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ORIGINAL RESEARCH

Major Nutrient Patterns and Bone Mineral Density among Postmenopausal Iranian Women

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Abstract Our understanding of the influence of overall nutrient intake on bone mineral density (BMD) is limited because most studies to date have focused on the intakes of calcium, vitamin D, or a few isolated nutrients. Therefore, we examined the association of major nutrient patterns with BMD in a sample of postmenopausal Iranian women. In this cross-sectional study, 160 women aged 50–85 years were studied and their lumbar spine and femoral neck BMDs were measured using dual-energy X-ray absorptiometry. Dietary intakes were assessed using a validated 168-item food frequency questionnaire, and daily intakes of 30 nutrients were calculated. All nutrient intakes were energy adjusted by the residual method and were submitted to principal component factor analysis to identify major nutrient patterns. Overall, three major nutrient patterns were identified, among which only the first pattern, which was high in folate, total fiber, vitamin B_6, potassium, vitamin A (as retinol activity equivalent), vitamin C, β-carotene, vitamin K, magnesium, copper, and manganese, had a significant association with BMD. After controlling for potential confounders, multivariate adjusted mean of the lumbar spine BMD of women in the highest tertile of the first pattern scores was significantly higher than those in the lowest tertile (mean difference 0.08; 95 % confidence interval 0.02–0.15; $P = 0.01$). A nutrient pattern similar to pattern 1, which is associated with high intakes of fruits and vegetables, may be beneficial for bone health in postmenopausal Iranian women.

Keywords Bone mineral density · Diet · Iran · Menopause · Nutrient patterns · Osteoporosis

Introduction

Postmenopausal osteoporosis and related fractures are serious public health concerns worldwide and are responsible for considerable morbidity, mortality, and health care costs [1, 2]. In Iran, osteoporosis is one of the most common diseases of postmenopausal women, contributing to over 36,026 lost years of healthy life (18,757 in men and 17,270 in women) in 2001 according to the disability-adjusted life-year index [3, 4]. Given the rapid rise in the prevalence of this debilitating disease and associated fractures [5], maintaining bone mineral density (BMD) in the first place seems necessary. BMD is largely affected by a variety of genetic, endocrine, mechanical, and nutritional factors, with extensive interactions between these factors [6]. Nutritional factors in particular play an important role in the optimization of bone health because they are modifiable [7].

However, our current understanding of the influence of nutrient intake on BMD is still limited because most studies to date have focused primarily on the intakes of calcium, vitamin D, or a few isolated nutrients [8] and have

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paid less attention to the contribution of overall nutrient intake to bone health. Because people eat meals consisting of complex combinations of nutrients, the traditional approach of evaluating diet–disease relationship, which focuses on highly correlated nutrients individually, may be inadequate for taking into account cumulative intercorrelations and interactive or synergistic effects on the bioavailability, circulating levels, metabolism, and excretion of nutrients [9, 10]. In addition, it is obvious that our exposure to a complex of nutrients interacts to affect bone health, and therefore, understanding the interactions of these nutrients within diets and even in the nutrient supplements seems crucial [11]. This goal can be achieved by studying overall nutrients as an exposure (i.e., nutrient patterns), rather than nutrients in isolation [12]. The main advantage of this approach is the ability to identify significant cumulative effects that may be too small to detect with individual nutrients [12]. Furthermore, analyzing a small number of patterns, rather than an array of individual nutrients, decreases the possibility of producing statistically significant associations by chance [10, 13]. Moreover, finding nutrient patterns that are associated with greater bone mass might be useful in designing nutrient supplements with highest possible protective effects on bone health.

Despite its prominence, to our knowledge, only Sugiura et al. [14] have previously assessed the attributes of nutrient patterns to BMD. They found an inverse association between a nutrient pattern of antioxidants characterized with high intakes of β-cryptoxanthin, vitamin C, β-carotene, lutein, and vitamin E and a risk of having low radial BMD in postmenopausal Japanese women. However, their study was limited to the intake patterns of a few antioxidant nutrients, and they did not assess the influence of overall nutrient intake on BMD. Therefore, we aimed to examine the relationship between major nutrient patterns and BMD in a sample of postmenopausal Iranian women.

Materials and Methods

Study Population and Sample

A total of 213 postmenopausal women aged 50–85 years who were admitted to a community-based outpatient bone densitometry center in Tehran, Iran (winter 2011), were consecutively enrolled for participation in this cross-sectional study. Menopause was defined as lack of any menstrual cycle during the preceding year. After excluding participants who were following a specific diet (e.g., weight-reducing diets) ($n = 11$); those who were ingesting alcohol or drugs that affect bone metabolism ($n = 29$) such as glucocorticoids, antacids, diuretics, thyroxin, calcitonin, anticonvulsants, and anticoagulants (except for antiresorptive medications); those with a diagnosed endocrine (e.g., abnormal menopause, diabetes), gastrointestinal, rheumatoid, or renal disorder ($n = 13$); those with >70 blank items on the food-frequency questionnaire (FFQ) ($n = 6$); and those who reported a total daily energy intake outside the range of 800–4,600 kcal (range, 3,347–19,246 kJ) ($n = 3$), 151 women (mean age 60.3 years) remained for the current analysis.

The ethics board of the National Nutrition and Food Technology Research Institute (World Health Organization Collaborating Center), Iran, approved the study protocol, and written informed consent was obtained from each participant after being informed of the purpose of this research. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 [15].

Measurements

Bone Mineral Density

BMD of the lumbar spine (L1–L4) and the left femoral neck (g/cm^2) were measured by a trained technician by dual-energy X-ray absorptiometry (Hologic Discovery W QDR Series, Hologic Inc., Bedford, MA, USA). The Hologic densitometer had been initially calibrated by the manufacturer, and it was calibrated consistently and automatically throughout the present study using an automatic internal reference system. Quality control measures were also performed automatically based on guidelines for standard operating procedures. The coefficient of variation of the BMD measurements was 1 %. All participants were scanned during the winter to control for the seasonal variation that may occur in BMD.

Dietary Intake

We used a semiquantitative FFQ for assessing participants' usual dietary intake during the past year. This FFQ has been reported to be a valid and reliable tool for assessing nutrient intakes in Tehranian adults [16]. It consisted of 168 food items (with standard serving sizes) commonly consumed by Iranians. All participants were asked to report their frequency of consumption for each food item on a daily (e.g., bread), weekly (e.g., rice, meat), or monthly (e.g., fish) basis. The daily intake of all food items was computed and then converted to daily grams of food intake using a manual for household measures [17]. Because the Iranian food composition table (IFCT) [18] is incomplete and contains information on a limited number of raw materials and a few nutrients, we used the USDA food composition data included in the Nutritionist 4 software (First Databank; Hearst, San Bruno, CA, USA) to calculate the energy and nutrient content of foods. However, for Iranian food items not included in Nutritionist 4, such as kashk (a dairy food), the IFCT was used [18]. It is noteworthy that the energy and macronutrient content of many of the food items in IFCT, such as breads and fruits, are almost similar to alternative food items in the USDA food composition table, with a correlation of >0.9 [16]. Daily intakes of nutrients were then calculated for each participant and were energy adjusted by the residual method [19]. The tools and procedures we used to compute participants' daily intakes of energy and nutrients were the same as those used in the validation study of the FFQ by Mirmiran et al. [16].

Anthropometric Variables

Weight was measured with digital scales (Seca 881, Germany) to the nearest 0.1 kg while the participants were wearing minimal clothing without shoes. Height was also measured without shoes and was recorded to the nearest 0.1 cm using a stadiometer (Seca 214 portable stadiometer). Participants' body mass index (BMI) was then calculated as weight in kilograms divided by height in meters squared.

Physical Activity

As reported previously [20], data on physical activity were obtained by using a valid self-reported questionnaire [21] and expressed as metabolic equivalent hours per day (MET·h·d). This questionnaire was previously used in a representative sample of Tehranian adult women, yielding in consistent results [22].

Other Variables

Additional covariate information on age (years), age at menarche (years), age at menopause (years), parity (*n*), lactation (months), sunlight exposure (less than an hour, an hour or more), smoking (yes, no), fragility fracture history (yes, no), history of hormone replacement therapy (HRT) (yes, no), supplement intake including calcium, vitamin D, multivitamins, minerals, glucoseamines, omega-3 fatty acids, and phytoestrogens (yes, no), antiresorptive drug use including bisphosphonates and selective estrogen receptor modulators (yes, no), and education (less than a high school diploma, high school diploma or more), as a marker of socioeconomic status, was obtained using general questionnaires.

A well-trained dietitian administered all questionnaires through face-to-face interviews and performed anthropometric measurements on the patients' admission day.

Statistical Analysis

All analyses were conducted by SPSS software, version 16 (IBM, Armonk, NY, USA), and a P value of <0.05 was considered statistically significant. The principal component analysis (a type of factor analysis) was administered to derive nutrient patterns (factors) based on a set of 30 major nutrients. This statistical procedure aggregates specific nutrients into nutrient patterns on the basis of the degree to which nutrients in the data set are correlated with one another [10]. Therefore, each nutrient pattern represents a list of specific nutrients, with intakes that have the largest correlation with each other in the population under study. In other words, the nutrients in a given pattern are frequently eaten together by the study subjects, and those nutrients not in that pattern are not consistently eaten with those in the pattern. In general, a minimum sample size of 100 subjects and a minimum of 5 subjects per variable (in our case, daily intake of each nutrient), regardless of the number of variables, was recommended by Gorsuch [23] and Hair et al. [24] as requirements for conducting factor analysis. Therefore, in order to fulfill these requirements, we were only able to include a maximum of 30 major nutrients in factor analysis.

Before running the analysis, the correlation matrix among the 30 nutrients was visually and statistically examined to justify undertaking factor analysis. The χ^2 for Bartlett's test of sphericity was statistically significant at $P < 0.001$, and the Kaiser–Meyer–Olkin measure of sampling adequacy resulted in a score of 0.70, indicating that the correlation among the variables was adequately strong for a factor analysis. In this study, we used varimax rotation (a type of orthogonal rotation) to identify optimal uncorrelated factors and to achieve a simple matrix with improved interpretability [25]. We chose to retain three major nutrient patterns with eigenvalues of >3 for current analysis combining the following criteria: factor eigenvalue greater than 1, Scree test, and natural interpretability of the factor [25]. Factor scores for each nutrient pattern and each subject were computed by summing the intake of each nutrient weighted by factor loading [25] and were then divided into three categories according to tertile ($n = 50$ in tertiles 1 and 3; $n = 51$ in tertile 2). Factor loading is the correlation coefficient between individual nutrients and each of the identified nutrient patterns. A large absolute loading in a factor indicates a strong relationship between a given nutrient and the factor, whereas the plus and minus signs refer to direct and inverse associations, respectively [25].

We also calculated the Pearson's correlation coefficients between nutrient pattern scores and log-transformed intakes of 25 predefined food groups. The details of categorizing 168 food items from FFQ into 25 predefined food groups are described elsewhere [20]. For continuous variables including BMD values, the normality assumption was initially assessed using the Kolmogorov–Smirnov test. Crude and multivariate adjusted means of the lumbar spine and femoral neck BMD were then computed and compared between tertiles of each nutrient pattern scores using one-way analysis of variance and analysis of covariance, respectively. Adjustments were done for age, BMI, physical activity, age at menarche, age at menopause, parity, lactation, sunlight exposure, smoking, education, fragility fracture history, history of HRT, supplement intake, and antiresorptive drug use in the analysis of covariance. Finally, pairwise differences in the means of the BMD between highest and lowest tertiles of each nutrient pattern scores were examined by the Bonferroni post hoc test to appropriately adjust for multiple comparisons.

Results

Table 1 presents the characteristics and dietary intakes of study participants. The mean BMD values at the lumbar spine and femoral neck among postmenopausal women were 0.86 and 0.67 g/cm^2, respectively.

Table 2 shows the nutrients used in the factor analysis and factor loadings for each of the identified nutrient patterns. The first pattern was abundant in folate, total fiber, vitamin B_6, potassium, vitamin A as retinol activity equivalent (RAE), vitamin C, β-carotene, vitamin K, magnesium, copper, and manganese. The second pattern was high in vitamin B_2, protein, calcium, phosphorus, zinc, vitamin B_{12}, and vitamin D and low in vitamin E. The third pattern was characterized by high intakes of total fat, monounsaturated fatty acids, saturated fatty acids, and polyunsaturated fatty acids and low intakes of carbohydrate and vitamin B_1. Overall, these three nutrient patterns explained 54.74 % of total variance in nutrient intakes.

Table 3 presents the Pearson's correlation coefficients of nutrient pattern scores with food group intakes. There were reasonable correlations between the first pattern scores and intakes of vegetables ($r = 0.55$, $P < 0.001$), fruits and fruit juices ($r = 0.44$, $P < 0.001$), nonrefined cereals ($r = -0.30$, $P < 0.001$), and refined cereals ($r = -0.30$, $P < 0.001$). The second pattern scores also had reasonable correlations with intakes of low-fat dairy products ($r = 0.56$, $P < 0.001$), fish ($r = 0.34$, $P < 0.001$), mayonnaise ($r = -0.33$, $P < 0.01$), and high-fat dairy products ($r = -0.31$, $P < 0.001$). In addition, the third pattern score was reasonably correlated with intakes of vegetable oils ($r = 0.42$, $P < 0.001$) and mayonnaise ($r = 0.37$, $P < 0.01$).

Table 4 shows the crude and multivariate adjusted means of the BMD by tertiles of scores for each nutrient pattern. Among the three nutrient patterns, we identified that only the first pattern had a significant association with BMD. After controlling for potential confounders, the multivariate adjusted mean of the lumbar spine BMD of women in the highest tertile of the first pattern scores was significantly higher than those in the lowest tertile (mean difference 0.08; 95 % confidence interval 0.02–0.15; $P = 0.01$). No significant association was observed between other patterns and lumbar spine BMD. In addition, none of the patterns was associated with femoral neck BMD.

Discussion

To our knowledge, this is the second investigation to report the association between nutrient patterns and BMD. Findings suggest that the first pattern (abundant in folate, total fiber, vitamin B_6, potassium, vitamin A as RAE, vitamin C, β-carotene, vitamin K, magnesium, copper, and manganese), which was associated with high intakes of fruits and vegetables and low intakes of cereals, had a significant positive relationship with BMD at the lumbar spine among a sample of postmenopausal Iranian women.

Our finding of a significant positive relationship between the first pattern and BMD at the lumbar spine, but not at the femoral neck, is in line with the 1997 study of New et al. [26], which reported a direct association of fiber, potassium, vitamin C, and magnesium intake with lumbar spine and not with femoral neck BMD. These interesting observations might be explained by the predominance of trabecular bone at the lumbar spine compared to the femoral neck, which contains a higher proportion of cortical bone. Although the trabecular bone constitutes only 20 % of the skeletal mass in a healthy adult skeleton, its surface area and rate of remodeling is greater than that of cortical bone [27]. Therefore, it seems that these characteristics might make the lumbar spine relatively more sensitive to changes in nutrient intake compared to the femoral neck.

The significant direct association of our first pattern with lumbar spine BMD is also consistent with the findings of Sugiura et al. [14]. This study reported an inverse association between a nutrient pattern characterized with high intakes of antioxidant nutrients found abundantly in fruits and vegetables (i.e., β-cryptoxanthin, vitamin C, β-carotene, lutein, and vitamin E) and risk of having low radial BMD among postmenopausal Japanese women. Generally, this finding could be simply justified by the fact that all nutrients in our first pattern are somehow important for bone health. Magnesium, copper, manganese, and vitamins A, K, C, B_6, and folate play key roles in the formation and maintenance of bone structure and are required for normal bone metabolism. Fiber and potassium intake also contribute to bone health for different reasons [28–30].

Table 1 Characteristics and dietary intakes of 151 study participants

Characteristic	Postmenopausal women
Patient characteristics	
Lumbar spine BMD (g/cm^2)	0.86 [0.84–0.89]
Femoral neck BMD (g/cm^2)	0.67 [0.65–0.69]
Age (years)	60.3 [59.1–61.6]
BMI (kg/m^2)	27.5 [26.8–28.2]
Physical activity (MET·h·d)	42.1 [41.3–42.9]
Age at menarche (years)	13.5 [13.2–13.7]
Age at menopause (years)	49.4 [48.4–49.9]
Parity (*n*)	3.7 [3.4–4.0]
Lactation (months)	26.0 [21.5–31.8]
Sunlight exposure (less than an hour)	107 (70.9)
Smoking	14 (9.3)
Education (high school diploma or more)	92 (60.9)
Fragility fracture history	8 (5.3)
History of HRT	7 (4.6)
Supplement intake	115 (76.2)
Antiresorptive drug use	27 (17.9)
Characteristics of dietary intake	
Energy intake (kcal/day)	2208.3 [2100.6–2321.6]
Carbohydrate (g/day)	350.7 [333.6–368.7]
Protein (g/day)	92.3 [87.0–97.5]
Total fat (g/day)	58.6 [55.1–62.2]
Saturated fatty acids (g/day)	16.4 [15.3–17.8]
Monounsaturated fatty acids (g/day)	17.3 [16.1–18.5]
Polyunsaturated fatty acids (g/day)	14.2 [13.1–15.5]
Total fiber (g/day)	29.1 [27.4–30.9]
Vitamin A as RAE (μg/day)	2,143.1 [1,919.8–2,392.3]
β-Carotene (μg/day)	1,199.9 [1,032.8–1,380.2]
Vitamin D (μg/day)	1.2 [0.9–1.6]
Vitamin E (mg/day)	5.3 [4.8–5.8]
Vitamin K (μg/day)	169.0 [151.4–186.8]
Vitamin B$_1$ (mg/day)	2.1 [2.0–2.2]
Vitamin B$_2$ (mg/day)	2.6 [2.5–2.8]
Vitamin B$_3$ (mg/day)	19.7 [18.5–20.9]
Vitamin B$_6$ (mg/day)	2.3 [2.2–2.5]
Folate (μg/day)	445.9 [419.9–473.4]
Vitamin B$_{12}$ (μg/day)	4.4 [4.1–4.9]
Vitamin C (mg/day)	347.2 [323.8–376.2]
Calcium (mg/day)	1,465.6 [1,366.5–1,587.6]
Phosphorus (mg/day)	1,495.2 [1,408.1–1,603.6]
Magnesium (mg/day)	407.5 [387.6–432.7]
Zinc (mg/day)	11.7 [11.0–12.3]
Iron (mg/day)	25.5 [23.8–27.7]
Copper (μg/day)	1,844.6 [1,754.6–1,958.6]
Manganese (mg/day)	5.4 [5.1–5.8]
Sodium (g/day)	3.8 [3.5–4.1]
Potassium (g/day)	5.7 [5.4–6.0]
Fluoride (mg/day)	1.7 [1.4–1.9]
Caffeine (mg/day)	121.5 [107.8–137.0]

Data are presented as mean [95 % confidence intervals] or *n* (%). Arithmetic and geometric means are reported for normally and nonnormally distributed variables, respectively

BMD bone mineral density, *BMI* body mass index, *MET* metabolic equivalent, *HRT* hormone replacement therapy, *RAE* retinol activity equivalent

Table 2 Nutrients used in the factor analysis and factor loadings for each of the identified nutrient patterns

Nutrient	Nutrient pattern		
	1	2	3
Folate	0.85	0.31	–
Total fiber	0.82	–	−0.33
Vitamin B$_6$	0.81	–	–
Potassium	0.79	0.48	–
Vitamin A (as RAE)	0.79	–	–
Vitamin C	0.75	–	–
β-Carotene	0.72	–	–
Vitamin K	0.72	–	–
Magnesium	0.72	0.62	–
Copper	0.71	0.33	–
Manganese	0.44	–	–
Vitamin B$_2$	0.31	0.90	–
Protein	–	0.87	–
Calcium	–	0.84	–
Phosphorus	–	0.82	–
Zinc	–	0.82	–
Vitamin B$_{12}$	–	0.67	–
Vitamin D	–	0.63	–
Vitamin E	0.32	−0.32	–
Iron	–	–	–
Total fat	–	–	0.94
Monounsaturated fatty acids	–	–	0.87
Carbohydrate	–	−0.42	−0.73
Saturated fatty acids	–	–	0.68
Vitamin B$_1$	–	–	−0.64
Polyunsaturated fatty acids	–	–	0.64
Sodium	–	–	–
Vitamin B$_3$	–	–	–
Fluoride	–	–	–
Caffeine	–	–	–
Explained variance (%)	29.15	14.68	10.90

Factor loadings of <0.3 are not shown for simplicity

RAE retinol activity equivalent

Different studies also support the positive association between bone density and intakes of potassium, magnesium [31], fiber [32], and vitamins C [33], K [34], A [35], B$_6$ [30], folate [36], and β-carotene [14] among postmenopausal women. Furthermore, supplementation with vitamins C [37] and K [38], and minerals potassium [39], magnesium [40], copper, and manganese [41] in postmenopausal women have been effective in increasing BMD and/or reducing the rate of bone loss.

Moreover, recent evidence suggests the contribution of oxidative stress to osteoporosis [42] through the involvement of reactive oxygen species and free radicals in osteoclastogenesis, in apoptosis of osteoblasts, and

Table 3 Pearson's correlation coefficients of nutrient pattern scores with food group intakes ($N = 151$)

Food group	Food items	Nutrient pattern score		
		Pattern 1	Pattern 2	Pattern 3
Nonrefined cereals	Dark breads (e.g., barbari, sangak, taftun), bran breads, others	−0.30**	–	–
Refined cereals	Lavash bread, baguette bread, rice, pasta, others	−0.30**	–	–
Vegetables	Cauliflower, carrot, tomato and its products, spinach, lettuce, cucumber, eggplants, onion, greens, green bean, green pea, squash, mushroom, pepper, corn, garlic, turnip, others	0.55**	–	–
Potatoes	Potatoes	–	–	–
Fruits and fruit juices	Melon, watermelon, honeydew melon, plums, prunes, apples, cherries, sour cherries, peaches, nectarine, pear, fig, date, grapes, kiwi, pomegranate, strawberry, banana, persimmon, berry, pineapple, oranges, dried fruits, all juices, others	0.44**	–	–
Red or processed meats	Beef and veal, lamb, minced meat, sausage, deli meat, hamburger	–	–	–
Organ meats	Heart, kidney, liver, tongue, brain, offal, rennet	–	–	–
Poultry	Chicken	–	–	–
Fish	All fish types	–	0.34**	–
Legumes	Lentils, split pea, beans, chickpea, fava bean, soy, others	–	–	–
Eggs	Eggs	–	–	–
Nuts	Almonds, peanut, walnut, pistachio, hazelnut, seeds, others	–	–	–
Low-fat dairy products	Low-fat milk, skim milk, low-fat yogurt, cheese, Kashk, yogurt drink, others	–	0.56**	–
High-fat dairy products	High-fat milk, high-fat yogurt, cream cheese, cream, dairy fat, ice cream, others	–	−0.31**	–
Hydrogenated fats	Hydrogenated vegetable oils, solid fats (animal origin), animal butter, margarine	–	–	–
Vegetable oils	Vegetable oils	–	–	0.42**
Mayonnaise	Mayonnaise	–	−0.33*	0.37*
French fries	French fries	–		–
Sweets and desserts	Cookies, cakes, muffins, pies, chocolates, honey, jam, sugar cubes, sugar, candies, sweet Tahini, others	–	–	–
Snacks	Biscuits, corn puffs, crackers, potato chips, others	–	–	–
Soft drinks	Soft drinks	–	–	–
Tea and coffee	Tea and coffee	–	–	–
Pickles	Pickles, sauerkraut	–	–	–
Salt	Salt	–	–	–
Condiments	Turmeric, pepper, others	–	–	–

Pearson's correlation coefficients of <0.3 are not shown for simplicity

* $P < 0.01$, ** $P < 0.001$

Table 4 Crude and multivariate adjusted means of the BMD by tertiles of scores for each nutrient pattern ($N = 151$)

BMD	First pattern scores			Pairwise difference between T3 and T1	Second pattern scores			Pairwise difference between T3 and T1	Third pattern scores			Pairwise difference between T3 and T1
	T1	T2	T3		T1	T2	T3		T1	T2	T3	
Lumbar spine (g/cm^2)												
Crude	0.84	0.84	0.91	0.06	0.85	0.88	0.86	0.01	0.85	0.87	0.87	0.03
	[0.80–0.89]	[0.80–0.87]	[0.87–0.95]*	[−0.01; 0.13]	[0.81–0.89]	[0.84–0.92]	[0.82–0.90]	[−0.06; 0.08]	[0.80–0.89]	[0.83–0.91]	[0.83–0.91]	[−0.04; 0.10]
Multivariate adjusted	0.83	0.85	0.91	0.08**	0.86	0.87	0.86	−0.01	0.86	0.85	0.87	0.02
	[0.79–0.87]	[0.81–0.88]	[0.88–0.95]**	[0.02; 0.15]	[0.82–0.90]	[0.83–0.91]	[0.82–0.89]	[−0.08; 0.06]	[0.82–0.90]	[0.82–0.89]	[0.84–0.91]	[−0.05; 0.08]
Femoral neck (g/cm^2)												
Crude	0.67	0.67	0.67	0.00	0.66	0.69	0.67	0.01	0.64	0.70	0.68	0.04
	[0.64–0.71]	[0.64–0.69]	[0.65–0.70]	[−0.05; 0.05]	[0.63–0.68]	[0.66–0.72]	[0.63–0.70]	[−0.04; 0.07]	[0.60–0.67]	[0.67–0.73]	[0.66–0.71]*	[−0.01; 0.10]
Multivariate adjusted	0.66	0.68	0.68	0.02	0.66	0.68	0.67	0.01	0.64	0.69	0.68	0.04
	[0.63–0.69]	[0.65–0.70]	[0.65–0.70]	[−0.03; 0.07]	[0.64–0.69]	[0.65–0.71]	[0.64–0.70]	[−0.04; 0.05]	[0.62–0.67]	[0.66–0.72]	[0.66–0.71]*	[−0.01; 0.09]

Data are presented as mean [95 % confidence interval]. Crude and multivariate adjusted means of the BMD were computed for and compared between tertiles of each nutrient pattern scores using one-way analysis of variance and analysis of covariance, respectively. Adjustments were done for age, BMI, physical activity, age at menarche, age at menopause, parity, lactation, sunlight exposure, smoking, education, fragility fracture history, history of hormone replacement therapy, supplement intake, and antiresorptive drug use in the analysis of covariance. Pairwise differences in the means of the BMD between highest (T3) and lowest (T1) tertiles of each nutrient pattern scores were examined by performing Bonferroni post hoc test. $n = 50$ in tertiles 1 and 3; $n = 51$ in tertile 2

BMD bone mineral density

* $P < 0.05$, ** $P \leq 0.01$

consequently in bone resorption [43, 44]. Therefore, the positive relationship between lumbar spine BMD and the first pattern in the present study might also be due to the higher intakes of antioxidant micronutrients found in high amounts in fruits and vegetables such as vitamins C, β-carotene, copper, and manganese in this pattern, because these nutrients play important roles in protecting bones against oxidative stress [45, 46]. For example, a high intake of vitamin C is especially of great importance when the connective tissue of bone is a target for oxidative damage because it is well clarified that vitamin C decreases oxidative stress by acting as an scavenger of singlet oxygen and peroxyl radicals [46]. In general, our finding is in line with those of the recent studies, which have reported inverse relationships between low BMD and antioxidant vitamins and carotenoid intakes among postmenopausal women [14, 47], and highlights the potential benefits of antioxidant micronutrients intake for bone health.

In addition, increasing evidence suggests that a mild and chronic metabolic acidosis resulting from a higher dietary acid load causes calcium loss from bone [48], inhibits osteoblast function, increases osteoclast activity, and in consequence limits bone formation and decreases its density [49], whereas the opposite is true of metabolic alkalosis [50]. As shown in Table 3, our first pattern was associated with high intakes of alkali-forming foods, such as fruits and vegetables, and low intakes of acid-forming foods, such as cereals. Furthermore, this pattern was characterized by high intakes of potassium and magnesium, which are major determinants of alkali load in a diet [51]. Therefore, the direct association of our first pattern with lumbar spine BMD might also stem from higher dietary alkali load as a result of higher intakes of these nutrients in this pattern.

In the present study, the second pattern was not associated with lumbar spine or femoral neck BMD. This observation was surprising for us because this pattern is characterized by high intakes of some essential nutrients for bone health, such as protein, calcium, phosphorus, zinc, and vitamin D [28], and we expected it to be positively associated with BMD among postmenopausal women of this study. Because the study of Sugiura et al. [14] was limited to the intake patterns of a few antioxidant nutrients, comparison of this finding with their study is not possible, and further studies are needed in this respect.

However, there may be some potential explanations for the lack of a significant association between our second pattern and BMD, and we believe that one of the most plausible explanations could be the low intake of vitamin E in this pattern. This assumption is supported by the fact that vitamin E supplementation has been demonstrated to be effective in increasing bone density and protecting against bone loss and damage caused by oxidative stress in rats [52]. Furthermore, according to the study of Pasco et al. [53], taking vitamin E supplements may suppress bone resorption in postmenopausal women. Generally, it seems that a vitamin E-deficient diet may cause bone damage, probably as a result of increased oxidative stress or impaired calcium absorption that leads to a state of calcium deficiency [52].

In addition, although higher protein levels have been associated with beneficial effects on bone, especially when they are consumed with adequate calcium, potassium, and other minerals [54], the influence of protein intake on bone health may depend on whether the whole diet is balanced in terms of its acid-generating potential [55]. This assumption is further supported by a positive association of a ratio of lower protein to higher potassium dietary intake (i.e., less dietary acid) with greater spine, hip, and forearm BMD among women in the study of New et al. [56]. Furthermore, a similar finding was observed in our previous dietary pattern research in the same study population, in which a dietary pattern characterized by a high intake of acid-forming foods such as meats and cereals, but which was also high in proteins from animal origin, had a significant inverse association with BMD [20]. Overall, according to the acid–base literature, protein and phosphorus are considered to be net acid-generating nutrients and therefore net negative risk factors for dissolution of bone minerals and bone resorption [48, 51]. The results of a review of acid–base literature by Arnett [57] also provide evidence that excessive protein intake results in a mild and chronic metabolic acidosis (pH change of about -0.02 to -0.05). Even this subtle change in pH may be sufficient to cause appreciable bone loss over time. Therefore, we cannot entirely exclude the possibility that high intakes of protein and phosphorus, which are major determinants of dietary acid load [51], in the second pattern of our study might have altered the acid–base balance, neutralized the potential beneficial effects of this pattern on BMD, and hence resulted in an overall insignificant association.

Moreover, despite the great importance of adequate phosphorus and zinc intakes for bone health and integrity [29], there are some concerns about potential undesirable effects of high intakes of these nutrients on bone, especially among women [58, 59]. Some evidence exists that high dietary phosphorus intake, by depressing ionized calcium, leads to an elevation in serum parathyroid hormone level and consequently increases bone resorption [60]. On the other hand, zinc supplementation has been sometimes reported to have adverse effects on calcium metabolism [61]. Furthermore, Nielsen and Milne [59] have demonstrated that a moderately high intake of zinc depresses magnesium balance and causes undesirable alterations in indices of bone turnover in postmenopausal women.

Finally, although substantial literature supports the beneficial effects of vitamin D supplementation on bone density [29], some points need to be considered about its dietary intake. It is generally accepted that the main source of vitamin D intake in humans is the exposure of skin to the UVB rays in sunlight [46]. Therefore, dietary intake of this vitamin may play only a partial role in the regulation of postmenopausal bone loss [62]. Furthermore, the vitamin D intake among postmenopausal women of this study was extremely low and probably insufficient to induce the expected protective effects of this vitamin on BMD. This extremely low intake might be another reason why we could not find any positive association between the second pattern and BMD.

We did not find any significant association between the third pattern and BMD. This finding could be partially attributed to the lower intakes of nutrients in the third pattern among postmenopausal women of our study; this pattern explained only 10.90 % of total variance in nutrient intakes. Additionally, the third pattern was characterized by high intakes of different types of dietary fat and low intakes of carbohydrate and vitamin B_1, which, by exerting different or even opposite effects on BMD, might have resulted in an overall insignificant association. For example, although it has been demonstrated that dietary fats can have important effects on bone health via several mechanisms including alterations in duodenal calcium absorption, osteoblast function, prostaglandin synthesis, and oxidation of lipids [63–66], depending on the type of dietary fat, these effects may be quite different. This assumption is further confirmed by the fact that higher intakes of total fat and saturated fatty acids have been inversely associated with BMD [67, 68], while positive relationships have been reported between monounsaturated and polyunsaturated fatty acid intake and bone density [69, 70]. Furthermore, previous studies have demonstrated a positive relationship between the intake of vitamin B_1 and BMD in postmenopausal women [71], while the effects of high carbohydrate intake on women's bone mass have been varied, depending on the type of carbohydrate [69, 72].

Some points must be considered in interpreting our findings. First, because of the cross-sectional design of the study, causality cannot be inferred between the nutrient patterns and BMD. Second, our sample size was relatively small, which might have attenuated our ability to detect potential significant associations. Third, several subjective or arbitrary decisions must be made by the investigators during the use of factor analysis, which may affect the results and their interpretation [73]. Fourth, factor analysis is sometimes criticized because of the dependence of identified factors on the population under study. Therefore, the validity and reliability of our findings require confirmation in other populations.

In summary, the results of the present study show that a nutrient pattern characterized by high intakes of nutrients abundantly found in fruits and vegetables including folate, total fiber, vitamin B_6, potassium, vitamin A (as RAE), vitamin C, β-carotene, vitamin K, magnesium, copper, and manganese is positively associated with BMD at the lumbar spine among postmenopausal Iranian women. This finding further confirms the hypothesis that high consumption of fruits and vegetables may be beneficial for bone health in postmenopausal women. However, further large-scale studies of sufficient methodological quality are needed to support our findings.

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Ethical Considerations All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 [15]. Informed consent was obtained from all patients for being included in the study.

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