest influence on BMD (Beta=-0.82), weaker in 1-2 t/w consumer subgroup (Beta=-0.49), while the weakest influence had meat intake on everyday basis (Beta=-0.43). Females on everyday meat consumption had significantly lower BMD for p<0,001(p=0,000) i.e. 0.38 g/cm^2 (B=-0.38) lower BMD compared to no consumers as well as all other MCF subgroups showed significantly lower BMD compared to no consumers. These results suggest that neither frequent nor rear consumption of meat was beneficial for BMD. Moreover, those females had significantly lower BMD compared to no consumer. Therefore, protein intake of those females might be insufficient from other food protein source than meat, which could be more beneficial for BMD, or they had lower calcium intake and higher calcium loss through urinary excretion. These results go in favor of the finding of Mauer et al (2003) who determined that Western-type diet (high in animal protein and fat) was associated with osteoporosis and urinary calcium loss. Spencer and Weaver (2003) found that increased urinary calcium was linked to acid-forming foods, such as meat, fish, eggs, and cereal, and negatively associated with plant foods and is likely determined by the acid-base status of the total diet. Moreover, it has been suggested that animal protein–based diets might have a greater negative effect on skeletal health than do vegetable-based diets (Sellimeyer et al. 2001) because dietary animal protein induces a greater increase in urinary calcium excretion than vegetable protein. Results of BMD and its correlation with MCF for each age group separately (40-49, 50-59, 60-69 and above 69) are presented in further tables bellow and they slightly differ from previously mentioned age no stratified results.

Females aged 40-49. No significant difference in average value of BMD (g/cm^2), (H=2.26) and p>0.05(p=0.52), related to MCF, was found. There was no significant difference (p>0.05) in the results of multiple comparison of p values related to BMD of females and MCF. The results presented in Table 3. bellow are related to the ratio between BMD in g/cm^2 like dependent phenomenon, and MCF on every day basis, 3-5 t/w and 1-2 t/w consumption; and the age of females like independent phenomena. Strong insignificant correlation for R=0.60 and p>0.05(p=0.48) was found. No consumers were used as referent category. The highest influence to this ratio had 3-5 t/w consumption (Beta=-0.51), than everyday consumption (Beta=-0.41), age had even weaker influence (Beta=-0.29), while the weakest influence had 1-2 t/w meat consumption (Beta=-0.23). Under condition of unchanged other parameters, females on 3-5 t/w meat consumption had averagely 0.21g/cm^2 (B=-0.21) insignificantly lower BMD than no consumers p> 0. 05 (p= 0. 42).The results between the other MCF and no consumers were similar.

Table 3. BMD/ Age & Meat Consumption Frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			2.29	1.27	1.80	0.11
Age	-0.29	0.36	-0.02	0.03	-0.81	0.44
Everyday	-0.41	0.44	-0.30	0.32	-0.93	0.38
3-5 t/w	-0.51	0.60	-0.21	0.24	-0.85	0.42
1-2 t/w	-0.23	0.55	-0.10	0.24	-0.41	0.70

All results suggest that MCF insignificantly correlated with BMD of females aged 40-49. Frequent meat consumption was associated with insignificantly lower BMD compared to 1-2 t/w meat

consumption or to no consumers BMD. Data about the link between MCF and BMD, and the range of protein intakes for optimizing bone health among premenopausal women is unclear. Kerstetter et al (2005) studied protein-induced effects on net bone balance in young women and showed increased gastrointestinal calcium absorption as well as insignificant trend toward decreased bone resorption with a high-protein diet. Data from one large population-based cohort study performed by Beasley et al (2010) provided evidence that protein intake in the upper range of typical consumption in the United States did not affect bone mass of premenopausal women negatively.
Females aged 50-59. There was significant difference for H=31.72 and p < 0. 00 1(p=0.000) of females BMD (g/cm^2) at age of 50-59 on 3-5 t/w consumption and those on 1-2 t/w consumption and no consumers (0.84 v. 1.01 v. 1.117). For p<0.001(p=0.000) females on 3-5 t/w meat consumption had significantly lower BMD compared to those on 1-2 t/w consumption and to no consumers for p<0.01(p=0.003). However, there was no significant BMD difference for p>0.05 (p=0.081) between no consumers and 1-2 t/w consumers.
The results presented in Table 4. bellow are related to the ratio between BMD in g/cm^2, and MCF (3-5 t/w and 1-2 t/w consumption and no consumption); and the age of females. Moderately strong significant correlation for R=0.67 and p< 0.001 (p=0.000) was found for this ratio. No consumer subgroup was a referent category. The highest influence to BMD values had 3-5 t/w consumption (Beta=-1.09), than 1-2 t/w consumption (Beta=-0.51), and age the weakest one (Beta=-0.11). Under unchanged other parameters, females on 3-5 t/w meat consumption had significantly lower BMD за p<0.001(p=0.000), compared to no consumers for 0.33 g/cm^2 (B=-0.33); as well as females on 1-2 t/w meat consumption for p<0.05 (p=0.03) i.e. 0.15 g/cm^2 (B=-0.15) lower BMD compared to no consumers, under unchanged other parameters; For each year increasing in age BMD was insignificantly lower for averagely 0,006 g/cm^2 (B=-0.006).

Table 4. BMD/ Age and Meat consumption frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			1.48	0.29	5.06	0.000
Age	-0.11	0.10	-0.006	0.01	-1.10	0.27
3-5 t/w	-1.09	0.23	-0.33	0.07	-4.74	0.000
1-2 t/w	-0.51	0.23	-0.15	0.07	-2.20	0.03

The results of postmenopausal women at 50-59 years age strongly suggest that frequent meat consumption correlated with lower BMD. Frequent consumption of meat (3-5 t/w) was followed with higher percentage of osteoporosis of the spine detected by densitometry compared to those on 1-2 t/w consumption (76.47% v. 26.92%). The calcium intake was not measured in this study, but these results are consistent with the finding of Fescanhich et al (1996) who suggested that, as a result of increased urinary calcium excretion with high protein intake, there was an increased risk of fractures or osteoporosis. May be females in our study did not consume enough vegetables and fruits in parallel with high meat consumption. Barzel et Massey (1988) suggest that excess in dietary protein can adversely affect the bone because a diet, which is high in protein and low in fruits and vegetables, generates a large amount of acid, mainly as sulfates and phosphates. The kidneys respond to this dietary acid challenge with net acid excretion. Concurrently, the skeleton supplies buffer by active resorption of bone. Calciuria is directly related to net acid excretion. On contrary, Heaney and Layman (2008) suggested that higher protein diets were actually associated with greater bone mass and fewer fractures when calcium intake was adequate.
Females aged 60-69. For H=13.67 and p<0.01(p=0.001), significant BMD (g/cm^2) difference in females aged 60-69 on 3-5 t/w meat consumption compared to those on 1-2 t/w consumption and no consumers was found (0.83 v. 0.97 v.1.15). Females no consumers, had significantly higher BMD for p<0,001(p=0,000) compared to BMD of 3-5 t/w consumers, while the difference of BMD between no consumers and 1-2 t/w meat consumers was insignificant for p>0.05(p=0.14). The results shown on Table 5. bellow are related to BMD; and MCF (3-5 t/w, 1-2 t/w & no consumers) & age of the females. Moderately strong significant correlation was found for this ratio for R=0.56 and

p<0.001(p=0.0006). No consumers subgroup was used as referent data. The highest influence to this ratio had 3-5 t/w meat consumption (Beta=-0.70), than 1-2 t/w consumption, and the weakest one had the females age (Beta=0.14). Under unchanged other parameters, females on 3-5 times/week meat consumption had 0.33 g/cm^2 (B=-0.33) lower BMD compared to no consumers, which was significant difference for p<0.001(p=0.000); Females on 1-2 t/w meat consumption had approximately 0.18 g/cm^2 (B=-0.18) lower BMD compared to no consumers, which was significant difference as well for p<0.01(p=0.005); and with each increasing of the age for one year, BMD was insignificantly higher for p>0.05(p=0.26).

Table 5. BMD / Age & Meat consumption frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			0.50	0.57	0.88	0.38
Age	0.14	0.12	0.01	0.01	1.14	0.26
3-5 t/w	-0.70	0.15	-0.33	0.07	-4.56	0.000
1-2 t/w	-0.44	0.15	-0.18	0.06	-2.96	0.005

Females at age of 60-69 on 3-5 t/w meat consumption had higher percentage of osteoporosis detected compared to those females on 1-2 t/w consumption (51% v. 42.85%). These results together with the previously mentioned results suggest that frequent meat consumption was not beneficial for BMD of older women above 60. It has to be noted that the quantity of meat intake could not be precisely estimated due to subjectivity of the participants, thus it was not known whether these females had abundant protein intake, accompanied with low vegetable intake. The calcium intake was not considered as well. It is interesting that these results are more consistent with older research findings. Sellmeyer et al (2001) and Dawson-Huges et al (1990) concluded that elderly women who had relatively high dietary animal protein intakes and limited vegetable protein intakes had more rapid bone loss at the femoral neck and a greater risk of hip fracture than did those with lower dietary animal protein intakes and higher vegetable protein intakes, thus suggesting that abundant dietary protein may also be harmful in older persons. Barzel and Massey (1998) noted that a high intake of dietary protein may adversely affect bone through effects on calcium excretion and acid-base metabolism.

Females aged over 69. For H=15.99 and p<0.01(p=0.001) a significant difference of BMD (g/cm^2) in females aged above 69 related to MCF on everyday basis compared to 3-5 t/w, 1-2 t/w and no consumers ((M=0.73 v. 0.84 v. 0.92 v. 0.95). In this age subgroup only the females above 69 years age who were on 1-2 t/w meat consumption had significantly higher BMD for p<0.01(p=0.002) compared to those who were on every day consumption. Therefore these results suggest that 1-2 t/w meat consumption is not harmful for BMD of females above 69 years old. Results presented on Table 6. are related to the ratio between BMD (g/cm^2) like dependent phenomenon and meat consumption frequency; and females age as independent phenomena. Moderately insignificant correlation for this ratio was found for R=0.44 and p<0.01(p=0.002). No consumer subgroup was used as referent data. The highest influence to this ratio had everyday meat consumption (Beta=-0.42), than 3-5 t/w consumption (Beta=-0.37), the age (Beta=0.08), while 1-2 t/w consumption had the weakest influence (Beta=-0.07). Under condition of unchanged other parameters, females on everyday meat consumption had significantly lower BMD for p<0.05 (p=0.04), i.e. 0,21 g/cm^2 (B=-0.21) lower BMD as compared to no consumers; 3-5 times/week meat consumers had averagely 0.10 g/cm^2 (B=-0.10) lower BMD compared to no consumers, which was insignificant difference for p>0.05(p=0.27; With each increasing in age for one year BMD was increased for averagely 0.003 g/cm^2 (B=0.003), insignificantly for p>0.05 (p=0.43); and 1-2 times/week meat consumers had averagely 0.02 g/cm^2 (B=-0.02) lower BMD compared to no consumers, insignificantly for p>0.05(p=0.84).

Table 6. BMD / Age & Meat Consumption Frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			0.75	0.27	2.76	0.007
Age	0.08	0.10	0.003	0.003	0.79	0.43
Everyday	-0.42	0.21	-0.21	0.10	-2.05	0.04
3-5 t/w	-0.37	0.33	-0.10	0.09	-1.11	0.27
1-2 t/w	-0.07	0.34	-0.02	0.09	-0.20	0.84

Females at age over 69 on 3-5 t/w meat consumption had higher percentage of osteoporosis detected compared to those females on 1-2 t/w consumption (58.33% v. 37.04%). The results of the females aged over 69 strongly suggest that frequent meat consumption on every day basis or 3-5 t/w is not beneficial for their BMD which is compatible with the findings of 60-69 years old subgroup analyzed previously and with the findings of Sellmeyer et al (2001), Dawson-Hughes et al (1990), and Barzel and Massey (1998).

Conclusions

Based upon the results of this study, frequent meat consumption on everyday basis and 3-5 t/w consumption was followed with higher incidence of osteoporosis, and significantly lower BMD compared to 1-2 t/w consumers and no consumers, thus it is not recommendable for prevention of osteoporosis, when females were not categorized in age subgroups. The results were slightly different when females were divided in subgroups according to their age: 40-49; 50-59; 60-69: and >69 years old. MCF was insignificantly correlated to BMD of females aged 40-49 suggesting that other factors like estrogens levels, physical activity, and lifestyle might played more important role rather than meat consumption. However, in age of 50-59 and 60-69 there was a moderately strong significant correlation between BMD and MCF, and higher incidence of osteoporosis, suggesting that frequent meat consumption had negative impact on BMD of these females. In the age over 69, despite the findings in this study of significantly higher BMD in 1-2 t/w meat consumption compared to BMD of females on everyday consumption, the influence of MCF on BMD of females over 69 age was insignificant, suggesting that other factors might have higher positive or negative impact on their BMD. Therefore we recommend avoidance of meat consumption or its decrease to 1-2 t/w combined with more fruit and vegetables with adequate food derived calcium intake in elderly population.

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