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Diet Modulates the Effects of Genetic Variants on the Vitamin D Metabolic Pathway and Bone Mineral Density in Mexican Postmenopausal Women

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ABSTRACT

Background: Macro- and micronutrients, such as proteins, vitamin D, and calcium (Ca), are important dietary factors that can modify bone mineral density (BMD). Genetic factors can interact with diet, affecting an individual's predisposition to osteoporosis.

Objectives: This study aimed to evaluate the associations between macro- and micronutrient intakes and BMD in Mexican postmenopausal women, and their interactions with genetic polymorphisms involved in the vitamin D metabolic pathway.

Methods: We analyzed data from 317 postmenopausal women from the Health Workers Cohort Study, a longitudinal cohort studied in Cuernavaca, Mexico. Postmenopausal women participated in 2 data collection waves (2004–2006 and 2010–2011), with a mean time of 6.4 years. Dietary intake was assessed with a semi-quantitative FFQ. BMD (femoral neck, hip, and lumbar spine) was measured by DXA. Hybrid mixed-effects regression models were used to assess the associations of dietary macro- and micronutrients on BMD, after adjusting for confounding factors and for diet and single nucleotide polymorphism interactions.

Results: At baseline, the median age was 57 years (IQR, 50–64). Mean femoral neck, hip, and lumbar spine BMDs decreased over time. We observed statistically significant longitudinal associations for diet (Ca, vitamin D, magnesium, phosphorus, and protein intake) and BMD. Increases of vitamin D, Ca, and protein intakes by 1 SD were associated with mean increases in the femoral neck BMD (0.083 SD, 0.064 SD, and 0.130 SD, respectively). Multiple significant interactions were identified between several loci (*CYP2R1*, *CYP24A1*, *CYP27B1*, *VDR,* and *DHCR7/NADSYN1*) and diet for BMDs (femoral neck, hip, and lumbar spine), mainly for protein intake.

Conclusions: Our data support associations of vitamin D, Ca, protein, phosphorous, and magnesium consumption with BMD in Mexican postmenopausal women and suggest possible gene-diet interactions. These results could facilitate future personalized nutrition recommendations to help prevent low BMD. *J Nutr* 2021;151:1726–1735.

Keywords: bone mineral density, gene-diet interaction, macronutrients, micronutrients, vitamin D

Introduction

A decrease in bone mineral density (BMD) and impaired bone quality are natural aging processes that predispose individuals, especially postmenopausal women, to osteoporosis (1). Osteoporosis is a chronic disease characterized by low bone mass and structural deterioration of bone tissue, resulting in increased bone fragility and susceptibility to fracture. Osteoporosis has significant economic and social impacts, including deterioration in the quality of life (2). In Mexico, the costs of managing osteopenia and osteoporosis account for up to 154.9 million USD, whereas costs related to fragility fractures

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(FF) reach 256.2 million USD in health-care services. Estimates from 2010 in Mexico list the forearm (45%) as the most frequent site of a fragility fracture, followed by the hip (21%), spine (19%), and humerus (15%). However, forearm fractures represented only 35% of FF expenses in Mexico during 2010. In contrast, hip and spine fractures accounted for approximately 36% and 20%, respectively, of the entire medical care costs. The current increase in life expectancy implies greater risks of FF, and therefore a major economic burden (3).

Although significant risk factors have been identified, dietary factors, specifically macronutrient and micronutrient intakes, represent a critical understudied area in osteoporosis research. Proteins, carbohydrates, fats, and dietary fibers are the macronutrients and vitamins and minerals [vitamin D, calcium (Ca), magnesium, and phosphorous] are the micronutrients most involved in bone health (4, 5). Nutrients associated with fruit and vegetable intake (in particular, potassium and magnesium) have been associated with high BMD in late premenopausal women (6). Specifically, nutrients such as vitamin C, niacin, protein, phosphorous, zinc, and folate have been associated with increased BMDs in postmenopausal women (7), while iron and magnesium (together with zinc for premenopausal women) were associated with more significant forearm bone mineral content (7, 8). A placebo-controlled trial in which postmenopausal women were supplemented with zinc, copper, and manganese, in addition to Ca, resulted in a small increase in BMD after 2 years (9). The results of another study suggested that although menopausal status and hormone replacement therapy (HRT) use are the overriding factors affecting bone loss in women in their early fifties, dietary Ca may help in preventing bone loss at the hip. However, intakes of monounsaturated and polyunsaturated fatty acids (and possibly vitamin A) appear to increase bone loss, and the detrimental effect of PUFAs is more pronounced when Ca intake is low (10). Although macro- and micronutrients have been shown to have beneficial effects on bone health, a clear relationship with bone metabolism has not been established (10–12). The development of degenerative bone diseases, and especially osteoporosis, is believed to be associated with genetic and environmental factors and their interactions. The study of gene-environment interactions has been present since the early days of genetic research, and is an active research field (13).

Examinations of single nucleotide polymorphisms (SNPs) in the genes relevant to dietary intake and vitamin D metabolism could provide mechanistic insights of the physiopathology of BMD loss (14). The concept that the interactions between diet and relevant genetic variants affect BMD has been previously reported. Previous studies in different populations indicate that Ca and vitamin D intakes may modify the effects of several gene variants (*VDR* and *GC*) on BMD (15, 16). However, to date, no studies have evaluated this association in the Mexican population (17, 18). The present study aimed to analyze whether specific dietary components modulate the effect of genetic variants on the vitamin D metabolic pathway, and consequently on BMD values.

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Supplemental Figure 1 and Supplemental Tables 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: AIMs, ancestry informative markers; BMD, bone mineral density; Ca, Calcium; FF, fragility fractures; HRT, hormone replacement therapy; HWCS, Health Workers Cohort Study; IMSS, Mexican Institute of Social Security; LTPA, leisure time physical activity; SNP, single nucleotide polymorphism; VKDP, VK-dependent protein.

Methods

Subjects and study design

We performed longitudinal and cross-sectional analyses with data from postmenopausal women belonging to the Health Workers Cohort Study (HWCS). The HWCS was designed to evaluate the associations between genetic and lifestyle factors on different health outcomes in the Mexican population. The study design and methodology of the HWCS have been previously published (19). Briefly, the HWCS is a dynamic, prospective, open-cohort study composed of employees from the Mexican Institute of Social Security (IMSS for its Spanish acronym) and their families in Cuernavaca, Morelos. The sample included in this analysis was composed of postmenopausal women who enrolled between 2010–2011, and represents 42% of the total postmenopausal women evaluated in the second wave of the HWCS (2010–2012). Postmenopausal women were defined as being older than 45 years old and having experienced 12 months without a menstrual period. For the present study, we included a sample of 317 postmenopausal women aged 45–92 years who had a complete diet assessment and BMD data at baseline (2004 to 2006) and follow-up (from 2010 to 2011). We also had information about ancestry informative markers (AIMs) to rule out false associations due to stratification of the population (**Supplemental Figure 1**) (20). There were no statistically significant differences between the postmenopausal women included in the analysis and those not included (data not shown).

Ethics approval

The National Research and Ethics Committee of the IMSS evaluated and approved all of the study procedures. All subjects were informed that their participation in the study was voluntary, and were asked to sign an informed consent form (19).

Data collection

BMD measurements.

Femoral neck BMD (g/cm^2), hip BMD (g/cm^2), and lumbar spine BMD (g/cm^2) measurements were obtained following standard procedures. BMDs were measured from the nondominant proximal femur, the lumbar spine (L1–L4), and the whole hip using a DXA Lunar DPX NT instrument (Lunar Radiation Corp.) (19). Daily quality control checks were conducted using the manufacturer phantom; the daily variation coefficient was within usual operational standards, and the in vivo variation coefficient was lower than 1.0%–1.5% (19).

Dietary assessment.

We used a semi-quantitative FFQ that was previously validated in a Mexican population (21). The questionnaire collects data regarding the consumption frequency of 116 food items during the previous year. The instrument specifies commonly used size portions. For each type of food or beverage, there are 10 frequency response categories. Average daily nutrient intakes were calculated by multiplying the frequency of consumption of each food by the nutrient content (19). We obtained the information on nutrient intake from a comprehensive database of food contents (22). In this study, the nutrients were divided into 2 categories: macronutrients (protein) and micronutrients (vitamin D, vitamin K, Ca, magnesium, potassium, phosphorous).

SNP genotyping.

Genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA Isolation Kit (QIAGEN systems Inc.), according to the manufacturer's instructions. In a previous study (23), a GoldenGate

design (Illumina) including a total of 384 SNPs was used to test for associations with BMD. The array contained 29 SNPs involved in the vitamin D metabolic pathway and 96 AIMs. The 29 SNPs related to vitamin D were selected based on public information available in dbSNP: a database of single nucleotide polymorphisms (Genome Build version 36.2; http://www.ncbi.nlm.nih.gov) using the following criteria: SNPs identified through genome-wide association studies of 25(OH)D serum concentrations, minor allele frequency >5% in Europeans, SNPs with functional relevance, and SNPs previously identified in association studies of 25(OH)D. To complete the design, TagSNPs were selected with the Tagger program (http://www.broad.mit.edu/mpg/tagger/) using pairwise tagging with a r^2 threshold of 0.8 (24). These SNPs were genotyped following the Illumina protocol in a sample of 400 postmenopausal women who participated in the second wave of the HWCS (23). Quality control exclusions were implemented, such as for those with a low SNP call rate (<97%), deviation from Hardy-Weinberg equilibrium ($P \leq 0.05$), related women, and gender concordance. Genotype data of these 29 SNPs were extracted for this analysis.

Other measurements.

Participants completed a self-administered questionnaire focused on characteristics such as birth date, education, past medical history, current medication use, and lifestyle information (e.g., diet, smoking status, alcohol consumption) (19). Physical activity among participants was measured through a self-administered questionnaire, validated in its Spanish-translated version and adapted to be used in a Mexican population (25). This questionnaire estimates leisure time physical activity (LTPA) in minutes per week during a typical week within the past year. The survey includes 16 items. To obtain the daily LTPA, the time and frequency spent on each activity were recorded and added, then the total time was divided by 7. At baseline, the questionnaire included 3 questions regarding use of dietary supplements. Multivitamin supplement use was assessed by asking the participants "during the past 4 years, have you taken multivitamins?" Women who confirmed their consumption were asked to provide the brand and amount taken per week. Anthropometric measurements and blood samples were collected by trained personnel using standardized techniques (19). For the classification of BMI status, we use the WHO cutoff points (26).

Statistical analysis

Descriptive statistical analyses of the main variables of interest by wave were performed using measures of central tendency for continuous variables and frequencies for categorical variables. The sign test of matched pairs (for continuous variables) and McNemar's test (for categorical variables) were used to evaluate differences at different time points. The associations between macro- and micronutrient consumption and BMDs (femoral neck, hip, and lumbar spine) were determined with a hybrid mixed-effects regression model. This model simultaneously estimates the coefficients of longitudinal associations within individuals and the coefficients of the cross-sectional associations between subjects (27). For each model, the coefficients were expressed in SDs for BMD and diet (i.e., SD change in BMD by the change in 1 SD of the specific nutrient).

The model is described below:

$$BMD_{it} = \beta_0 + \beta_1(diet_{it} - \overline{diet_i}) + \beta_2\overline{diet_i} + \beta_3 Confounder_i + \sum_{k=4}^{k} \beta_k Confounder_{k,it} + b_i + \varepsilon_{it} \quad (1)$$

Here, i indicates subjects and t represents period 0, 1. Diet represents the nutrients, evaluated separately (Ca, vitamin D, phosphorus, potassium, magnesium, vitamin K, and protein intake); β_1 represents the estimated longitudinal associations; β_2 represents cross-sectional associations; $confounder_i$ includes baseline age and baseline dietary supplement consumption; and $confounder_{it}$ includes BMI, alcohol intake, smoking, use of HRT, and LTPA. For the Ca intake model, confounders were adjusted for consumption of Ca supplements. Finally, ε_{it} indicates the error term and b_i indicates subject-level random effects. It is assumed that b_i and ε_{it} are independently and normally distributed, with means of 0 and constant variance (27). It has been reported that adjusting for total energy intake in nutritional epidemiology can control for confounding (28). Various adjustment methods that can be used include the residual method, which provides an estimation of nutrient intake uncorrelated with total energy. To be consistent with other diet and BMD studies, we performed a sensitivity analysis adjusting for energy intake by including it as a covariate (the standard multivariate method of energy adjustment) and using the nutrient residuals method (28). Furthermore, we explored intra-individual associations between changes in macro- and micronutrient intake categories, defined by tertiles and BMDs (g/cm^2; femoral neck, hip, and lumbar spine), using the fixed effects regression model. These models were adjusted by BMI, alcohol intake, smoking, and LTPA. Analyses to evaluate the differences across time and associations between diet and BMD with a P value < 0.05 were considered statistically significant.

We also performed interaction analyses to explore whether the intake of specific nutrients modifies the effects of some polymorphisms on BMDs (femoral neck, hip, and lumbar spine). This analysis was performed by introducing the interaction term (specific nutrient x specific SNP) as a covariate in the hybrid mixed-effects models. The associations between diet and BMDs (femoral neck, hip, and lumbar spine) for the heterozygous genotype and homozygous mutant genotype were estimated with the linear combination of the corresponding coefficients (main coefficient + interaction coefficient for each genotype). Ancestry estimates were evaluated through a principal component analysis, using PLINK software and the smartpca program in EIGENSOFT v3.0 package (29), and were included in the interaction models to control for the effect of false associations due to population stratification (30). A Bonferroni adjustment was used to correct for gene-nutrient interactions (α/406); therefore, significance was inferred when a P value was <0.0001. The Hardy-Weinberg equilibrium was tested for each SNP using a chi-square test, and the SNPs were in equilibrium (P values > 0.1). All analyses were performed using STATA 13.0 (31).

Results

This study included 317 nonrelated postmenopausal women. The average time between the baseline and follow-up assessments was 6.4 years (SD, 0.5). At baseline, the average age was 57 years (IQR, 50–64), 25.2% confirmed dietary supplement consumption, 55.5% had an elementary or secondary education, 13.9% completed high school, and 27.4% had a higher education level. Nearly 44% of the sample had overweight and 26.5% had obesity. The mean femoral neck BMD decreased from 0.921 g/cm^2 at baseline to 0.873 g/cm^2 at follow-up. The mean hip and lumbar spine BMDs also decreased over time (from 0.959 to 0.917 and from 1.035 to 0.999 g/cm^2, respectively). However, no significant change was observed in the proportion of women with a normal femoral neck BMD between baseline and follow-up (43.2% compared with 42.0%, respectively). The proportion of women with a normal hip BMD decreased significantly from baseline to follow-up (69.7% compared with 58.1%, respectively). The same trend was observed for the lumbar spine BMD, with the proportion of women with a normal value decreasing significantly between baseline and follow-up (36.7% compared with 28.5%, respectively). Furthermore, the frequency of osteopenic hip BMDs increased from baseline to follow-up (37.4% compared with 26.8%, respectively).

Median energy and nutrient intakes decreased significantly between baseline and follow-up. At baseline, median levels of macro- and micronutrient intakes were 210 IU/day (IQR,

TABLE 1 Descriptive statistics of the 317 postmenopausal women from Health Workers Cohort Study

Values are mean (SD) or median [25th, 75th percentile]	Baseline	Follow-up	*P* value[1]
Age, years	57 [50, 64]	63 [57, 70]	
BMI, kg/m^2	27.1 [24.6, 30.1]	27.1 [24.8, 30.0]	0.09
Nutritional status			
Overweight, %	43.9	48.3	0.11
Obesity, %	26.5	25.9	0.86
Smoker status			
Current, %	11.7	6.9	0.001
Past, %	24.9	30.3	0.001
Body fat proportion	45.0 [40.1, 48.6]	45.3 [41.6, 49.2]	0.02
Leisure time physical activity, min/day	13.0 [3.2, 47.1]	9.7 [1.4, 33.2]	0.10
Femoral neck BMD, g/cm^2	0.921 (0.135)	0.873 (0.127)	<0.001
Z-score femoral neck	0.358 (0.926)	0.235 (0.740)	<0.001
T-score femoral neck	−1.134 (0.906)	−1.172 (0.905)	<0.001
Femoral neck BMD status			
Normal, %	43.2	42.0	0.22
Osteopenic, %	50.1	51.3	0.45
Osteoporotic, %	6.7	6.7	1.00
Hip BMD, g/cm^2	0.959 (0.140)	0.917 (0.137)	<0.001
Z-score hip	0.389 (0.932)	0.419 (0.903)	0.13
T-score hip	−0.396 (1.143)	−0.721 (1.085)	<0.001
Hip BMD status			
Normal, %	69.7	58.1	<0.001
Osteopenic, %	26.8	37.4	<0.001
Osteoporotic, %	3.5	4.5	0.38
Lumbar spine BMD, g/cm^2	1.035 (0.171)	0.999 (0.893)	<0.001
Z-score lumbar spine	0.358 (0.926)	0.235 (0.740)	0.04
T-score lumbar spine	−1.355 (1.370)	−1.645 (1.246)	<0.001
Lumbar spine BMD status			
Normal, %	36.7	28.5	0.0007
Osteopenic, %	42.1	45.9	0.15
Osteoporotic, %	21.2	25.6	0.02
Diet			
Total energy, kcal/day	1890 [1480, 2420]	1670 [1190, 2140]	<0.001
Alcohol, g/day	0.8 [0, 1.8]	0.5 [0, 1.5]	0.006
Vitamin D intake, IU/day	210 [140, 320]	150 [90, 260]	<0.001
Vitamin K intake, μg/day	77.3 [45.4, 116]	75.0 [43.2, 110]	0.29
Calcium intake, mg/day	930 [710, 1300]	820 [520, 1130]	<0.001
Phosphorus intake, mg/day	1300 [1010, 1680]	1080 [820, 1480]	<0.001
Magnesium intake, mg/day	360 [270, 470]	310 [230, 420]	<0.001
Potassium, mg/day	3540 [2490, 4860]	3010 [2050, 4200]	<0.001
Total protein intake, g/day	66.4 [51.1, 86.0]	50.1 [37.8, 66.9]	<0.001
Hormone replacement therapy, %	7.8	9.5	0.008

Abbreviation: BMD, bone mineral density.
[1] *P* values from sign test of matched pairs (continuous variables) or McNemar's test (categorical variables).

140–320) for vitamin D, 930 mg/day (IQR, 710–1300) for Ca, 1300 mg/day (IQR, 1010–1680) for phosphorus, 360 mg/day (IQR, 270–470) for magnesium, and 66.4 g/day (IQR, 51.1–86.0) for protein (Table 1).

In both adjustment sets, we observed within-individual associations of vitamin D, Ca, phosphorous, magnesium, potassium, and protein intakes with BMD (femoral neck, hip, and lumbar spine) changes over time ($P < 0.05$). For example, a 1-SD increase in vitamin D consumption between the baseline and follow-up assessments (~170 IU/day) was associated with an average increase of 0.083 SD (0.010 g/cm^2) in femoral neck BMD. However, no statistically significant differences in between-individual associations were observed (Tables 2 and 3).

A sensitivity analysis showed statistically significant, positive intra-individual associations between diet (vitamin D, phosphorus, and protein) and BMDs (femoral neck and hip) using both energy adjustment methods. However, we observed differences for Ca, vitamin K, magnesium, phosphorous, and potassium intakes (**Supplemental Tables 1** and **2**). These differences could explain why, in the residual model, the variables of nutrient residual and total energy intakes are no longer correlated; therefore, we avoided collinearity problems (28).

Additionally, in the models that accounted for energy adjustment with the nutrient residual method, we observed associations between individuals for protein (β, 0.124; 95% CI: 0.010–0.237) and magnesium (β, 0.115; 95% CI: 0.007–0.225) intakes in femoral neck BMD. For hip BMD, we observed

TABLE 2 Within- and between-subject associations for specific nutrient intakes and femoral neck and hip BMD from Health Workers Cohort Study

	Outcome							
	Femoral neck BMD				Hip BMD			
	Model 1[2]		Model 2[3]		Model 1[2]		Model 2[3]	
Nutrient intakes (SD[1])	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Within-subject associations								
Vitamin D (176 IU/day)	0.083 (0.034–0.132)	0.001	0.083 (0.034–0.132)	0.001	0.066 (0.023–0.110)	0.003	0.066 (0.023–0.110)	0.003
Calcium[4] (601 mg/day)	0.064 (0.013–0.114)	0.01	0.064 (0.013–0.114)	0.01	0.051 (0.007–0.096)	0.02	0.051 (0.007–0.096)	0.02
Vitamin K (119 μg/day)	− 0.034 (−0.083 to 0.016)	0.18	− 0.034 (−0.083 to 0.016)	0.18	− 0.034 (−0.077 to 0.010)	0.13	− 0.034 (−0.077 to 0.010)	0.13
Phosphorus (624 mg/day)	0.094 (0.044–0.144)	0.0002	0.094 (0.044–0.144)	0.0002	0.077 (0.033–0.122)	0.001	0.077 (0.033–0.122)	0.001
Magnesium (185 mg/day)	0.068 (0.013–0.121)	0.02	0.067 (0.013–0.120)	0.02	0.056 (0.009–0.104)	0.02	0.056 (0.009–0.104)	0.02
Potassium (2109 mg/day)	0.071 (0.016–0.126)	0.01	0.071 (0.016–0.126)	0.01	0.064 (0.015–0.112)	0.01	0.064 (0.015–0.112)	0.01
Protein (31.8 g/day)	0.130 (0.081–0.179)	1.9×10^{-7}	0.130 (0.081–0.179)	1.9×10^{-7}	0.113 (0.070–0.156)	3.1×10^{-7}	0.113 (0.070–0.156)	3.1×10^{-7}
Between-subject associations								
Vitamin D (176 IU/day)	− 0.020 (−0.134 to 0.093)	0.73	− 0.019 (−0.132 to 0.094)	0.74	− 0.091 (−0.201 to 0.026)	0.13	− 0.088 (−0.205 to 0.028)	0.14
Calcium[4] (601 mg/day)	− 0.075 (−0.192 to 0.041)	0.21	− 0.071 (−0.187 to 0.045)	0.23	− 0.122 (−0.242 to −0.002)	0.05	− 0.127 (−0.247 to −0.007)	0.04
Vitamin K (119 μg/day)	− 0.082 (−0.195 to 0.031)	0.16	− 0.0929 (−0.207 to 0.021)	0.11	− 0.124 (−0.241 to −0.007)	0.04	− 0.132 (−0.249 to −0.014)	0.03
Phosphorus (624 mg/day)	− 0.071 (−0.190 to 0.049)	0.25	− 0.068 (−0.188 to 0.051)	0.26	− 0.106 (−0.229 to 0.0176)	0.09	− 0.102 (−0.225 to 0.021)	0.10
Magnesium (185 mg/day)	− 0.070 (−0.184 to 0.043)	0.23	− 0.074 (−0.188 to 0.040)	0.20	− 0.091 (−0.209 to 0.026)	0.13	− 0.091 (−0.209 to 0.026)	0.13
Potassium (2109 mg/day)	− 0.084 (−0.200 to 0.030)	0.15	− 0.091 (−0.205 to 0.023)	0.12	− 0.105 (−0.223 to 0.012)	0.08	− 0.109 (−0.226 to 0.009)	0.07
Protein (31.8 g/day)	− 0.052 (−0.173 to 0.068)	0.40	− 0.054 (−0.174 to 0.067)	0.38	− 0.077 (−0.202 to 0.047)	0.23	− 0.077 (−0.201 to 0.048)	0.23

Abbreviation: β, beta coefficient; BMD, bone mineral density; Ca, calcium.

[1]Parameter coefficients are expressed for a change of 1 SD in nutrient intake and BMD.

[2]Model 1 was adjusted for age (years), BMI (kg/m^2), alcohol consumption (g/day), smoking status (nonsmoker, smoker, ex-smoker) and leisure time physical activity (minutes per day).

[3]Model 2 was additionally adjusted for supplement intakes and use of hormone replacement therapy.

[4]Additional adjustment for the consumption of Ca supplements.

TABLE 3 Within- and between-subject associations for specific nutrient intakes and lumbar spine BMD from Health Workers Cohort Study

	Model 1[2]		Model 2[3]	
Nutrients (SD[1])	β (95% CI)	*P* value	β (95% CI)	*P* value
Within-subject associations				
Vitamin D (176 IU/day)	0.072 (0.016–0.127)	0.01	0.072 (0.017–0.126)	0.011
Calcium[4] (601 mg/day)	0.065 (0.007–0.122)	0.03	0.065 (0.008–0.122)	0.03
Vitamin K (119 μg/day)	− 0.032 (−0.090 to 0.027)	0.29	− 0.032 (−0.090 to 0.027)	0.29
Phosphorus (624 mg/day)	0.097 (0.042–0.153)	0.001	0.097 (0.042–0.153)	0.001
Magnesium (185 mg/day)	0.082 (0.022–0.141)	0.007	0.082 (0.022–0.141)	0.007
Potassium (2109 mg/day)	0.082 (0.021–0.142)	0.008	0.081 (0.021–0.142)	0.009
Protein (31.8 g/day)	0.137 (0.083–0.191)	6.7×10^{-7}	0.137 (0.083–0.191)	6.7×10^{-7}
Between-subject associations				
Vitamin D (176 IU/day)	0.039 (−0.089 to 0.166)	0.55	0.046 (−0.080 to 0.126)	0.48
Calcium[4] (601 mg/day)	− 0.008 (−0.139 to 0.123)	0.91	− 0.018 (−0.15.0 to 0.114)	0.79
Vitamin K (119 μg/day)	0.002 (−0.126 to 0.130)	0.97	− 0.007 (−0.135 to 0.121)	0.91
Phosphorus (624 mg/day)	0.011 (−0.112 to 0.146)	0.87	0.018 (−0.116 to 0.152)	0.79
Magnesium (185 mg/day)	0.020 (−0.108 to 0.147)	0.77	− 0.109 (−0.226 to 0.009)	0.07
Potassium (2109 mg/day)	0.009 (−0.119 to 0.138)	0.89	0.005 (−0.123 to 0.133)	0.94
Protein (31.8 g/day)	0.037 (−0.100 to 0.173)	0.60	0.037 (−0.100 to 0.172)	0.60

Abbreviation: β, beta coefficient; BMD, bone mineral density; Ca, calcium.
[1] Parameter coefficients are expressed for a change of 1 SD in nutrient intake and BMD.
[2] Model 1 was adjusted for age (years), BMI (kg/m^2), alcohol consumption (g/day), smoking status (nonsmoker, smoker, ex-smoker), and leisure time physical activity (minutes per day).
[3] Model 2 was additionally adjusted for supplement intakes and use of hormone replacement therapy.
[4] Additional adjustment for the consumption of Ca supplements.

associations between individuals for protein intake (β, 0.124; 95% CI: 0.010–0.237) in Model 1 (Supplemental Table 1). However, we did not observe associations between individuals for lumbar spine BMD (Supplemental Table 2).

In comparison, we observed statistically significant within-individual associations between changes in macro- and micronutrient intake categories (defined by tertiles) and BMDs (femoral neck, hip, and lumbar spine; **Supplemental Tables 3** and **4**).

There were no significant gene-diet interactions for between-individual associations with BMD (data not shown). Notably, we did not observe statistically significant interactions between vitamin K intake and SNPs for within- and between-individual associations (data not shown).

Conversely, we observed statistically significant interactions between the SNPs involved in the vitamin D metabolic pathway and the consumption of Ca, vitamin D, protein, phosphorus, potassium, and magnesium for within-subject associations with BMDs (femoral neck, hip, and lumbar spine; **Table 4**; **Supplemental Tables 5–9**).

We observed statistically significant interactions between vitamin D intake and the SNPs of the *CYP2R1*, *CYP24A1*, and *DHCR7/NADSYN1* genes in femoral neck and hip BMDs, but for lumbar spine BMD we only observed interaction with the *CYP2R1* gene. For example, under a codominant model, in women with the AA genotype of rs10766197 in *CYP2R1*, with each additional standard unit of vitamin D consumption ($\sim$ 170 IU/day) there was an increase of 0.421 SD in the femoral neck BMD. In contrast, among women with the wild-type GG genotype, we observed an increase of 0.091 SD on BMD (*P* for interaction = 0.026; Supplemental Table 5). Interactions with Ca consumption were observed for variants in *CYP27B1* with the femoral neck BMD, variants in *CYP24A1* with the hip BMD, and variants in *CYP2R1* with the lumbar spine BMD (Supplemental Table 6). Additionally, we found intra-individual interactions between *CYP24A1*, *DHCR7/NADSYN1*, and *CYP27B1* SNPs and protein consumption for the femoral neck and hip BMDs (Table 3).

Statistically significant interactions with phosphorus and potassium consumption were observed for SNPs in *CYP24A1* and *CYP27B1* in femoral neck and hip BMDs (Supplemental Tables 7 and 8), and with magnesium consumption for SNPs in *CYP2R1*, *CYP24A1*, *CYP27B1*, and *VDR* in femoral neck and hip BMDs (Supplemental Table 9). For the lumbar spine BMD, we observed interactions between the rs2244719 variant in *CYP24A1* and phosphorus intake; interactions between variants in *CYP27B1*, *CYP2R1*, *DHCR7/NADSYN1*, and *VDR* and potassium intake; and interactions between variants in *CYP24A1* and *CYP27B1* and magnesium intake (Supplemental Tables 7–9). Additionally, we explored the adjustment for multivitamin intake at baseline and HRT; however, the results of the interactions did not change (data not shown).

Associations derived from the interactions were not statistically significant at the Bonferroni-corrected significance threshold (<0.0001), except for the associations with protein intake ($P < 2 \times 10^{-5}$). We also explored the effect of energy adjustment in the interaction models and the results did not change, except for Ca and magnesium in the calorie adjustment model (data not shown).

Discussion

The present study results indicate that there is a longitudinal association between the intakes of macro- and micronutrients, including protein, vitamin D, Ca, phosphorous, potassium, and magnesium, and BMD among Mexican postmenopausal women. It is important to note that a decrease was observed in the number of participants who had a BMD within the healthy range for the hip and lumbar spine; this observation agrees with previous work reporting that BMD decreases with age (1, 2).

TABLE 4 Interactions between protein intake, genetic polymorphisms on the vitamin D metabolic pathway, and BMD from Health Workers Cohort Study

				Outcome					
				Femoral neck BMD		Hip BMD		Lumbar spine BMD	
Gene	SNP	Genotype	n	Protein (31.8 g/day),[1] β (95% CI)[2]	*P* value	Protein (31.8 g/day),[1] β (95% CI)[2]	*P* value	Protein (31.8 g/day),[1] β (95% CI)[2]	*P* value
CYP24A1	rs2244719	CC	178	0.075 (0.004–0.145)	0.04	0.067 (0.005–0.128)	0.03	0.100 (0.021–0.178)	0.012
		CT	125	0.144 (0.068–0.220)	0.0002	0.119 (0.053–0.185)[3]	0.0004	0.119 (0.034–0.204)[3]	0.006
		TT	14	0.295 (0.161–0.429)[4]	$P^5 = 1.5 \times 10^{-5}$	0.294 (0.175–0.413)[4]	$P^5 = 1.3 \times 10^{-6}$	0.343 (0.196–0.489)[4]	$P^5 = 4.5 \times 10^{-6}$
CYP24A1	rs2296241	GG	115	0.104 (−0.029 to 0.179)	0.007	0.088 (0.023–0.154)	0.008	—	
		GA	148	0.122 (0.052–0.192)[3]	0.001	0.110 (0.049–0.171)[3]	0.0004	—	
		AA	48	0.294 (0.137–0.452)[4]	0.0002	0.268 (0.127–0.409)[4]	0.0002	—	
	rs7944926	AA	86	0.203 (0.106–0.300)	$P^5 = 4 \times 10^{-5}$	0.198 (0.114–0.283)	$P^5 = 4.4 \times 10^{-6}$	—	
DHCR7/NADSYN1									
		AG	149	0.130 (0.060–0.201)	0.0003	0.103 (0.042–0.165)	0.001	—	
		GG	76	0.059 (−0.037 to 0.156)[4]	0.23	0.057 (−0.027 to 0.141)[4]	0.18	—	
	rs4944957	AA	87	0.205 (0.106–0.305)	$P^5 = 5 \times 10^{-5}$	0.198 (0.111–0.285)	$P^5 = 7.4 \times 10^{-6}$	—	
DHCR7/NADSYN1									
		AG	151	0.133 (0.062–0.204)	0.0003	0.105 (0.043–0.168)	0.001	—	
		GG	79	0.062 (−0.030 to 0.154)[4]	0.19	0.062 (−0.019 to 0.142)[4]	0.13	—	
	rs12800438	GG	85	0.204 (0.104–0.303)	$P^5 = 6 \times 10^{-5}$	0.196 (0.110–0.283)	$P^5 = 8.9 \times 10^{-6}$	—	
DHCR7/NADSYN1									
		GA	155	0.130 (0.060–0.200)[3]	0.0002	0.103 (0.042–0.164)	0.001	—	
		AA	71	0.062 (−0.035 to 0.159)[4]	0.21	0.064 (−0.020 to 0.149)[4]	0.14	—	
CYP27B1	rs4646536	AA	117	0.192 (0.109–0.275)	$P^5 = 6.2 \times 10^{-6}$	0.164 (0.091–0.237)	$P^5 = 1.1 \times 10^{-5}$	—	
		AG	152	0.144 (0.074–0.214)[3]	$P^5 = 5 \times 10^{-5}$	0.124 (0.063–0.186)[3]	$P^5 = 7.3 \times 10^{-5}$	—	
		GG	42	−0.0169 (−0.130 to 0.097)[4]	0.77	0.004 (−0.096 to 0.104)[4]	0.93	—	
CYP27B1	rs703842	AA	115	0.190 (0.106–0.274)	$P^5 = 8.7 \times 10^{-6}$	0.163 (0.090–0.237)	$P^5 = 1.3 \times 10^{-5}$	—	
		AG	156	0.136 (0.068–0.205)[3]	0.0001	0.121 (0.061–0.182)	$P^5 = 7.9 \times 10^{-5}$	—	
		GG	40	−0.006 (−0.126 to 0.113)[4]	0.92	0.001 (−0.104 to 0.107)[4]	0.99	—	
GC	rs7041	TT	29	—		—		0.123 (0.021–0.226)	0.018
		TG	145	—		—		0.082 (0.007–0.157)[3]	0.031
		GG	77	—		—		0.296 (0.181–0.411)[4]	$P^5 = 4.5 \times 10^{-7}$

Abbreviation: β, beta coefficient; BMD, bone mineral density; SNP, single nucleotide polymorphism.
[1]Parameter coefficients are expressed for a change of 1 SD in protein intake and BMD.
[2]Model adjusted for: age (years), ancestry (3 components), BMI (kg/m^2), alcohol consumption (g/day), smoking status (nonsmoker, smoker, ex-smoker) and leisure time physical activity (min/day).
[3]Contrast *P* value < 0.05: heterozygous genotype compared with homozygous mutant genotype.
[4]Contrast *P* value < 0.05: wild-type genotype compared with homozygous mutant genotype.
[5]Statistically significant coefficients after applying the Bonferroni adjustment ($P < 0.0001$).

Even though the data were derived from the FFQ, we observed that a high percentage of postmenopausal women had micronutrient intakes below the RDAs (32): 85.2% of the postmenopausal women had a vitamin D intake <400 IU/day, 68.4% had a Ca intake of <1200 mg/day, 72.6% consumed <4700 mg/day of potassium, and 37.8% had a magnesium intake of <320 mg/day.

According to the literature, the relationship between Ca, vitamin D, and BMD has been extensively studied, suggesting that high consumption of these nutrients is associated with higher BMD values (17, 33). However, unlike the findings of our study, which support a longitudinal association even after adjusting for fixed variables and variables that change over time, 2 recent meta-analyses did not show conclusive results (34, 35).

Around 85% of the body's phosphorus is found in bone mineral content, and phosphorus is a main component required for hydroxyapatite formation (36). The results of our study show an association between phosphorus consumption and a change in BMD over time. Some cross-sectional studies have also observed that higher phosphorus intake is associated with higher BMD levels in postmenopausal women (37, 38). In contrast, another study found that high phosphorus consumption is associated with an increase in fractures, although this analysis was not adjusted for potential confounders such as sex, tobacco consumption, alcohol consumption, and physical activity (39).

Magnesium positively affects the function of osteoblasts and osteoclasts, and it also stabilizes amorphous Ca phosphate, slowing its transformation to hydroxyapatite and making bones stronger (40). Studies have shown positive associations between magnesium consumption and BMD (41, 42). Additionally, a meta-analysis reported a marginal association between magnesium and femoral neck BMD (β, 0.14; 95% CI: 0.001–0.28), with high heterogeneity between studies (43). Our findings also support a longitudinal association between magnesium consumption and higher BMD values.

Protein constitutes one-third of total bone mass, of which type 1 collagen fibers account for the majority (44). Therefore, adequate protein intake is likely to play an essential role in bone resistance (45). Additionally, protein provides the structural matrix of bone, optimizes insulin-like growth factor levels, and increases intestinal absorption of Ca (46). Surprisingly, the impact of dietary protein intake on osteoporosis-related phenotypes in humans is a long-standing debate. Meta-analysis results indicate that protein consumption is associated with increased BMD at the lumbar spine, but not at the femoral neck or with total BMD (47). The Framingham study showed a more significant loss of femoral neck BMD after a 4-year follow-up among individuals in the lowest quartile of protein consumption compared to those in the highest quartile (48). The Framingham results are similar to those reported in the present study.

Previous studies have reported that vitamin K may affect BMD and the fracture incidence (49). Vitamin K is an essential coenzyme for the gamma-glutamyl carboxylase enzyme, which converts glutamic acid residues to gamma-carboxyglutamic acid within Vitamin K-dependent proteins (VKDPs). Osteocalcin belongs to the VKDPs; it regulates the transcription of osteoblastic markers, the formation of osteoclasts, and bone resorption (50). A meta-analysis including randomized control trials reported no evidence of vitamin K affecting BMD or the vertebral fractures incidences in post-menopausal or osteoporotic patients. Booth et al. (51) did not find a longitudinal association between vitamin K intake and BMD. Similar results were seen in our study; however, additional longitudinal studies are needed to confirm these findings.

We observed longitudinal associations between potassium intake and BMD. Zhu et al. (52) reported that after a 5-year follow-up, women in the highest quartile of urinary potassium excretion had a higher BMD than those in the lowest quartile. However, a previous study found no association between the baseline potassium intake and longitudinal changes in BMD (42). The opposite results may be related to measurement errors or adjustments for confounding factors.

Few studies have evaluated gene-diet interactions, and most of them have focused on the *VDR* and *GC* genes and the intakes of Ca and vitamin D. Stathopoulou et al. (15) reported that the Cdx-2, TaqI, and Bsml variants were associated with lumbar spine BMD only in postmenopausal women with lower Ca intake (<680 mg/d), while the TaqI and Bsml variants were associated with osteoporosis (ORs 2.32 and 2.18, respectively). Fang et al. (16) observed an interaction between the haplotype *GC*-1 (composed by the rs4588 and rs7041 SNPs) and low Ca consumption (<1.09 g/day) on the risk of osteoporosis, but found no interaction with the haplotype 1-*VDR* gene (BsmI-ApaI-TaqI). Our study did not observe an interaction between diet and SNPs in the *GC* gene on BMD. These differences may be due to sample size, uncontrolled environmental factors [Fang et al.'s study (16) only adjusted for age and sex], and differences in linkage disequilibrium patterns.

We observed significant interactions between magnesium intake and genetic variants in the *CYP2R1, CYP24A1, CYP27B1,* and *VDR* genes on the association with BMD changes over time. It has been reported that approximately 50–60% of the total body magnesium content is stored in bone. In bone, magnesium binds on the surfaces of hydroxyapatite crystals to determine its size. Magnesium deficiency is relatively common in the population and may be associated with osteoporosis (53). The interaction between magnesium intake and cholecalciferol causes Ca deposition in bone (54–56) and helps in the activation of vitamin D, regulation of Ca, and phosphate homeostasis to influence bone growth and maintenance (57). All of the enzymes that metabolize vitamin D seem to require magnesium, which acts as a cofactor in the enzymatic reactions in the liver and kidneys (57). Vitamin D, either cholecalciferol or ergocalciferol, does not have a significant biological activity. Vitamin D needs to be processed further in the liver and kidneys to generate the biologically active form 1,25-dihydroxyvitamin D. The enzymatic activity of both the hepatic 25-hydroxylase (CYP2R1) and the renal 1α-hydroxylase (CYP27B1) requires magnesium (58). However, the mechanism by which magnesium intake could regulate BMD through the *CYP2R1*, *CYP24A1*, *CYP27B1*, and *VDR* genes remains unclear and requires additional research.

Previous studies in vitro (59, 60) indicate that a high dietary Ca intake decreases the activity of 1-α hydroxylase (encoded by the *CYP27B1* gene) in the kidneys, while its activity in bone increases, promoting the incorporation of Ca in bone (59). Additionally, in primary human osteoblasts, high Ca concentrations increase the expression of *CYP27B1* (61). Our results support the role of the *CYP27B1* gene in bone and demonstrate interactions between SNPs and Ca intake in BMD. Additional studies are needed to identify the functionality of genes in BMD and their interactions with Ca.

To the best of our knowledge, this is the first study examining the interaction between protein intake and genetic variants in the *CYP24A1*, *CYP27B1*, and *DHCR7/NADSYN1* genes and BMD. This represents an initial effort to evaluate gene-nutrient interactions for genetic variants involved in the metabolism of vitamin D. These findings may provide the basis for developing

specific dietary interventions according to the genetic variants present in an individual.

An important strength of this analysis is the use of a hybrid mixed-effects model. This model controls for unobserved, time-invariant confounding factors with time-invariant effects when examining longitudinal associations (27).

This study has some limitations that should be addressed. First, the women in this study are probably more educated and healthier than the general population of Mexico. Therefore, our findings related to sociodemographic and dietary variables cannot predict associations at the national level. However, we believe that the HWCS population could properly represent adults living in urban areas of central Mexico. Although only 42% of the postmenopausal women who arrived in the second cohort measurement were followed-up, we did not observe differences between the included and excluded women; therefore, our results are less prone to selection bias. Second, after adjusting for multiple comparisons, only the protein intake interactions remained statistically significant, probably due to our limited sample size. However, we cannot rule out the possibility that the observed associations with the other nutrients are present; it must be considered that the adjustment by multiple tests controls the global type I error, but considerably inflates the type II error. Therefore, more studies focusing on genetic associations are needed to assess their interactions with other genetic and environmental factors. Third, we cannot rule out a possible measurement error when assessing dietary intake using the FFQ. However, this questionnaire has been validated in Mexican women and has been demonstrated to provide reasonable estimates for the nutrients evaluated (correlation 0.40 or higher between FFQ compared with 24-hour recalls), similar to those reported in other studies (21, 62). We assume that our study's measurement error is nondifferential, thus leading to attenuation of the associations. Fourth, the time elapsed between the baseline and follow-up assessments is long (average time 6.4 years), which possibly weakened some observed associations. Fifth, age is a significant cause of low BMD. Therefore, given our small sample size, we could not explore the associations between diet and BMD in the different age groups.

In conclusion, this study suggests that interactions between gene and diet influence BMD, which could facilitate the development of personalized nutrition programs to prevent or reduce the risk of osteoporosis. However, additional longitudinal studies in the Mexican adult population are required to confirm these results.

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The authors' responsibilities were as follows: RV-C, JS, and BR-P: designed the research, had primary responsibility for the final content, and conducted research; BR-P and RV-C: wrote the manuscript; BR-P and ADQ-S: analyzed the data or performed the statistical analysis; and all authors: drafted and edited the manuscript, and read and approved the final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, RV-C, upon reasonable request.

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