

Vitamin D metabolism-related genetic variants, dietary protein intake and improvement of insulin resistance in a 2 year weight-loss trial: POUNDS Lost

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Abstract

Aims/hypothesis Vitamin D and related genetic variants are associated with obesity and insulin resistance. We aimed to examine whether vitamin D metabolism-related variants affect changes in body weight and insulin resistance in response to weight-loss diets varying in macronutrient content.

Methods Three vitamin D metabolism-related variants, *DHCR7* rs12785878, *CYP2R1* rs10741657 and *GC* rs2282679, were genotyped in 732 overweight/obese participants from a 2 year weight-loss trial (POUNDS Lost). We assessed genotype effects on changes in body weight, fasting levels of glucose and insulin, and HOMA-IR at 6 months (up to 656 participants) and 2 years (up to 596 participants) in response to low-protein vs high-protein diets, and low-fat vs high-fat diets.

Results We found significant interactions between *DHCR7* rs12785878 and diets varying in protein, but not in fat, on

changes in insulin and HOMA-IR at both 6 months (p for interaction <0.001) and 2 years (p for interaction ≤ 0.03). The T allele (vitamin-D-increasing allele) of *DHCR7* rs12785878 was associated with greater decreases in insulin and HOMA-IR ($p < 0.002$) in response to high-protein diets, while there was no significant genotype effect on changes in these traits in the low-protein diet group. Generalised estimating equation analyses indicated significant genotype effects on trajectory of changes in insulin resistance over the 2 year intervention in response to high-protein diets ($p < 0.001$). We did not observe significant interaction between the other two variants and dietary protein or fat on changes in these traits. **Conclusions/interpretation** Our data suggest that individuals carrying the T allele of *DHCR7* rs12785878 might benefit more in improvement of insulin resistance than noncarriers by consuming high-protein weight-loss diets.

Trial registration: ClinicalTrials.gov NCT00072995

Qibin Qi and Yan Zheng contributed equally to this work.

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Keywords Diet intervention · Genetic variants · Insulin resistance · Vitamin D · Weight loss

Abbreviations

DHCR7	7-Dehydrocholesterol reductase
GWAS	Genome-wide association study
25(OH)D	25-Hydroxyvitamin D
POUNDS Lost	Preventing Overweight Using Novel Dietary Strategies Trial
SNP	Single nucleotide polymorphism

Introduction

Vitamin D has a pleiotropic role in various metabolic functions beyond regulation of calcium and bone metabolism [1].

Compelling evidence has shown that low blood levels of 25-hydroxyvitamin D (25(OH)D), an indicator of circulating vitamin D levels, are associated with obesity, insulin resistance and an increased risk of type 2 diabetes [2–8]. A recent genome-wide association study (GWAS) identified that variants in several genes involved in vitamin D metabolism, such as *DHCR7*, *CYP2R1* and *GC*, were associated with 25(OH)D levels [9]. The *DHCR7* gene encodes 7-dehydrocholesterol reductase (DHCR7), which converts 7-dehydrocholesterol (a precursor both for vitamin D and for cholesterol) to cholesterol, thereby reducing the availability of this precursor for cutaneous synthesis into vitamin D by the action of sunlight [10]. The enzyme encoded by *CYP2R1*, cytochrome P450, family 2, subfamily R, polypeptide 1, is a key vitamin D 25-hydroxylase [11]. The *GC* gene encodes vitamin D binding protein, which plays a major role in vitamin D transport and storage [12]. However, the causal relationships between vitamin D levels and cardiometabolic diseases inferred by Mendelian randomisation studies remain unclear [4, 13–15]. For example, a vitamin D genetic score (based on variants in *DHCR7* and *CYP2R1*) was found to be associated with blood pressure and hypertension [13], but this score was not associated with obesity in a previous analysis [4]. Afzal et al [14] reported that genetic variants associated with lower plasma 25(OH)D concentrations are associated with an increased risk of type 2 diabetes, while another study did not observe such associations [15]. Thus, examining whether vitamin D metabolism-related genetic variants affect long-term changes in body weight, glucose metabolism and insulin resistance may help explain the role of vitamin D in cardiometabolic diseases.

It has been shown that *DHCR7* activity and *DHCR7* gene expression could be regulated by vitamin D or other dietary nutrients [16–19], suggesting potential interactions between dietary factors and vitamin D metabolism-related genes. Protein-rich and fat-rich foods are the main sources of naturally occurring cholecalciferol (vitamin D₃) [20]; therefore, we hypothesised that diets varying in protein or fat content might differentially affect the effect of vitamin D metabolism-related variants on body weight and related metabolic traits. Thus, we tested this hypothesis among 732 participants in a 2 year randomised dietary intervention weight-loss trial, the Preventing Overweight Using Novel Dietary Strategies Trial (POUNDS Lost) [21]. We examined the effects of three GWAS-identified vitamin D metabolism-related variants in the *DHCR7*, *CYP2R1* and *GC* genes on weight loss and improvement of glucose and insulin metabolism in response to diets varying in protein or fat content.

Methods

Study participants POUNDS Lost is a randomised intervention trial in which 811 overweight or obese participants aged

30–70 years were assigned to one of four energy-limited diets over a 2 year period, in order to compare the effects of energy-reduced diets with different compositions of fat, protein and carbohydrate on weight loss. The detailed study design and methods have been described before [21]. Participants were excluded from this trial if they had diabetes treated with medication or unstable cardiovascular disease, if they used medications influencing body weight or if they expressed insufficient motivation at baseline examination. According to the two-by-two factorial design, two diets were low fat (20% of energy derived from fat) and two were high fat (40% of energy derived from fat), and two were average protein (15% of energy derived from protein) and two were high protein (25% of energy derived from protein). All these diets consisted of similar foods and met guidelines for cardiovascular health. The caloric prescription for each participant represented a deficit of 3,138 kJ (750 kcal)/day from baseline, as calculated from the participant's resting energy expenditure and activity level.

After 2 years, 80% of the participant ($n=645$) completed the trial. The main results of weight loss in POUNDS Lost have been reported before [21]. Most of the weight loss occurred in the first 6 months, and the amount of weight loss after 2 years was similar among different diet groups. Both self-reported dietary data and biomarkers confirmed that participants modified their intake of macronutrients in the direction of the assigned goals [21]. In the current study, a total of 732 participants with genotype data available were included (90% of the participants in POUNDS Lost). At 6 months, 656 participants with body weight measurement and 626 participants with blood samples were included for the analyses. At 2 years, 596 participants with body weight measurements and 533 participants with blood samples were included for the analyses.

The study was approved by the human participants committee at the Harvard T.H. 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 provided by all participants.

Measurements At baseline examination and at 6 month and 2 year visits, body weight for each participant was measured in the morning before breakfast. Height was measured at baseline only. BMI was presented as weight/height² (kg/m²). In the current study, ethnicity was self-reported and grouped as white, black and others. Fasting blood samples were collected at baseline and at 6 month and 2 year visits. Fasting glucose and insulin were measured using an immunoassay with chemiluminescent detection on the Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). Analyses of glucose and insulin were performed at the

Clinical Laboratory at Pennington Biomedical Research Center. Insulin resistance was estimated by HOMA-IR [22]: fasting glucose (mmol/l) × fasting insulin (pmol/l)/156.3. Dietary intake was assessed by a review of the 5 day diet records at baseline and by 24 h recall during a telephone interview on three nonconsecutive days at 6 months and at 2 years [21].

Genotyping DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA, USA). Three lead single nucleotide polymorphisms (SNPs) in vitamin-D-related loci, *DHCR7* rs12785878 ($p=2.1 \times 10^{-27}$), *CYP2R1* rs10741657 ($p=3.3 \times 10^{-20}$) and *GC* rs2282679 ($p=1.9 \times 10^{-109}$), identified through a previous large-scale GWAS for 25-hydroxyvitamin D concentrations (up to 33,996 individuals) [9], were selected in the current study. Genotyping was performed using the OpenArray SNP Genotyping System (BioTrove, Woburn, MA, USA). The genotype success rate was 99%. Replicate quality control samples (10%) were included and genotyped with >99% concordance. The genotype frequencies in all participants or in white participants were in Hardy–Weinberg equilibrium ($p>0.05$).

Statistical analysis General linear models for continuous variables and χ^2 tests for categorical variables were applied for the comparison according to genotype groups at baseline. To improve normality, fasting insulin and HOMA-IR were \log_{10} transformed before analyses. The primary outcomes were changes in body weight, fasting insulin, glucose and HOMA-IR over the time the participants remained in the trial. General linear models were applied to compare changes from baseline in these outcomes across genotype groups according to diet groups (high-protein vs low-protein diets and high-fat vs low-fat diets) at 6 month and 2 year visits, adjusted for age, sex, ethnicity and baseline value for the respective outcome. We used additive inheritance models (e.g. *DHCR7* rs12785878 genotype groups were coded as 0, 1 and 2 in continuous form for GG, GT and TT) in the analyses. To test potential gene–diet intervention interactions, a genotype-by-diet interaction term (e.g. *DHCR7* rs12785878 genotype × high-/low-protein diet group) was included in the models. In addition, generalised estimating equation methods were applied to test genotype effects on the trajectory of changes in fasting insulin and HOMA-IR over the 2 year intervention. As HOMA-IR was calculated on the basis of glucose and insulin, and the endpoints at 6 months and 2 years were correlated, we did not treat them as independent variables. All statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA).

Bonferroni's adjustment was applied to adjust p values for 18 independent tests (3 independently measured traits [body weight, glucose and insulin] × 3 genetic variants × 2 diet comparisons) at each time point ($p<0.0028$). We used Quanto 1.2.4 (University of Southern California, Los Angeles, CA, USA;

<http://biostats.usc.edu/Quanto.html>) to estimate the detectable effect sizes of genotype-by-diet interactions. For the *DHCR7* rs12785878 variant, the study had 80% power to detect gene–diet interaction effect sizes of 2.55 and 3.53 kg for weight loss, 0.22 and 0.25 mmol/l for changes in fasting glucose levels, 0.22 and 0.24 log-transformed unit for changes in fasting insulin levels, and 0.24 and 0.26 log-transformed unit for changes in HOMA-IR at 6 months and 2 years, respectively, at a $p<0.0028$ level. More power calculation results are shown in electronic supplementary material (ESM) Table 1.

Results

Baseline characteristics Table 1 shows the baseline characteristics of participants according to the genotypes of *DHCR7* rs12785878, *CYP2R1* rs10741657 and *GC* rs2282679. For all three genetic variants, the genotype frequencies were similar between men and women, high- and low-protein diet groups and high- and low-fat diet groups, while they differed among ethnic groups ($p\leq 0.05$). In addition, the *CYP2R1* rs10741657 variant was associated with fasting glucose levels at baseline.

Weight loss and dietary intake Consistent with the entire POUNDS Lost [21], the amount of weight loss was similar between the low- and high-protein diet groups (6 months: 6.5 vs 6.6 kg, $p=0.71$; 2 years: 3.6 vs 4.5 kg, $p=0.11$) and between the low- and high-fat diet groups (6 months: 6.7 vs 6.4 kg, $p=0.52$; 2 years: 4.1 vs 3.9 kg, $p=0.76$) among study participants (ESM Table 2). The reported dietary intakes confirmed that participants modified their intake of macronutrients in the direction of the intervention (ESM Table 3). Participants in the high-protein diet groups and in the high-fat diet groups reported higher dietary protein intake and higher fat intake than those in the low-protein diet groups and in the low-fat diet groups, respectively (all $p<0.05$).

Genotypes and changes in weight, glucose and insulin resistance by diet groups We observed significant interactions between *DHCR7* rs12785878 and high-/low-protein diet intervention on changes in fasting insulin (p for interaction=0.0008) and HOMA-IR (p for interaction=0.0009) at 6 months, adjusted for age, sex, ethnicity and the baseline value for the respective outcome trait (Table 2). In the high-protein diet groups, the T-allele of *DHCR7* rs12785878 was associated with greater decreases in fasting insulin ($p=0.0009$) and HOMA-IR ($p=0.002$; Table 2 and Fig. 1). These genotype–diet interactions and genotype effects remained significant after correcting for multiple testing ($p<0.0028$). In the low-protein diet groups, there was no significant genotype effect on changes in fasting insulin or HOMA-IR ($p\geq 0.14$). Results were similar but attenuated at 2 years (Table 2 and Fig. 1), and there were nominally significant genotype–diet interactions on changes in fasting

Table 1 Participant characteristics according to the genotypes of *DHCR7* rs12785878, *CYP2R1* rs10741657 and *GC* rs2282679 at baseline^a

Characteristic	<i>DHCR7</i> rs12785878				<i>CYP2R1</i> rs10741657				<i>GC</i> rs2282679				
	TT	TG	GG	GG	GG	GA	AA	AA	AA	AA	AC	CC	<i>p</i>
<i>n</i>	345	291	96	96	290	335	96	96	416	258	49		
Age, years	51.9±8.9	50.3±9.3	49.4±10.0	49.4±10.0	51.1±9.4	50.7±9.3	51.2±8.7	51.2±8.7	50.8±9.3	50.9±9.4	52.4±8.6	0.54	
Sex, <i>n</i> (%)													0.39
Men	146 (51.0)	112 (39.2)	28 (9.8)	28 (9.8)	102 (36.2)	144 (51.1)	36 (12.8)	36 (12.8)	155 (54.6)	107 (37.7)	22 (7.7)		
Women	199 (44.6)	179 (40.1)	68 (15.3)	68 (15.3)	188 (42.8)	191 (43.5)	60 (13.7)	60 (13.7)	261 (59.5)	151 (34.4)	27 (6.2)		
Race or ethnic group, <i>n</i> (%)													<0.0001
White	328 (56.2)	217 (37.2)	39 (6.7)	39 (6.7)	219 (38.0)	275 (47.7)	82 (14.2)	82 (14.2)	293 (50.9)	235 (40.8)	48 (8.3)		
Black	10 (8.9)	57 (50.9)	45 (40.2)	45 (40.2)	58 (52.7)	41 (37.3)	11 (10.0)	11 (10.0)	97 (87.4)	13 (11.7)	1 (0.9)		
Hispanic or other	7 (19.4)	17 (47.2)	12 (33.3)	12 (33.3)	13 (37.1)	19 (54.3)	3 (8.6)	3 (8.6)	26 (72.2)	10 (27.8)	0 (0.0)		
Diet groups varying in protein, <i>n</i> (%)													0.56
Low (15% of energy)	167 (45.3)	151 (40.9)	51 (13.8)	51 (13.8)	147 (40.4)	162 (44.5)	55 (15.1)	55 (15.1)	216 (58.9)	124 (33.8)	27 (7.4)		
High (25% of energy)	178 (49.0)	140 (38.6)	45 (12.4)	45 (12.4)	143 (40.1)	173 (48.6)	41 (11.5)	41 (11.5)	200 (56.2)	134 (37.6)	22 (6.2)		
Diet groups varying in fat, <i>n</i> (%)													0.68
Low (20% of energy)	166 (45.5)	152 (41.6)	47 (12.9)	47 (12.9)	139 (38.8)	180 (50.3)	39 (10.9)	39 (10.9)	208 (57.9)	123 (34.3)	28 (7.8)		
High (40% of energy)	179 (48.8)	139 (37.9)	49 (13.4)	49 (13.4)	151 (41.6)	155 (42.7)	57 (15.7)	57 (15.7)	208 (57.1)	135 (37.1)	21 (5.8)		
Body weight, kg	92.9±15.9	94.2±15.7	91.9±14.4	91.9±14.4	92.4±15.0	94.0±16.0	92.8±15.5	92.8±15.5	93.4±14.8	93.5±16.8	90.6±15.2	0.22	
BMI, kg/m ²	32.5±3.9	32.8±3.9	32.8±3.8	32.8±3.8	32.6±3.9	32.8±3.8	32.5±4.0	32.5±4.0	32.8±3.8	32.6±4.0	32.1±3.6	0.59	
Glucose, mmol/l	5.2±0.7	5.1±0.6	5.0±0.7	5.0±0.7	5.2±0.7	5.1±0.6	5.0±0.6	5.0±0.6	5.1±0.7	5.1±0.6	5.2±0.7	0.74	
Insulin, pmol/l ^b	73.6 (50.0–103.5)	75.0 (47.2–113.2)	68.1 (45.1–106.3)	68.1 (45.1–106.3)	75.0 (49.3–107.6)	70.8 (47.2–104.2)	77.8 (53.5–110.4)	77.8 (53.5–110.4)	74.3 (48.6–107.0)	71.5 (48.6–109.0)	68.8 (47.2–91.7)	0.38	
HOMA-IR ^b	2.5 (1.6–3.5)	2.4 (1.5–3.8)	2.2 (1.3–3.6)	2.2 (1.3–3.6)	2.5 (1.5–3.7)	2.2 (1.5–3.5)	2.5 (1.6–3.6)	2.5 (1.6–3.6)	2.4 (1.5–3.7)	2.3 (1.5–3.6)	2.2 (1.5–3.0)	0.47	

Throughout the table the percentages indicate the relative proportion of each genotype at each variant

^aData are *n* (%), means ± SD or median (interquartile range). *p* values were calculated by χ^2 test for categorical variables and *F* tests after adjusted for age, sex and ethnicity for continuous variables

^bThese variables were log₁₀ transformed before analysis

Table 2 Effects of *DHCR7* rs12785878, *CYP2R1* rs10741657 and *GC* rs2282679 on changes in body weight, glucose, insulin and HOMA-IR in response to low-/high-protein diets^a

Characteristic	<i>DHCR7</i> rs12785878				<i>CYP2R1</i> rs10741657				<i>GC</i> rs2282679			
	6 months		2 years		6 months		2 years		6 months		2 years	
	$\beta \pm \text{SE}$	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>
Change in body weight, kg												
Low-protein diet	-0.62±0.51	0.22	-0.50±0.69	0.46	-0.33±0.45	0.46	-0.43±0.64	0.50	0.89±0.52	0.08	1.05±0.73	0.15
High-protein diet	-1.18±0.47	0.01	-0.96±0.66	0.14	0.19±0.43	0.66	0.73±0.58	0.21	0.40±0.48	0.41	0.19±0.65	0.78
<i>p</i> for interaction	0.25		0.52		0.48		0.19		0.68		0.41	
Change in glucose, mmol/l												
Low-protein diet	0.05±0.04	0.23	0.08±0.05	0.08	-0.02±0.04	0.57	0.01±0.04	0.91	0.01±0.04	0.84	0.01±0.05	0.94
High-protein diet	-0.02±0.04	0.68	-0.05±0.05	0.35	-0.03±0.04	0.42	0.04±0.05	0.44	-0.01±0.04	0.95	-0.01±0.05	0.95
<i>p</i> for interaction	0.30		0.07		0.95		0.71		0.82		0.93	
Change in log insulin, pmol/l												
Low-protein diet	0.05±0.04	0.19	0.05±0.04	0.25	0.04±0.04	0.32	0.004±0.04	0.92	0.05±0.04	0.24	-0.01±0.05	0.80
High-protein diet	-0.13±0.04	0.0009	-0.06±0.04	0.15	0.02±0.04	0.56	0.04±0.04	0.34	0.04±0.04	0.29	0.01±0.04	0.72
<i>p</i> for interaction	0.0008		0.03		0.74		0.63		0.91		0.54	
Change in log HOMA-IR												
Low-protein diet	0.06±0.04	0.14	0.06±0.05	0.19	0.03±0.04	0.47	0.005±0.05	0.92	0.05±0.04	0.25	-0.02±0.05	0.76
High-protein diet	-0.13±0.04	0.002	-0.06±0.05	0.16	0.02±0.04	0.70	0.04±0.04	0.30	0.04±0.04	0.35	0.02±0.05	0.74
<i>p</i> for interaction	0.0009		0.02		0.8		0.57		0.99		0.51	

^aData are $\beta \pm \text{SE}$, representing effect size for each vitamin-D-increasing allele of *DHCR7* rs12785878 (T allele), *CYP2R1* rs10741657 (A allele) and *GC* rs2282679 (A allele), respectively, on change in each trait, adjusted for age, sex, ethnicity and the baseline value for the respective outcome trait

insulin (*p* for interaction=0.03) and HOMA-IR (*p* for interaction=0.02). These results did not change materially after further adjustment for weight loss (ESM Table 4).

For genetic variants in *CYP2R1* and *GC*, we did not find significant genotype–diet interactions or genotype effects on changes in body weight, fasting glucose, fasting insulin or HOMA-IR at 6 months or 2 years (all *p*>0.05; Table 2).

We also examined interaction between genetic variants and high-/low-fat diet interventions on changes in these metabolic traits (ESM Table 5). There were nominally significant interactions between *CYP2R1* rs10741657 and dietary fat on changes in fasting insulin and HOMA-IR at both 6 months and 2 years (*p* for interaction <0.05). In the low-fat diet groups, the A allele of *CYP2R1* rs10741657 showed a trend to be associated with less decreases in fasting insulin and HOMA-IR at both 6 months (*p*=0.04) and 2 years (*p*=0.03), while no genotype effect was observed in the high-fat diet groups. However, these results did not pass a threshold of *p*<0.0028 after correcting for multiple testing.

As the majority of participants in this study were white (~80%), we further performed sensitivity analyses by excluding other ethnic groups. Similar results were observed when the analyses were restricted to the white participants.

Trajectory of changes in insulin resistance by *DHCR7* rs12785878 in response to high-/low-protein diets We then assessed the genotype of *DHCR7* rs12785878 by time effect on improvement of insulin resistance over the 2 year intervention in the participants assigned to the high- and low-protein diets (Fig. 2). In the high-protein diet group, participants carrying the T-allele of *DHCR7* rs12785878 (TT and TG genotypes) had a greater improvement of fasting insulin (*p*<0.0001) and HOMA-IR (*P*=0.0009) than non-T-carriers (GG genotype) across the 2 year intervention. As expected, the genetic associations with changes in fasting insulin and HOMA-IR appeared to be more pronounced at 6 months than at 2 years. In the low-protein diet group, there was no significant genotype effect on the trajectory of changes in insulin resistance over the 2 year intervention (*p*≥0.11). Similar results were observed when the analyses were restricted to the white participants.

Discussion

In this large, 2 year randomised dietary weight-loss intervention trial, we found significant interactions between a vitamin-D-related genetic variant *DHCR7* rs12785878 and dietary protein intake on changes in fasting insulin and HOMA-IR;

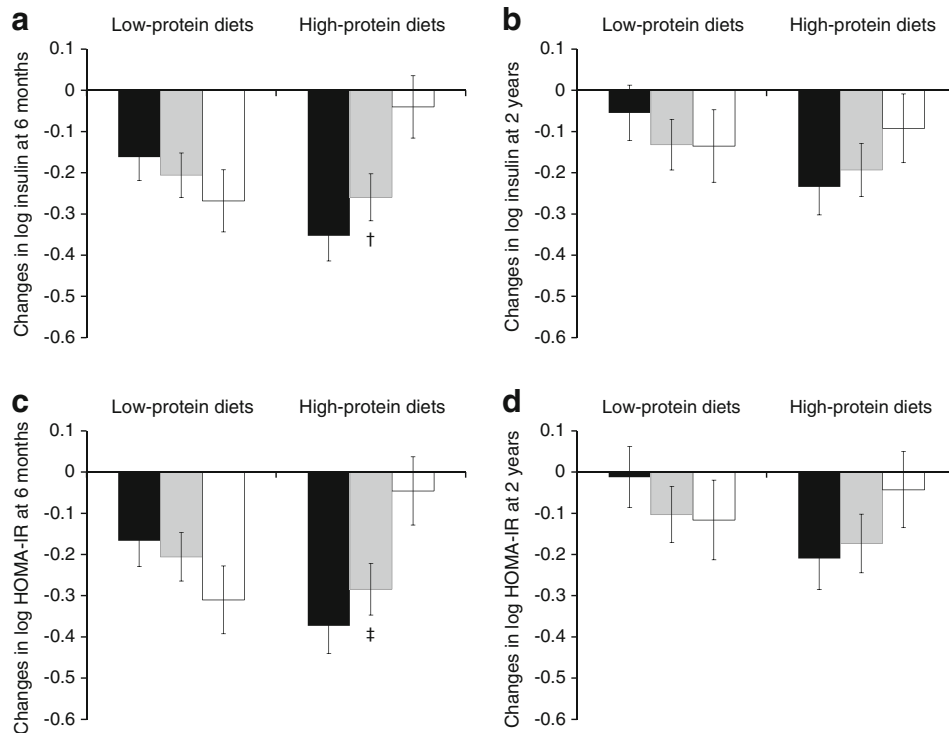
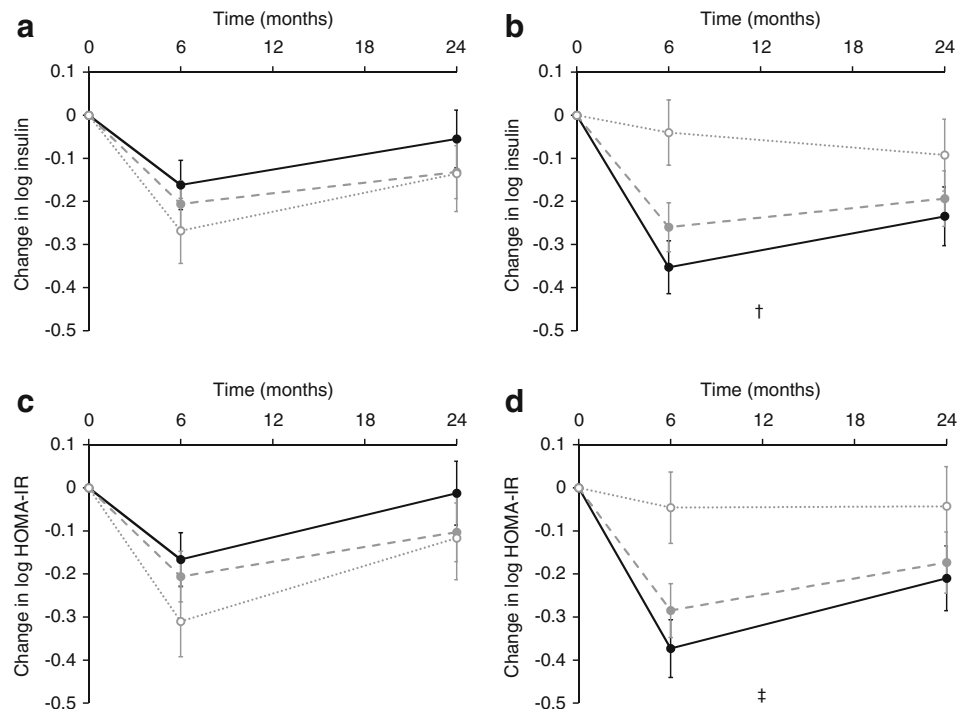


Fig. 1 Changes in insulin (**a**, **b**) and HOMA-IR (**c**, **d**) by *DHCR7* rs12785878 genotype in response to low-protein diets and high-protein diets at 6 months and 2 years. Black bars, *DHCR7* rs12785878 TT genotype; grey bars, *DHCR7* rs12785878 TG genotype; white bars, *DHCR7* rs12785878 GG genotype. Data are means \pm SE, adjusted for age, sex, ethnicity and baseline value for the respective outcome traits. $p=0.0008$ and 0.03 for interactions between *DHCR7* rs12785878 and diet

intervention on changes in fasting insulin at 6 months (**a**) and 2 years (**b**), respectively. $p=0.0009$ and 0.02 for interactions between *DHCR7* rs12785878 and diet intervention on changes in HOMA-IR at 6 months (**c**) and 2 years (**d**), respectively. † $p=0.0009$ and ‡ $p=0.002$ for genotype effects on changes in insulin (**a**) and HOMA-IR (**c**), respectively, at 6 months in the high-protein diet groups

Fig. 2 Trajectory of changes in insulin and HOMA-IR by *DHCR7* rs12785878 genotype in response to low-protein diets (**a**, **c**) and high-protein diets (**b**, **d**) over a 2 year intervention. Black circle and solid line, *DHCR7* rs12785878 TT genotype; grey circle and dashed line, *DHCR7* rs12785878 TG genotype; white circle and grey dotted line, *DHCR7* rs12785878 GG genotype. Data are means \pm SE, adjusted for age, sex, ethnicity and baseline value for the respective outcome traits. † $p<0.0001$ and ‡ $p=0.0009$ for genotype effects on changes in insulin (**b**) and HOMA-IR (**d**), respectively, in the high-protein diet groups



furthermore, results were consistent for short-term (6 months) and long-term (2 years) changes. The T-allele of *DHCR7* rs12785878 was associated with greater improvement of insulin resistance among individuals who were assigned to the high-protein diets.

A large body of evidence implicates the *DHCR7* gene in insulin resistance through effects on vitamin D levels. The T allele of *DHCR7* rs12785878 was strongly associated with higher circulating 25(OH)D levels in GWAS ($p=2.1 \times 10^{-27}$) [9], while lower 25(OH)D levels have been associated with obesity, insulin resistance, systemic inflammation and the risk of diabetes [2–8]. A recent Mendelian randomisation study found that the *DHCR7* allele score but not the *CYP2R1* allele score was associated with the risk of type 2 diabetes [14]. Several indices of insulin resistance were found to be significantly different across *DHCR7* rs12785878 genotypes in women with polycystic ovary syndrome [23]. In addition, *DHCR7* genetic variation was recently reported to interact with type 2 diabetes status to influence subclinical atherosclerosis [24]. However, no studies have investigated the interactions between *DHCR7* genetic variants and diet/lifestyle factors in relation to cardiometabolic traits.

Although the mechanism underlying the observed *DHCR7* rs12785878 gene–dietary protein interaction is unclear, several potential biological processes might be implicated. A perfect proxy SNP of *DHCR7* rs12785878 ($r^2=1$) has been found to modulate *DHCR7* mRNA levels in liver [24]. In addition, protein-rich foods (e.g. fish, dairy if fortified and meat to a lesser extent) are the main sources of naturally occurring cholecalciferol (vitamin D₃) [20], and cholecalciferol could suppress *DHCR7* activity in human skin cells [19]. In POUNDS Lost, the difference between high- and low-protein diets is mainly from the difference in consumption of skinless turkey, lean beef and skimmed/nonfat milk, while the difference between high- and low-fat diets is mainly from the difference in consumption of walnuts and olive oil [21]. Thus, there might be a greater difference in vitamin D content between high- and low-protein diets than that between high- and low-fat diets. However, we could not exclude the possibility that other dietary nutrients might be also involved, since it has been suggested that *DHCR7* gene expression might be regulated by dietary alpha-tocopherol [16], cholestyramine-plus-Lovastatin diet [17] and soy protein [18] in rats. In addition, dietary protein and amino acids are important modulators of glucose metabolism and insulin resistance [25–27]. Taken together, we speculate that cholecalciferol and/or other nutrients from protein-rich foods may interact with *DHCR7* rs12785878 (or a causal variant indicated by this SNP) to influence *DHCR7* function, thereby affecting vitamin D levels and eventually modifying the effects on changes in insulin resistance.

Diminished adherence occurred between 6 months and 2 years in POUND Lost [21]; therefore, it was not surprising

that the genetic effects on changes in insulin resistance were attenuated at 2 years. Improvement of insulin resistance has been associated with changes in body weight in this intervention study. However, we did not find significant gene–diet interaction or genetic effects on changes in body weight, although it has been previously suggested that adiposity might be involved in the relationships between vitamin-D-related variants, 25(OH)D levels and type 2 diabetes [14]. Moreover, the genetic effects on improvement of insulin resistance were independent of weight loss, suggesting other pathways might be involved.

We did not find strong evidence for interactions between the other two vitamin-D-related genetic variants, *CYP2R1* rs10741657 and *GC* rs2282679, and dietary interventions on changes in body weight or glucose/insulin metabolism in this 2 year weight-loss trial. Both genes have been largely implicated in vitamin D metabolism, and *CYP2R1* rs10741657 ($p=3.3 \times 10^{-20}$) and *GC* rs2282679 ($p=1.9 \times 10^{-109}$) were also strongly associated with circulating 25(OH)D levels in GWAS [9]. However, a recent study found that the expression levels of *CYP2R1* in adipose tissues did not change during diet-induced weight loss [28]. Nevertheless, further studies are warranted to validate the observed nominally significant interactions between *CYP2R1* rs10741657 and dietary fat on changes in fasting insulin and HOMA-IR. It has been suggested that the quality of dietary fat influences glucose metabolism and insulin resistance [29]. However, it should be noted that diets varying in fat content (low-fat diets or high-fat diets) are not emphasised in the nutritional therapy recommendations for diabetes [30], and both extremely high- and low-fat diets might not be beneficial regarding glucose metabolism in diabetes [29].

To the best of our knowledge, this is the first study to show significant interactions between vitamin-D-related genetic variation and dietary protein on improvement of insulin resistance in a large and long-term randomised trial. Previous evidence has suggested the important implications of vitamin D in insulin resistance and type 2 diabetes. Our findings provide new insight into the utility of using knowledge of vitamin-D-related genetic variation to improve personalised dietary interventions. However, several limitations of this study should be addressed. We did not measure circulating vitamin D or 25(OH)D concentrations in this study, which limited our analysis to examine the role of vitamin D levels in the gene–diet interaction and explore potential underlying mechanisms. Although our major findings remained significant after adjustment for multiple tests, replication of these results is needed in future. In addition, the majority of participants are white (80%) in our study and thus further studies are needed in other ethnic groups, since significant ethnic differences in circulating vitamin D levels and related genetic variants have been acknowledged [31].

In summary, our data indicate that dietary protein may modify the effect of the *DHCR7* rs12785878 variant on changes in insulin resistance over a 2 year dietary intervention. Individuals carrying the T allele (vitamin-D-increasing allele) of *DHCR7* rs12785878 might benefit more in improvement of insulin resistance than noncarriers by consuming high-protein weight-loss diets. Our findings of gene–protein dietary interaction are biologically plausible, provide additional evidence for the roles of vitamin D in insulin resistance, and suggest new insights into effective strategies for type 2 diabetes prevention and intervention.

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