Genetically determined vitamin D levels and change in bone density during a weight-loss diet intervention: the Preventing Overweight Using Novel Dietary Strategies (POUNDS Lost) Trial

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ABSTRACT

Background: Obesity is closely associated with bone health. Although diet and weight loss produce many metabolic benefits, studies of weight loss diets on bone health are conflicting. Genetic variations, such as vitamin D levels, may partly account for these conflicting observations by regulating bone metabolism.

Objective: We investigated whether the genetic variation associated with vitamin D concentration affected changes in bone mineral density (BMD) in response to a weight-loss diet intervention.

Design: In the 2-y Preventing Overweight Using Novel Dietary Strategies (POUNDS Lost) trial, BMD was measured for 424 participants who were randomly assigned to 1 of 4 diets varying in macronutrient intakes. A genetic risk score (GRS) was calculated based on 3 genetic variants [i.e., 7-dehydrocholesterol reductase (*DHCR7*) rs12785878, cytochrome P450 2R1 (*CYP2R1*) rs10741657 and group-specific component globulin (*GC*) rs2282679] related to circulating vitamin D levels. A dual-energy X-ray absorptiometry scan was performed to assess changes in whole-body BMD over 2 y. The final analysis included 370 participants at baseline.

Results: We found a significant interaction between dietary fat intake and vitamin D GRS on 2-y changes in whole-body BMD (P-interaction = 0.02). In the high-fat diet group, participants with higher GRS showed significantly less reduction in whole-body BMD than those with lower GRS, whereas the genetic associations were not significant in the low-fat diet group. We also found a significant interaction between dietary fat intake and the GRS on 6-mo change in femur neck BMD (P-interaction = 0.02); however, the interaction became nonsignificant at 2 y.

Conclusion: Our data indicate that dietary fat intake may modify the effect of vitamin D–related genetic variation on changes in BMD. Overweight or obese patients predisposed to sufficient vitamin D may benefit more in maintaining BMD along with weight loss by eating a low-fat diet. This trial was registered at clinicaltrials.gov as NCT03258203. *Am J Clin Nutr* 2018;108:1129–1134.

Keywords: Vitamin D, bone health, weight loss, diet

INTRODUCTION

Body mass has been linked to bone health (1–4). Weight-loss diets have shown metabolic benefits, but the data relating weight loss to bone health are controversial (5). Several studies have shown that a low BMI is a risk factor for osteoporosis (6, 7). Diet-induced weight loss has been linked to a decrease in bone mineral density (BMD) in some studies (8, 9); however, inconsistent results were also reported (10). We hypothesized that such inconsistent observations might be partly due to genetic variations.

Vitamin D is closely related to bone health (11–13), by promoting calcium absorption and acting on osteoblasts and osteoclasts to modulate calcium metabolism (13). Circulating 25-hydroxyvitamin D [25(OH)D] is the most suitable indicator of vitamin D status. Three serum vitamin D–associated genetic variants, 7-dehydrocholesterol reductase (*DHCR7*) rs12785878,

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Supplemental Figure 1 is 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/ajcn/.

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Abbreviations used: BMD, bone mineral density; *CYP2R1*, cytochrome P450 2R1; *DHCR7*, 7-dehydrocholesterol reductase; *GC*, group-specific component globulin; GRS, genetic risk score; SNP, single-nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

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cytochrome P450 2R1 (*CYP2R1*) rs10741657, and group-specific component globulin (*GC*) rs2282679, were identified in a recent genome-wide association study (14). The genetic variants have been also related to BMD (15, 16). However, no study has assessed whether vitamin D genetic variations affect changes in BMD in weight-loss intervention trials.

In this study, we examined the relations between vitamin D genetic variations and changes in BMD in a 2-y dietary weight-loss intervention trial, called Preventing Overweight Using Novel Dietary Strategies Trial (POUNDS Lost) (17). Because intervention studies suggest that dietary factors produce different effects on bone health (18, 19), we examined in particular the potential interactions between vitamin D genetic variants and dietary interventions on BMD.

METHODS

Study participants

Information on study design and methods has been given elsewhere (**Supplemental Figure 1**) (17). In brief, a total of 811 overweight and obese participants were randomly assigned to 1 of 4 weight-loss diets that varied in macronutrient composition for 2 y. The percentages of energy derived from fat, protein, and carbohydrates in the 4 diets were 20%, 15%, and 65% (low-fat, average-protein diet); 20%, 25%, and 55% (low-fat, high-protein diet); 40%, 15%, and 45% (high-fat, average-protein diet); and 40%, 25% and 35% (high-fat, high-protein diet), respectively. Thus 2 diets were low fat (20%) and 2 diets were high fat (40%), and 2 diets were average protein (15%) and 2 diets were high protein (25%). A total of 370 participants with both BMD measurement and genotyping data were included in the final analysis.

The study was approved by the human participants committee at the Harvard TH Chan School of Public Health and Brigham and Women's Hospital, Boston, MA, and the Pennington Biomedical Research Center, Baton Rouge, LA, and by a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. Written informed consent was obtained from all participants.

Measurements of BMD and covariates

The primary outcome of the study was the change in body weight, and the secondary outcome was the change in BMD. The details of bone measurements have been described in full elsewhere (20). Briefly, a random sample of 50% of the 811 enrolled participants ($n = 424$) in the POUNDS Lost trial were selected to undergo repeated BMD measurement by dual-energy X-ray absorptiometry (Hologic QDR-4500A bone densitometer; Hologic, Inc.). The BMDs of whole-body, femoral neck, total hip, and spine were measured at baseline, 6 mo ($n = 296$), and 2 y ($n = 213$). Measurements were carried out by investigators and staff who were unaware of the diet assignment of the participants. Body weight was measured with the use of calibrated hospital scales in the morning before breakfast and after urinating, with participants wearing a hospital gown (17). Body weight was measured in the morning before breakfast on 2 nonconsecutive days at baseline, 6 mo, and 2 y. Height was measured at baseline. BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Dietary intake was assessed in a random sample of 50% of the participants; at baseline from 5-d diet records and at 6 and 24 mo from 24-h recalls collected by telephone on 3 nonconsecutive days. The average daily intake over each period was calculated (21).

Genotyping

DNA was extracted from the buffy coat fraction of centrifuged blood with the use of a QIAmp Blood Kit (Qiagen). Genotyping was performed among all 811 participants with the OpenArray SNP Genotyping System (BioTrove, Woburn, MA). The genotype success rates were ~99% in available DNA samples. Replicated quality-control samples (10%) were included in every genotyping plate with >99% concordance (22). The genotype frequencies in all participants were consistent with Hardy-Weinberg equilibrium ($P > 0.05$).

Three single-nucleotide polymorpisms (SNPs) from a previous large-scale genome-wide association study for serum 25(OH)D levels were selected, including rs2282679, rs12785878, and rs10741657, located in the *GC*, *DHCR7*, and *CYP2R1* genes, respectively (14). The SNP data were available for 370 of the 424 participants for whom BMD measurements were available. Genetic risk score (GRS) was calculated by ($\beta 1 \times$ SNP1 + $\beta 2 \times$ SNP2 + $\beta 3 \times$ SNP3) $\times$ (3/sum of the β coefficients) (14). A higher GRS indicates a lower serum 25(OH)D level.

Statistical analyses

At baseline, general linear models for continuous variables and chi-square test for categoric variables were performed for comparison of characteristics by tertiles of vitamin D GRS. We used a generalized linear model to test changes in BMD, nutrient intakes, and biomarkers of adherence across vitamin D GRS tertiles. Generalized linear model was also used to test gene $\times$ diet intervention interactions by including the GRS-by-diet intervention interaction term, adjusted for age, sex, ethnicity, baseline values of the respective outcomes, baseline BMI, and weight loss at each intervention time. We also performed a sensitivity analysis in white participants. Statistical analyses were performed with SAS version 9.4 (SAS Institute, Inc., Cary, NC). All reported P values were 2-sided. $P < 0.05$ was used as the significance level. Power calculations were performed with Quanto1.2.4 (http://biostats.usc.edu/Quanto.html). The study had 80% power to detect a gene-diet interaction effect size of 0.021 g/cm^2 in whole-body BMD change under an additive model.

RESULTS

The baseline characteristics of participants are shown in **Table 1**. The distribution of GRS (in tertiles) was marginally significant across ethnicity and gender. No significant difference was observed for age, BMI, height, dietary intervention, baseline whole-body BMD, and femoral neck BMD across the tertiles of GRS. The nutrient intakes or biomarkers of adherence did not differ according to the GRS. (**Table 2**).

At 2 y, the change of whole-body BMD was 0.001 g/cm^2 ($P = 0.51$). No significant differences in BMD changes were

TABLE 1
Baseline characteristics according to the vitamin D GRS[1]

	Tertile1	Tertile 2	Tertile 3	*P*
n	66	171	133	
Age, y	53 ± 9	53 ± 8	52 ± 10	0.82
Female, %	30 (45)	104 (61)	69 (52)	0.07
Race/ethnicity				0.05
Black, %	4 (6)	30 (18)	15 (11)	
White, %	62 (94)	141 (82)	118 (89)	
BMI, kg/m^2	32 ± 4	33 ± 4	33 ± 4	0.99
Height, cm	171 ± 10	169 ± 8	170 ± 8	0.64
Weight, kg	94 ± 15	94 ± 16	94 ± 16	0.99
Femoral neck BMD, g/cm^2	0.83 ± 0.13	0.85 ± 0.13	0.84 ± 0.12	0.56[2]
Whole-body BMD, g/cm^2	1.15 ± 0.11	1.12 ± 0.11	1.14 ± 0.11	0.62[2]
Dietary intake/d				
Energy, kcal	2058 ± 592	1941 ± 541	2005 ± 539	0.80
Carbohydrate, %	45 ± 8	45 ± 8	44 ± 7	0.17
Fat, %	36 ± 6	37 ± 6	38 ± 5	0.12
Protein, %	18 ± 3	18 ± 3	18 ± 3	0.93
Urinary nitrogen, g	12.7 ± 5.0	12.3 ± 4.2	12.6 ± 4.3	0.62
Respiratory quotient	0.84 ± 0.05	0.84 ± 0.04	0.84 ± 0.05	0.53

[1]Values are means ± SDs unless otherwise indicated. Vitamin D GRSs were treated as continuous variables to calculate the *P* values. BMD, bone mineral density; GRS, genetic risk score.
[2]Adjusted for age, sex, ethnicity, and BMI.

observed between diet groups (high fat compared with low fat; high protein compared with average protein) (all $P > 0.05$). We did not find a significant main effect of the GRS on change in whole-body BMD during the 2-y intervention after adjustment for age, sex, ethnicity, BMI at baseline, weight change, fat diet group, and baseline value for the respective outcome (**Table 3**).

We then analyzed the interactions between vitamin D GRS and diet interventions on changes in BMD. We found that the GRS significantly interacted with dietary fat intake on change in whole-body BMD ($P = 0.02$) at 2 y after adjustment for age, sex, ethnicity, baseline BMI, weight change, and baseline values for whole-body BMD. In the high-fat diet group, participants in the highest tertile of GRS showed a more significant improvement in whole-body BMD than those in other tertiles, whereas participants showed no significant difference across the tertiles of GRS when assigned to a low-fat diet (**Figure 1**). We also found a similar interaction between GRS and dietary fat intake on change in femoral neck BMD at 6 mo ($P = 0.02$). However, the interaction attenuated and was not significant at 2 y.

In addition, we did not find any interaction between the GRS and dietary protein intake on change in BMD. From the results of a sensitivity analysis, we found similar interactions in white

TABLE 2
Nutrient intake and biomarkers of adherence according to vitamin D GRS at 2 y[1]

	Tertile 1 ($n = 41$)	Tertile 2 ($n = 96$)	Tertile 3 ($n = 76$)	*P*
Low-fat group				
n	22	46	38	
Energy, kcal	1576 ± 525	1527 ± 436	1612 ± 495	0.72
Carbohydrate, %	55 ± 12	53 ± 9	51 ± 10	0.18
Fat, %	26 ± 8	26 ± 5	30 ± 10	0.07
Protein, %	20 ± 4	20 ± 4	20 ± 4	0.96
Biomarkers of adherence				
Urinary nitrogen, g	12.6 ± 4.6	11.3 ± 4.2	12.3 ± 4.1	0.98
Respiratory quotient	0.85 ± 0.04	0.83 ± 0.04	0.84 ± 0.04	0.21
High-fat group				
n	19	50	38	
Energy, kcal	1498 ± 499	1442 ± 499	1503 ± 494	0.86
Carbohydrate, %	42 ± 9	46 ± 8	48 ± 12	0.09
Fat, %	34 ± 6	35 ± 7	33 ± 10	0.41
Protein, %	22 ± 6	20 ± 6	20 ± 5	0.16
Biomarkers of adherence				
Urinary nitrogen, g	14.3 ± 5.8	10.9 ± 4.6	11.6 ± 4.8	0.14
Respiratory quotient	0.82 ± 0.05	0.83 ± 0.04	0.82 ± 0.04	0.58

[1]Values are means ± SDs. General linear models (PROC GLM) were applied for the comparison according to groups. GRS, genetic risk score.

TABLE 3
Effect of dietary groups and GRS on change in whole-body BMD at 2 y[1]

	ΔBMD, g/cm^2	*P*
All	0.001 ± 0.028	0.51
Fat	—	0.11[2]
Low fat ($n = 106$)	0.005 ± 0.030	
High fat ($n = 107$)	−0.002 ± 0.026	
Protein	—	0.12[2]
Average protein ($n = 93$)	−0.002 ± 0.028	
High protein ($n = 120$)	0.004 ± 0.028	
GRS	—	0.75[2]
Tertile 1 ($n = 41$)	0.002 ± 0.038	
Tertile 2 ($n = 96$)	−0.001 ± 0.026	
Tertile 3 ($n = 76$)	0.003 ± 0.025	

[1] *t* Test was used to calculate the *P* value. BMD, bone mineral density; GRS, genetic risk score.
[2] General linear models (PROC GLM) were applied to test the effect of diet intake and GRS on BMD change.

participants. No significant interaction was found in total hip and spine BMD.

DISCUSSION

In this 2-y randomized, weight-loss intervention trial, we found that genetic variations related to vitamin D levels, assessed as a GRS, showed a significant interaction with dietary fat intake in relation to changes in whole-body BMD ($P = 0.02$). Participants in the highest tertile of the GRS showed more significant improvements in whole-body BMD than those in other tertiles when assigned to a high-fat diet. Our findings indicate that dietary fat intake might modify the effect of vitamin D–related genetic variation on changes in BMD, providing evidence for the benefits of personalized dietary intervention to improve bone health in weight-loss interventions.

Similar changes in whole-body BMD were observed among different dietary intervention groups, consistent with previous findings (20).

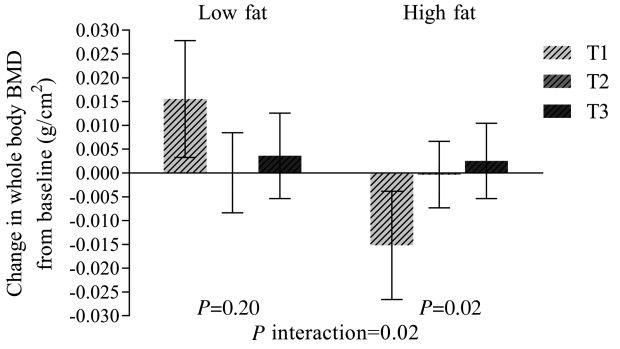

FIGURE 1 Effect of vitamin D GRS and dietary fat on whole-body BMD change during the 2-y intervention. General linear models (PROC GLM) were applied to test the effect of diet intake and GRS on BMD change. Values were expressed as adjusted least square means ± 95% CIs for changes in BMD. *P* values were adjusted for age, sex, ethnicity, baseline BMI, weight change, and baseline values for respective phenotypes. The lowest tertile (T1) represents the highest serum 25-hydroxyvitamin D level. For the low-fat group: T1, $n = 22$; T2, $n = 46$; T3, $n = 38$. For the high-fat group: T1, $n = 19$; T2, $n = 50$; T3, $n = 38$. BMD, bone mineral density; GRS, genetic risk score; T, tertile.

Weight-loss diets showed favorable metabolic effects, such as improved lipid profile and glycemic status (23, 24); however, several (9, 25, 26) studies have shown that weight-loss diets were associated with a decrease in BMD, though other studies did not find such effects (27, 28). Bone mineral content is determined by environmental and genetic factors, and ∼70–80% of peak bone mass is genetically determined (29). We selected 3 genetic variants and calculated a GRS as a predictor of serum vitamin D levels. The *DHCR7* gene encodes 7-dehydrocholesterol reductase, which converts 7-dehydrocholesterol, a precursor for both vitamin D and cholesterol, into cholesterol, thus reducing the availability of this precursor to synthesize vitamin D in the skin (30). The enzyme encoded by *CYP2R1* is a key vitamin D 25-hydroxylase affecting vitamin D metabolism (31). The *GC* gene encodes vitamin-binding protein, which plays a major role in vitamin D transport and storage (32). Our data suggest that genetic variation may partly account for the diverse response in BMD in response to dietary weight-loss interventions.

We found that the vitamin D GRS significantly modified the effect of fat intake on BMD changes; the highest tertile was related to a lower reduction in BMD in response to a high-fat diet. Vitamin D plays a central role in determining bone health (11), and vitamin D deficiency causes secondary hyperparathyroidism that mobilizes calcium from the skeleton and decreases BMD. Vitamin D is a fat-soluble nutrient, and dietary fat may promote vitamin D absorption (33, 34). Moreover, dietary fat supplies essential fatty acids, which may affect bone health by enhancing the effect of vitamin D activity and increasing calcium absorption in the gut (35).

There is evidence that a high-fat diet may be detrimental to bone health when combined with a sufficient calcium intake (36–38). Thus, our results suggest that a high-fat diet may balance its negative effect on BMD by improving vitamin D absorption and metabolism when the vitamin D is insufficient. However, once the benefit of vitamin D has been realized, a high-fat diet may adversely affect bone strength. Because direct evidence is limited, further studies are warranted to unravel the biological basis underlying the observed interaction between the vitamin D–related GRS and dietary fat intake.

Our study has several strengths. To our knowledge, this is the first study to investigate the interactions between the vitamin D–associated GRS and dietary fat on change in BMD in this, the largest and longest weight-loss diet-intervention trial yet reported. Our findings provide new insights into the potential role of genetic variation in modifying the change in BMD in weight-loss intervention trials. In addition, compared with observational studies, our study design allowed for controlling potential confounders to minimize the possibility of bias and provide a biologically plausible mechanism. Several limitations also need to be considered when interpreting our findings. First, the relatively small sample size of the subgroups might have limited power to detect moderate gene-diet interactions. Second, we did not assess the serum concentration of 25(OH)D, which limited our ability to explore potential underlying mechanisms. Third, we tested the interaction between the GRS and BMD changes, leading to multiple statistical comparisons. However, the a priori hypothesis-driven examination was based on the effects of vitamin D concentration on bone health, which is biologically plausible. Fourth, information about vitamin D and calcium intake was not available in our study. Last, because this is

a not an a priori–registered endpoint, our results are preliminary and require verification.

In conclusion, our results indicated that dietary fat may modify the effect of vitamin D–related genetic variation. Participants with a genetic predisposition to insufficient vitamin D might have better bone health when eating a high-fat diet in a long-term weight-loss diet. And for those predisposed to adequate vitamin D, avoiding a high-fat diet might be a good choice.

The authors' responsibilities were as follows—TZ, FMS, and LQ: designed the research; TZ, GAB, FMS, and LQ: conducted the research; TZ, DS, YH, and LQ: analyzed the data or performed statistical analysis; TZ and LQ: wrote the manuscript; LQ: had primary responsibility for the final content; and all authors: critically reviewed the manuscript and approved submission. The authors have no competing interests or conflicts of interest related to this study.

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