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VEGETABLE CONSUMPTION AND BONE MINERAL DENSITY IN FEMALES

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Abstract

Vegetables consisted of wide spectrum of nutrients are linked with lower bone turnover, especially bone resorption. For osteoporosis, the evidence from a combination of observational, experimental, clinical, and intervention studies strongly suggest a positive link between vegetable consumption and indexes of bone health. Objective of our research was to investigate the impact of the frequency of vegetable 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 vegetables on: daily bases; 3 - 5 times/week; 1 - 2 times/week; and non-consumers. Data analysis was performed by statistical program Statistica 7.1 for Windows and SPSS Statistics 17.0. The significance was determined by $p < 0.05$.

0 - 49 and above 69 years old females, did not have significant differences in BMD no matter of vegetable consumption. 50 - 59 years and 60 - 69 years old females on everyday consumption had significantly higher BMD compared to 3 - 5 and 1 - 2 weekly consumers ($p < 0.001$). However BMD between 3 - 5 and 1 - 2 weekly consumers did not differ significantly. The highest influence on BMD/consumption/age ratio had every day consumption than 3 - 5 weekly consumption, while age had the weakest influence. Every day consumers, no matter of age, had a lower percentage of osteoporosis and heavy forms of osteoporosis compared to no consumers (10.9% v. 50% and 17.2% v. 25%). Normal level of BMD was not identified in no consumers group.

Everyday consumption of vegetables has significantly positive impact on BMD of 50 - 59 and 60 - 69 years old females, and it can be considered as a natural option for Osteoporosis prevention, as well as protection factor of critical BMD lowering to fracture threshold. However in 40 - 49 and above 69 years of age the frequency of vegetable consumption have no such influence.

Key words: *Vegetables, Bone Mineral Density, Osteoporosis, Females, Prevention.*

1. Introduction

Consumption of vegetables is a significant part of a healthy diet, because they contain a wide spectrum of nutrients in small and large quantities, such as: minerals, vitamins, phytochemicals (flavonoids), antioxidants and fibers. Several studies suggest that a diet rich in fruits and vegetables (FV) can: prevent bone mineral density (BMD) reduction, osteoporosis occurrence and its progression [1]; reduce a risk of coronary heart disease [2]; stroke [3]; obesity [4]; most type of cancer [5]; and cataract formation [6].

Osteoporosis is a progressive inflammation related systemic-skeleton chronic disease, which is characterized by bone micro architecture disorder, low bone mineral mass and density, leading to bone fragility, disability and fracture diathesis. This nowadays civilization disease has a higher incidence in western European countries, in postmenopausal women (postmenopausal osteoporosis) and in elder population (senile osteoporosis) [7]. The number of over 100 million people worldwide, 22 million females and 5.5 million males in EU, suggests epidemic character of this disease [8].

Scientific evidence indicates that diet plays a significant role in the multi factorial etiology of osteoporosis beside genetics, BMD peak i.e. the maximum BMD which is reached at age of 30, sun exposure (maintaining vitamin D levels), lifestyle and exercise [9]. Dietary approaches to stop hypertension (DASH) intervention study strongly suggest that a diet high in fruit and vegetables accompanied with sodium reduction significantly improve markers of bone turnover and calcium metabolism in adults [10]. However, fruit and vegetable consumption is influenced by gender, age, income, education, family origin and socio demographic factors [11,12].

Population-based studies have also concluded that FV intake is associated with healthy bones and adequate bone mineral density [13,14]. Diet rich in vegetables and fruits is considered as beneficial factor for BMD and should be incorporated in public health policy recommendations [15]. The high potassium consumption [16] and favorable sodium/potassium ratio which reduces dietary acidity [17] and consequently reduces hypercalciuria [18], phytochemicals [19], and fibers [20] through vegetables consumption, accompanied with decreased sodium intake [21] are considered as possible factors responsible for the positive impact on bone mineral density.

Based upon observational intervention and experimental studies, investigations at molecular level, and clinical trials, there are proposed mechanisms trying to elucidate the manner of vegetable bioactive components action in maintaining bone integrity.

The acid-base hypothesis is supported by the findings of New *et al.,* [22], who suggests that metabolic breakdown products from Western diets (high in grains/protein, low in fruit/vegetables) contribute to increased bone loss, due to bone calcium mobilization in order to balance the metabolic acidosis in the organism. It is believed that fruits and vegetables are rich in precursors to bicarbonate ions which serve to buffer acids in the body, therefore inadequate bicarbonate ions concentration will initiate the body to mobilize alkaline calcium salts from the bone in order to neutralize acids consumed in the diet and those generated by metabolism. Increased consumption of fruits and vegetables reduces the net acid content of the diet and may preserve calcium in bones which might otherwise be mobilized to maintain pH homeostasis [23].

Mühlbauer *et al.,* [24] used urinary excretion of [^{3}H]-tetracycline following pre-labeling of rat bones to measure bone resorption in response to serial exposures to each food type comparing it with onion treated group as positive refference (because of onion's strong effect to inhibit bone resorption). Half of the food items tested showed bone turnover inhibitory capacity.

A doubt about postulated acid-base mechanisms of plant food action and extra potassium benefit is raised by the finding of Mühlbauer *et al.,* [25], who suggested that bone resorption suppression activity was not altered after correcting for the alkaline load of a mixed plant diet with potassium citrate. A meta-analysis of human studies showed no relation between dietary acid load and osteoporotic bone disease as well [26], nor did potassium intake influenced calcium balance [27].

Flavonoids, found in a wide diversity of plant foods from fruits and vegetables, herbs and spices, essential oils, and beverages, have the most potential of dietary components for promotion of bone health beyond calcium and vitamin D. Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8,000 individual compounds known. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions. However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and scavenge free radicals thus protect against inflammation related chronic diseases such as osteoporosis [28]. Flavonoids as a class of phytochemicals have been considered as bone loss protectors, likely related in part to their anti-inflammatory properties.

In the light of recent studies, we are discovering, that apart from quenching oxygen radicals, more subtle mechanism exist by which natural compounds act through gene expression and cell signaling pathways to activate enzymes that eliminate the mediators of inflammation - oxygen radicals [29].

In a large observational study in Scotland, total flavonoid intake was positively associated with BMD and increase in BMD of the spine and hip. Flavonoids in the catechin family had the strongest association with bone [30]. The relationship between flavonoid intake and bone health was stronger than what has been reported previously for fruits and vegetables. Moreover, no single mechanism has been put forward for flavonoid actions on bone.

Recent research has identified molecular targets in cell signaling pathways that affected bone. Some flavonoids appear to have bone anabolic activity, which has exciting implications beyond merely inhibiting bone resorption through suppressing osteoclast activation. Bioactive flavonoids are being assessed for properties beyond their chemical anti-oxidant capacity, including anti-inflammatory actions. Some have been reported to enhance bone formation and to inhibit bone resorption through their action on cell signaling pathways that influence osteoblast and osteoclast differentiation. Increased phytochemical intake may reduce bone loss associated with ageing, due to changed ratio between proinflammatory cytokines such as Tumor Necrosis Factor (TNF), Interleukin (IL)-1 and IL-17 which are triggers of osteoclast activation; and suppressor cytokines IL-12, IL-18, IL-33 and Interferons (IFN) which are strong suppressors of osteoclast differentiation and inhibit bone loss, in favor of the latter ones [31].

In addition, Mühlbauer *et al.,* [25, 32] threw more light on bone metabolism modulation of phytochemicals at molecular level. He concluded that bone resorption was reduced in animals fed particulary with vegetables/herbs/fruit, suggesting that bone resorption inhibiting properties (BRIPs) are due to pharmacologically-active phytochemicals rather than their base excess. Huang *et al.,* [33], concluded that onion decreased the ovariectomy-induced osteopenia in young adult rats. Rutin was identified initially as the bioactive compound, but later it was reported that the likely bioactive constituent in onion using bioactivity directed fractionation is Γ-L-glutamyl peptide [34]. Matheson *et al.,* [35], confirmed the association between onion consumption and BMD in perimenopausal and postmenopausal women. Other vegetables in the onion family, including garlic and leek, and members of the celeriac family, including cabbage, lettuce, and green beans, were also effective in suppressing bone resorption [19].

Several studies concluded that higher intakes of fruits and vegetables have been associated with improved BMD or bone mineral content in female population [14, 22 and 36]. It seems that the magnitude of flavonoid effect appears to depend on its source, context of exposure (pure compound vs. diet), route of exposure, and age and sex of the target population. Further observational, experimental, molecular, clinical, randomized, and intervention studies should bring us more clarified insights of the mechanisms by which bioactive FV substances manifest there positive effects on bone as well as which human population sex and age category have the highest benefit. However, the effects of various rich flavonoid vegetables intake in prospective long period follow up studies is hard to conduct in humans.

Based upon all previously mentioned studies about the beneficial effect of vegetables and fruits on BMD and overall human health it is strongly suggested that FV should be a significant part of world public health policy and recommendations. Education of population about the beneficial role of fruits and vegetables on human health should be priority of each country, especially because this type of food is considered as a potential natural answer of osteoporosis prevention and other chronic diseases.

Considering that no evidence data exist about the impact of nutrition on BMD, nor specific clinical trials conducted about the association of vegetable consumption and BMD of Macedonian population, the aim of this study was to investigate the link between the frequency of vegetable consumption and BMD in female population of Republic of Macedonia, and possibly answer to the question whether frequent vegetable consumption, might be recommended for prevention of osteoporosis.

2. Materials and Methods

BMD (in g/cm^2 and t-score) at lumbar spine was measured by Dual Energy X-ray Absorpciometry (DEXA) denzitometar 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: those consuming vegetables on daily bases; 3 - 5 times/week; 1 - 2 times/week; and no consumers (used as reference data). Data analysis was performed by statistical program Statistics 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 intensity of vegetable consumption as independent phenomena ratio was tested by Multiple Regression analysis (R). The significance was determined by $p < 0.05$.

3. Results and Discussion

3.1 BMD & vegetable consumption frequency

Females categorized in 4 subgroups according to the frequency of vegetable consumption were analyzed in context of number/percentage (%) of females within a frequency subgroup who had normal level 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 lower than -2.5 to -3.5): and a heavy form of osteoporosis prone to spontaneous fractures (lower than -3.5 to -5) presented in Table 1 below.

Everyday consumers had higher percentage of participants with a normal level of BMD, compared to 3 - 5 times/per week, 1 - 2 times/per week and no consumers (26.6 v. 20 v. 19.5 v. none). Normal level of BMD was not present in the group of no consumers, but the number of participants was small. Everyday consumers had osteoporosis identified in lower percentage compared to 3 - 5 times/week, 1 - 2 times/week and no consumers (10.9 v. 16.7 v. 36.6 v. 25 respectively). Summarizing the percentage of participants with osteoporosis and heavy form of osteoporosis, everyday consumers had much lower incidence of these disorders when compared to other three subgroups (28.1 v. 36.7 v. 53.7 v. 75) which suggests that 1 - 2 times consumers and no consumers are highly prone to osteoporosis and its heavy form.

These results are compatible with the finding of Qiu *et al.,* [36] who suggested that greater Intake of fruit and vegetables is associated with greater BMD and lower osteoporosis risk. Høstmark *et al.,* [37], reported an association between hip fractures and a low intake of vegetables and fruits.

The results related to investigated ratio between BMD in g/cm^2 as dependent phenomenon and vegetable consummation frequency (every day, 3 - 5 times/week, 1 - 2 times/week and no consumers) like independent phenomena are presented in Table 2.

Medium significant correlation of the ratio investigated for R = 0.47 and $p < 0.001$ (p = 0.000) was detected. No consumer females were used as referent group. The highest influence to the ratio investigated did have everyday consumption, than 3-5 times/week consumption, while the weakest influence did have 1 - 2 times/week consumption (Beta - 0.56 v. 0.18 v. 0.06 respectively).

Females on everyday vegetable consumption had 0.21 g/cm^2 (B = 0.21) in average, higher BMD compared to no consumers, which was significant difference - $p < 0.01$ (p = 0.008). This result is compatible with the finding of Ebrahimov *et al.,* [38] who suggested that high consumption of vegetables positively affected bone mineral density in rural women in Iran, and daily intake of at least 1.5 servings of vegetables was recommended for osteoporosis prevention.

However, our results showed that BMD among other subgroups of vegetable consumers less than everyday did not differ significantly. Namely, 3 - 5 times/week consumers had no significant BMD difference - $p > 0.05$ (p = 0.38), i.e. in average they had 0.07 g/cm^2 (B = 0.07) higher BMD compared to no consumers. No significant difference in BMD - $p > 0.05$ (p = 0.79) was detected between 1 - 2 times/week consumers and no consumers as well (B = 0.02). Høstmark *et al.,* [37, 39] suggested that rare intake of fruits and vegetables might have a negative influence on the skeleton, but the mechanisms involved are not clarified.

Results of BMD and its correlation with vegetable consumption frequency for each age group separately (40 - 49, 50 - 59, 60 - 69 and above 69 years old) are presented in further tables bellow and they slightly differ from previously mentioned results, when BMD and its association with consumer frequency was analyzed for the whole investigated female group no matter of age.

Table 1. Level of BMD in relation with vegetable consumption frequency

Vegetable consumption frequency	Level of BMD									
	Normal BMD		Osteopenia		Osteoporosis		Heavy form of Osteoporosis		Total	
	n.	%*	n.	%	n.	%	n.	%	n.	%
Everyday	17	26.6	29	45.3	7	10.9	11	17.2	64	100
3 - 5 times/week	12	20	26	43.3	10	16,7	12	20	60	100
1 - 2 times/week	16	19.5	22	26.8	30	36.6	14	17.1	82	100
No consumers		/	1	25	2	50	1	25	4	100

* number and % of participants.

Table 2. BMD and vegetable consumption frequency

Vegetable consumption	Beta	Std.Err. of Beta	B	Std.Err. of B	t (206)	p-level
Intercept			0.84	0.08	10.99	0.000
Everyday	0.56	0.21	0.21	0.08	2.67	0.008
3 - 5 times/week	0.18	0.20	0.07	0.08	0.89	0.38
1 - 2 times/week	0.06	0.22	0.02	0.08	0.26	0.79

3.2 Females at age of 40 - 49

There was no significant difference for N = 5.99 and p > 0.05 (p = 0.11) between BMD in g/cm^2 of everyday consumers and 3 - 5 times/week, as well as between 1 - 2 times/week and no consumers (1.28 v. 1.11 v. 0.96 v. 0.86 respectively) -Table 3.

Multiple p values comparison did not show significant difference (p > 0.05) of BMD between everyday consumers and other consumers subgroups (Table 4).

The results shown on Table 5 were related to the ratio between BMD in g/cm^2 as dependent phenomenon and the vegetable consumption frequency subgroups (everyday, 3 - 5 times/week, 1 - 2 times/week and no consumers); and the age of females as independent phenomena. Strong not significant correlation, R = 0.70 and p > 0.05 (p = 0.26) for this ratio was detected. A subgroup of no consumers was a referent category. Everyday consumption had the highest, but still insignificant influence to this ratio (Beta = 0.70), 3 - 5 times/week vegetable consumption (Beta = 0.57) showed weaker influence as well as the age (Beta = -0.33), while 1 - 2 times/week vegetable consumption had the weakest influence (Beta = 0.30).

In condition when other parameters were not changed: everyday vegetable consumers had insignificantly p > 0.05 (p = 0.15) higher BMD for 0.38 g/cm^2, (B = 0.38) compared to no consumers as well as 3 - 5 times/week consumers had insignificantly - p > 0.05 (p = 0.30) higher BMD for in average of 0.23 g/cm^2 (B = 0.23) compared to no consumers; Similar results were obtained from 1 - 2 times/week consumers who had insignificant 0.3 g/cm^2 (B = 0.13) higher BMD compared to no consumers; and each increasing of age for one year was followed by insignificant decrease - p > 0.05 (p = 0.30) of BMD for 0.03 g/cm^2 (B = -0.03).

This group of females is in premenopausal period having a regular menstrual period, thus they are still protected with estrogen, although estrogen level might be fluctuating. Estrogen plays crucial role in women's bone health. BMD-mediated causal relationship between estrogen and fracture in women has been well established. Deficiency of estrogen leads to increased bone loss, lower bone mineral density (BMD), and increased fracture risk [40]. In women with reduced bone density and/or an existing fracture, treatment with estrogen could reduce bone loss, and lower fracture risk [41]. The finding of Ho-Pham *et al.,* [42] suggested that estradiol, but not testosterone, was a significant determinant of bone mineral density in Asian women, but relative contributions of estradiol to the between-individual variation in bone density was likely to be modest for men.

Therefore, we suppose that 40 - 49 years old females under physiological estrogen levels protection might have greater consumption of fruits and milk and dairy products, greater physical activity or may posses other factors which contribute to BMD maintenance. In systematic review of Hamidi *et al.,* [43] bone health and its association with FV intake in women aged ≥ 45 years was investigated, but based on limited evidence, the benefits of FV on BMD remained unclear.

Table 3. Vegetables consumption frequency and BMD

Vegetable consumption frequency	Code	Valid N	Sum of Ranks
Everyday	1	2	21.00
3-5 times/week	2	5	38.00
1-2 times/week	3	4	16.50
No consumers	4	1	2.50

Table 4. Multiple comparisons of p values

Vegetable consumption frequency	{1} R:10.50	{2} R:7.60	{3} R:4.13	{4} R:2.50
Everyday {1}		1.00	0.25	0.42
3 - 5 times/week {2}	1.00		0.90	1.00
1 - 2 times/week {3}	0.25	0.90		1.00
No consumers {4}	0.42	1.00	1.00	

Table 5. BMD / Age and vegetable consumption frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			2.07	1.10	1.88	0.10
Age	-0.33	0.30	-0.03	0.02	-1.11	0.30
Everyday	0.70	0.43	0.38	0.23	1.62	0.15
3-5 times/week	0.57	0.51	0.23	0.21	1.11	0.30
1-2 times/week	0.30	0.50	0.13	0.21	0.60	0.57

3.3 Females at age of 50 - 59

Women are vulnerable to increased bone loss after menopause which starts after age of 50 approximately. Everyday consumption of vegetables at this female age group showed a positive effect on their BMD according to the results obtained.

Everyday vegetable consumers at age of 50 - 59 had significantly higher BMD ($p < 0.001$, $p = 0.000$) compared to 3 - 5 times/week consumers and to 1 - 2 times/week consumers. BMD expressed in g/cm^2 for everyday consumers compared to other two vegetable consumption subgroups was 1.09 v. 0.87. v. 0.85 respectively. However, there was insignificant difference of BMD between 3 - 5 times/week consumers and 1 - 2 times/week consumers ($p > 0.05$, $p = 1.00$). The results are presented in Table 6 bellow.

The results shown on Table 7 bellow related to the ratio between BMD in g/cm^2 as dependent phenomenon and the vegetable consumption frequency subgroups (everyday, 3 - 5 times/week, 1 - 2 times/week and no consumers); and the age of females as independent phenomena are presented. Strong significant correlation for this ratio is detected, $R = 0.72$ and $p < 0.001$ ($p = 0.000$). Females, 1 - 2 times/week consumers were referent subgroup. The highest influence on this ratio did have everyday vegetable consumption (Beta = 0.75), the weaker one had 3 - 5 times/week consumption (Beta = 0.08), while age parameter had the weakest influence (Beta = - 0.05).

In condition where other parameters were not changed: females who consume vegetables on everyday basis had 0.24 g/cm^2 (B = 0.24) higher BMD compared to 1 - 2 times/week consumers, which was significant difference ($p < 0.001$, $p = 0.000$); However, 3 - 5 times/week vegetable consumers had insignificantly ($p > 0.05$, $p = 0.42$) higher BMD for 0.03 g/cm^2 (B = 0.03) compared to 1 - 2 times/week consumers; and with each age increasing for one year, BMD was insignificantly decreased ($p > 0.05$, $p=0.61$) for 0.03 g/cm^2 (B = -0.03).

Our results for the beneficial effect of frequent vegetable consumption on BMD in postmenopausal women are confirmed by several studies [6, 10, 14, 16, and 44]. Different results were suggested by Liu *et al.,* [45]. These authors suggested that a high frequency of vegetable intake could increase the prevalence of osteoporosis among some Chinese post-menopausal women. Their possible explanation why the frequency of vegetable intake was independently and significantly associated with osteoporosis was that dark green vegetables such as: collard greens, kale, spinach, mustard leaves, turnip leaves, cabbage, broccoli, potatoes, pulses, and soybeans are common dietary sources of calcium, but the calcium they contain has low bioavailability, on contrary of dairy products calcium which has high bioavailability. Other kinds of vegetables, particularly those containing calcium oxalic acid, phytic and tannic acid, combine with the calcium in food and form insoluble calcium salt, so that the body cannot absorb calcium.

3.4 Females at age of 60 - 69

For N = 13.10 and $p < 0.01$ ($p = 0.001$), there was a significant difference of BMD in this age group of 60 - 69 in relation to everyday, 3 - 5 times/week, 1 - 2 times/week vegetable consumption frequency, i.e. the values of BMD in g/cm^2 were 1.13 v. 0.88 v. 0.90 respectively (See Table 8 bellow).

Table 6. Multiple comparison of p values

Vegetable consumption frequency	{1} R:49.79	{2} R:26.95	{3} R:22.13
Everyday {1}		0.000	0.000
3-5 times/week {2}	0.000		1.00
1-2 times/week {3}	0.000	1.00	

Table 7. BMD / Age & Vegetable Consumption Frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			0.99	0.27	3.63	0.0006
Age	-0.05	0.09	-0.003	0.005	-0.52	0.61
Everyday	0.75	0.10	0.24	0.03	7.40	0.000
3 - 5 times/week	0.08	0.10	0.03	0.03	0.82	0.42

Table 8. BMD and vegetable consumption frequency

Vegetable consumption frequency	Code	Valid N	Sum of Ranks
Everyday	1	18	651,50
3 - 5 times/week	2	9	182,50
1 - 2 times/week	3	24	492,00

Females who have consumed vegetables on everyday basis had significantly higher BMD compared to 3 - 5 times/week consumers ($p < 0.05$, $p = 0.03$) as well as compared to 1 - 2 times/week consumers ($p < 0.01$, $p = 0.002$). Interesting result was obtained when comparing the BMD of 3 - 5 times/week and 1 - 2 times/week vegetable consumption subgroups, 1 - 2 times/week consumers had insignificantly ($p > 0.05$, $p = 1.00$) higher BMD compared to 3 - 5 times/week consumers (Table 9 below).

The results on Table 10 (bellow) are related to the ratio between BMD in g/cm^2 like dependent phenomenon; and every day, 3 - 5 times/week, and 1 - 2 times/week vegetable consumption frequency and the age of females, as independent phenomena. A moderately significant correlation was notified ($R = 0.56$ and $p < 0.001$, $p = 0.000$). Vegetable consumers on 1 - 2 times/week basis were referent category. The highest influence to this ratio had everyday vegetable consumption (Beta = 0.54), 3 - 5 times/week consumption had a weaker influence (Beta = -0.035), while the weakest influence was manifested by age (Beta = 0.033).

In condition of unchanged other parameters it was notified that: Females who consume vegetables on everyday basis had 0.23 g/cm^2 (B = 0.23) higher BMD compared to 1 - 2 times/week consumers, which was significant difference ($p < 0.001$, $p = 0.000$); but females on 3 - 5 times/week consumption had insignificantly lower BMD ($p > 0.05$, $p = 0.9$) for 0.02 g/cm^2 compared to 1 - 2 times/week consumers.

Our results strongly suggest that vegetable intake on everyday bases is highly recommended for postmenopausal women at age of 60 - 69. Lyn *et al.,* [10] examined the effects of two dietary patterns of and three sodium levels on bone and calcium metabolism in a randomized feeding study on a total of 186 adults, aged 23 - 76 years. The authors suggested that DASH diet and reduced sodium intake significantly reduced bone turnover, which was sustained, may improved bone mineral status and ultimately reduced the risk of osteoporosis. This beneficial effect on bone health might have resulted from a variety of factors including higher calcium, potassium and magnesium intakes, its lower acidity and possibly fruit and vegetable-derived antioxidants and phytochemicals. For the elderly who have a higher risk of osteoporosis and often a change in taste acuity, the DASH diet rich in FV may be an effective alternative.

3.5 Females at age > од 69

There was no significant difference in BMD of females 69 years old in relation to vegetable consumption frequency (N = 7.49 and $p > 0.05$, $p = 0.06$). BMD of every day vegetable consumers was in average (M) 0.93 g/cm^{2,}, compared to 3 - 5 times/week consumers (M = 0.90 g/cm$^{2)}$), compared to 1 - 2 times/week consumers (M = 0.82 g/cm^2), and to no consumers (M = 0.83 g/cm^2) -Table 11.

Multiple comparisons of p values related to BMD of females who consumed vegetables on everyday basis,

Table 9. Multiple comparisons of p values

Vegetable consumption frequency	{1} R:36.19	{2} R:20.28	{3} R:20.50
Everyday {1}		0.03	0.002
3 - 5 times/week {2}	0.03		1.00
1 - 2 times/week {3}	0.002	1.00	

Table 10. BMD / Age & Vegetable consumption frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			0.75	0.57	1.32	0.19
Age	0.03	0.12	0.002	0.01	0.27	0.79
Everyday	0.54	0.13	0.23	0.06	4.19	0.000
3 - 5 times/week	-0.03	0.13	-0.02	0.07	-0.27	0.79

Table 11. Vegetable consumption frequency & BMD

Vegetable consumption frequency	Code	Valid N	Sum of Ranks
Everyday	1	24	1231.50
3 - 5 times/week	2	21	966.50
1 - 2 times/week	3	36	1290.50
No consumers	4	3	81.50

Table 12. Multiple comparisons of p values

Vegetable consumption frequency	{1} R:51.31	{2} R:46.02	{3} R:35.85	{4} R:27.17
Everyday {1}		1.00	0.10	0.64
3 - 5 times/wee {2}	1.00		0.77	1.00
1 - 2 times/week {3}	0.10	0.77		1.00
No consumers {4}	0.64	1.00	1.00	

Table 13. BMD / Age and vegetable consumption frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			0.56	0.28	1.99	0.04
Age	0.10	0.11	0.003	0.003	0.96	0.34
Everyday	0.36	0.27	0.11	0.08	1.36	0.18
3 - 5 times/week	0.28	0.26	0.09	0.08	1.09	0.28
1 - 2 times/week	0.03	0.29	0.009	0.08	0.11	0.91

3 - 5 times/week, 1 - 2 times/week and no consumers did not show significant difference ($p > 0.05$) - Table 12.

The results presented in Table 13 are related to BMD (g/cm^2) like dependent phenomenon; and vegetable consumption frequency (3 - 5 times/week, 1 - 2 times/week and no consumers) and age, like independent phenomena. Moderately significant correlation was detected (for $R = 0.36$ and $p < 0.05$ $p = 0.02$). Females no consumers of vegetable were referent category. Everyday consumption of vegetables showed the highest influence on BMD (Beta = 0.36), 3 - 5times/week consumption manifested weaker influence (Beta = 0.28), возраста (Beta = 0.10), but the weakest influence did have 1 - 2 times/week consumption (Beta = 0.03).

In condition of unchangeable other parameters the results showed that: Females on everyday consumption had insignificantly ($p > 0.05$, $p = 0.18$) higher BMD of 0.11 g/cm^2 (B = 0.11) in average compared to no consumers; 3 - 5 times/week vegetable consumers had 0.09 g/cm^2 (B = 0.09) higher BMD compared to no consumers which was insignificant difference ($p > 0.05$, $p = 0.28$); and the difference of 0.009 g/cm^2 (B = 0.009), higher BMD in 1 - 2 times/week consumers compared to no consumers was insignificant ($p > 0.05$, $p = 0.91$).

Our results in this age group of > 69 showed that vegetable consumption frequency had moderately significant correlation with BMD (for $R = 0.36$ and $p < 0.05$) which was weaker compared to results of 60 - 69 years old females ($R = 0.56$ and $p < 0.001$), and to 50 - 59 years old females who had strong significant correlation between vegetable consumption frequency and BMD ($R = 0.72$ and $p < 0.001$). However > 69 years old female group had higher correlation of BMD with vegetable frequency consumption than 40 - 49 years old females, where strong not significant correlation ($R = 0.70$ and $p > 0.05$) was detected.

Tuker *et al.,* [46] found that potassium, magnesium and FV intakes were associated with greater BMD in elderly men and women. Qui *et al.,* [36] performed a population-based cross-sectional study with 2083 women and 1006 men aged 40 - 75 years involved. Greater intake of FV was independently associated with a higher BMD and a lower presence of osteoporosis in middle-aged and elderly Chinese subjects with lower body mass index (BMI). Fruit tended to have more contribution to the favorable association than vegetables.

4. Conclusions

- Based upon the results of this study, vegetable consumption frequency significantly correlated with BMD in females, lower percentage of osteoporosis and lower percentage of heavy forms of osteoporosis (which can lead to fractures) when females were not categorized in age subgroups. There was no evidence of normal value of BMD in no consumers group, but the number of participants was small, thus further studies with higher number of females who do not consume vegetables should be performed.

- 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. Everyday consumption of vegetables had highly significant positive impact on BMD of 50 - 59 years old females, while in 60 - 69 and > 69 years old females it had moderately significant effect, thus it can be considered as a natural option for osteoporosis prevention, as well as protection factor of critical BMD lowering to fracture threshold. However, in females aged 40 - 49 the frequency of vegetable consumption had no such influence on their BMD.

- Education of a wider population at any age about the dietary habits and the beneficial role of fruits and vegetables on bone mineral density and overall human health should be implemented in each country. Health policy strategies should include recommendation for frequent vegetable and fruit consumption as a remarkable part of healthy nutrition.

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