Effect of the interaction between diet composition and the *PPM1K* genetic variant on insulin resistance and β cell function markers during weight loss: results from the Nutrient Gene Interactions in Human Obesity: implications for dietary guidelines (NUGENOB) randomized trial

Leticia Goni,[1,2] Lu Qi,[3–6] Marta Cuervo,[1,2,7,8] Fermín I Milagro,[1,2,7,8] Wim H Saris,[9] Ian A MacDonald,[10] Dominique Langin,[11–13] Arne Astrup,[14] Peter Arner,[17] Jean-Michel Oppert,[18] Mathilde Svendstrup,[15,19] Ellen E Blaak,[9] Thorkild IA Sørensen,[1,16,20] Torben Hansen,[15] and J Alfredo Martínez[1,2,7,8]

[1]Department of Nutrition, Food Sciences and Physiology, and [2]Center for Nutrition Research, Faculty of Pharmacy and Nutrition, University of Navarra, Pamplona, Navarra, Spain; [3]Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA; Departments of [4]Nutrition and [5]Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA; [6]Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [7]Biomedical Research Center Network in Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, Madrid, Spain; [8]Navarra Institute for Health Research, Pamplona, Navarra, Spain; [9]Department of Human Biology, School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, Netherlands; [10]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom; [11]National Institute of Health and Medical Research, UMR1048, Institute of Metabolic and Cardiovascular Diseases, Toulouse, France; [12]University of Toulouse, UMR1048, Institute of Metabolic and Cardiovascular Diseases, Paul Sabatier University, Toulouse, France; [13]Laboratory of Clinical Biochemistry, Toulouse University Hospitals, Toulouse, France; [14]Department of Nutrition, Exercise and Sports, and [15]Novo Nordisk Foundation Center for Basic Metabolic Research and [16]Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [17]Department of Medicine, Karolinska Institute at Karolinska University Hospital, Huddinge, Stockholm, Sweden [18]Department of Nutrition, Pitie-Salpetriere University Hospital; University Pierre et Marie Curie-Paris 6; Institute of Cardiometabolism and Nutrition; Center for Research on Human Nutrition Ile-de-France; Paris, France; [19]Danish Diabetes Academy, Odense, Denmark; and [20]Department of Clinical Epidemiology (formerly Institute of Preventive Medicine), Bispebjerg and Frederiksberg Hospitals, The Capital Region, Copenhagen, Denmark

ABSTRACT

Background: Circulating branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) have been shown to be associated with insulin resistance and diabetes risk. The common rs1440581 T allele in the protein phosphatase Mg2+/Mn2+ dependent 1K (*PPM1K*) gene has been related to elevated BCAA concentrations and risk of type 2 diabetes.

Objective: In the present study, we tested whether dietary fat and carbohydrate intakes influenced the association between the rs1440581 *PPM1K* genetic variant and glucose-metabolism traits during weight loss.

Design: The rs1440581 *PPM1K* genetic variant was genotyped in a total of 757 nondiabetic individuals who were randomly assigned to 1 of 2 energy-restricted diets that differed in macronutrient composition (low-fat diet: 20–25% fat, 15% protein, and 60–65% carbohydrate; high-fat diet: 40–45% fat, 15% protein, and 40–45% carbohydrate). The changes in fasting glucose, fasting insulin, insulin resistance (homeostasis model assessment of insulin resistance) and homeostasis model assessment of β cell function (HOMA-B) were measured after a mean $\pm$ SD weight loss of 6.8 $\pm$ 3.4 kg over 10 wk and analyzed according to the presence of the T allele of rs1440581.

Results: The rs1440581 T allele was associated with a smaller improvement in glucose concentrations after the 10-wk dietary intervention ($\beta \pm$ SE: 0.05 $\pm$ 0.02 mg/dL; $P = 0.03$). In addition, significant gene-diet interactions were shown for the rs1440581 *PPM1K* genetic variant in relation to changes in insulin and HOMA-B (P-interaction = 0.006 and 0.002, respectively). In response to the high-fat diet, the T allele was associated with a higher reduction of insulin ($\beta \pm$ SE: -0.77 ± 0.40 μU/mL; $P = 0.04$) and HOMA-B ($\beta \pm$ SE: -13.2 ± 3.81; $P = 0.003$). An opposite effect was observed in the low-fat diet group, although in this group the T allele was marginally ($P = 0.10$) and not significantly ($P = 0.24$) associated with insulin and HOMA-B, respectively.

Conclusion: *PPM1K* rs1440581 may affect changes in glucose metabolism during weight loss, and this effect is dependent on dietary fat and carbohydrate intakes. This trial was registered at controlled-trials.com as ISRCTN25867281. *Am J Clin Nutr* 2017;106:902–8.

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Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

Address correspondence to JAM (e-mail: jalfmtz@unav.es).

Abbreviations used: AAA, aromatic amino acid; BCAA, branched-chain amino acid; BCKD, branched-chain α ketoacid; HOMA-B, homeostasis model assessment of β cell function; NUGENOB, Nutrient Gene Interactions in Human Obesity: implications for dietary guidelines; POUNDS LOST, Preventing Overweight Using Novel Dietary Strategies; *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K; T2D, type 2 diabetes.

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INTRODUCTION

Metabolomics studies have shown that elevated circulating amino acids, particularly branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs), might be associated with obesity, insulin resistance, and a higher risk of type 2 diabetes (T2D) (1–4). In addition, a previous study reported that weight-loss diets in overweight and obese subjects may have long-term effects on circulating BCAA and AAA concentrations (5). Although it remains unknown whether BCAAs and AAAs are causally implicated in metabolic disturbances, the results of a recent Mendelian randomization study, which included a large-scale human genetic and a metabolomics study, provided evidence of a possible causal role of BCAA metabolism in the etiology of T2D (6). In contrast, Mahendran et al. (7) concluded that higher plasma BCAA concentrations had no causal effect on insulin resistance.

Several genome-wide association studies have revealed common genetic variants that determine plasma amino acid concentrations (6, 8–13). Of these, the T allele of the protein phosphatase Mg2+/Mn2+ dependent 1K (*PPM1K*) rs1440581 genetic variant has been associated with higher valine and leucine concentrations, in addition to other amino acids (6, 9, 11) as well as Fischer's ratio of BCAAs to AAAs (characteristic of liver fibrosis) (9, 14). The same polymorphism was defined by a systems genetics approach as a genetic variant involving susceptibility to T2D (15). Subsequently, Lotta et al. (6) observed an OR of 1.04 per risk allele (95% CI: 1.02, 1.07 per risk allele) of the *PPM1K* rs1440581 polymorphism for T2D risk. In the POUNDS LOST (Preventing Overweight Using Novel Dietary Strategies) trial, carriers of the risk allele of rs1440581 showed higher weight loss and better improvement in insulin sensitivity than did individuals without this allele when the participants consumed a high-fat diet that was prescribed for weight loss (16).

In the present study, we analyzed the association between the *PPM1K* rs1440581 genetic variant and changes in glucose-metabolism–related traits [fasting glucose, fasting insulin, HOMA-IR, and homeostasis model assessment of β cell function (HOMA-B)] in response to 2 energy-restricted diets with different relative amounts of fat compared with carbohydrates. In addition, potential gene-diet interactions over the course of the intervention were examined.

METHODS

Study participants

A total of 757 subjects from the NUGENOB (Nutrient Gene Interactions in Human Obesity: implications for dietary guidelines) study, which was a randomized, parallel, 2-arm, open-label, 10-wk dietary intervention that compared the effects of 2 hypoenergetic diets with either a low- or high-fat content, were included in the present study (controlled-trials.com; ISRCTN25867281) (**Supplemental Figure 1**). The NUGENOB study was conducted at 8 sites in 7 European countries [United Kingdom, Netherlands, France (2 centers), Spain, Czech Republic, Sweden, and Denmark].

The study design and methods have been described in detail elsewhere (www.nugenob.org) (17, 18). Briefly, the inclusion criteria were being aged from 20 to 50 y of age with BMI (in kg/m^2) $\geq$30. The exclusion criteria were as follows: weight change $>$3 kg $\leq$3 mo before the beginning of the study; drug-treated hypertension, diabetes, or hyperlipidemia; untreated thyroid disease; surgically treated obesity; pregnancy; alcohol or drug abuse; and participation in other simultaneous ongoing trials. The study protocol was approved by the ethics committee of each center and country. All participants gave written informed consent after they were informed of the nature and risks of the experimental procedure.

Dietary intervention

The macronutrient composition of the 2 diets was as follows: low-fat diet: 20–25% of total energy from fat, 15% of total energy from protein, and 60–65% of total energy from carbohydrate; and high-fat diet: 40–45% of total energy from fat, 15% of total energy from protein, and 40–45% of total energy from carbohydrate. Both diets were designed to provide 600 kcal/d less than the individually estimated daily energy requirement on the basis of an initial resting metabolic rate that was multiplied by 1.3 as described elsewhere (17).

To assess the dietary adherence across the intervention, a 3-d weighed food record of 2 weekdays and 1 weekend day was performed at baseline and the final week of the intervention (17). Moreover, 1-d weighed food records were completed in the second, fifth, and seventh weeks. The dietary records were analyzed with the use of the food-nutrient database that is routinely used in each center or country.

Measurements

Body weight and height were measured with calibrated scales and stadiometers, respectively, according to a commonly shared standardized protocol. Waist circumference was measured with the participant wearing only nonrestrictive underwear. The mean of 3 measurements was used for each variable. BMI was calculated as weight divided by height squared.

Venous samples were collected after an overnight fast of 12 h and after participants rested in a supine position for 15 min. Fasting plasma glucose and lipid concentrations were measured in a central laboratory with the use of standard enzymatic techniques on a COBAS FARA centrifugal spectrophotometer (Roche Diagnostica) (glucose HK 125, ANX Diagnostics; triglycerides, Sigma; total cholesterol: cholesterol 100, ABX Diagnostics; and HDL cholesterol, Roche). Fasting plasma LDL was calculating according to Friedewald's equation (19). Fasting plasma insulin concentrations were measured with a double antibody radioimmunoassay (Insulin RIA 100; Kabi-Pharmacia). Insulin resistance was estimated via the HOMA-IR with the use of the following equation (20–22):

$$[\text{Fasting insulin } (\mu\text{L/mL})] \times [\text{fasting glucose (mg/dL)} \div 18.01] \div 22.5 \quad (1)$$

β cell function was estimated via the HOMA-B as follows (20–22):

$$[20 \times \text{fasting insulin } (\mu\text{L/mL})] \div \{[\text{fasting glucose (mg/dL)} \div 19.01] - 3.5\} \quad (2)$$

Genotyping

DNA was extracted from buffy coats at the Steno Diabetes Center. Extracted DNA samples were diluted in Tris/EDTA

buffer to a stock a DNA solution of 100 ng/μL and a working DNA solution of 10 ng/μL. A total of 771 subjects were randomly assigned to one of the 2 diets. Of these subjects, 14 individuals were not successfully genotyped because of insufficient samples or technical errors. Thus, the *PPM1K* rs1440581 genetic variant was successfully genotyped in 757 individuals by the LCG group with the use of the KASPTM genotyping assay.

Statistical analyses

The primary outcomes of the present study were changes in overnight fasting glucose-metabolism traits (fasting glucose, fasting insulin, HOMA-IR, and HOMA-B) from baseline to completion of the 10-wk dietary intervention. To compare baseline characteristics across genotypes, an ANOVA test was used for continuous variables, and chi-square tests were used for categorical variables. Chi-square tests were also used to test for any genetic deviation from the Hardy-Weinberg equilibrium. General linear models that were adjusted for age and sex were applied to compare energy and nutrient intakes across the genotypes during the intervention. Multivariate general linear models were used to test the changes in the primary endpoints according to genotype groups after adjustment for covariates (model 1 was adjusted for age, sex, diet group, and the respective baseline variables; model 2 was adjusted as for model 1 and for BMI at baseline). All outcomes (fasting glucose, fasting insulin, HOMA-IR, and HOMA-B) were log transformed before analyses because of skewed distributions. Thus, the reported means ± SDs or βs ± SEs are in the original scale, whereas the *P* values relating to the variables are log transformed. The interaction term was included in the models (e.g., *PPM1K* genotype × high- or low-fat diet group) to test for potential gene-diet interactions (high-fat diet compared with low-fat diet). Additive and codominant genetic models were used in the analysis. STATA/SE version 12.0 software (StataCorp LP) was used for the statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

The *PPM1K* rs1440581 allele frequencies were consistent with Hardy-Weinberg equilibrium. The minor-allele frequency (T allele) was 0.51 in the study population. Baseline characteristics of the volunteers are described by *PPM1K* genotype in **Table 1**. There was no significant difference in the genotype distribution by sex or diet groups. The rs1440581 polymorphism was marginally positively associated with age ($P = 0.05$) but not with dietary intake or anthropometric measurements. Baseline concentrations of glucose, insulin resistance, and β cell function markers did not differ across *PPM1K* rs1440581 genotypes.

Diet adherence and weight loss

The targets of macronutrient intakes during the intervention were achieved (**Table 2**). The reported fat intake was 24.7% in the low-fat diet group (target: 20–25%) and 40.3% in the high-fat diet group (target: 40–45%) with a group difference of 15.6% ($P < 0.001$; 95% CI: 14.9, 16.3%). There were no significant differences in energy and macronutrient intakes across the *PPM1K* rs1440581 genotype and within diet groups (Table 2).

TABLE 1
Baseline characteristics of study participants categorized according to the *PPM1K* rs1440581 genetic variant[1]

	CC (n = 183)	CT (n = 380)	TT (n = 194)	P
Age, y	36.0 ± 7.9[2]	37.3 ± 8.0	38.0 ± 7.5	0.05
Sex, n (%)				0.80
M	47 (25.7)	97 (25.5)	45 (23.2)	
F	136 (74.3)	283 (74.5)	149 (76.8)	
Dietary group, n (%)				0.43
Low fat	92 (50.3)	184 (48.4)	105 (54.1)	
High fat	91 (49.7)	196 (51.6)	89 (45.9)	
Dietary intake, /d				
Energy, kcal	2225 ± 662	2206 ± 741	2135 ± 623	0.40
Protein, %	16.3 ± 3.3	16.3 ± 3.7	16.4 ± 3.4	0.52
Fat, %	36.6 ± 7.0	36.6 ± 7.8	35.6 ± 7.4	0.34
Carbohydrate, %	45.2 ± 8.0	45.0 ± 9.1	46.0 ± 7.8	0.46
Body weight, kg	100.5 ± 15.5	100.6 ± 17.1	99.8 ± 15.1	0.86
BMI, kg/m^2	35.5 ± 4.8	35.5 ± 4.9	35.5 ± 4.4	0.99
WC, cm	105.9 ± 12.3	105.8 ± 13.4	106.4 ± 11.9	0.90
Glucose, mmol/L	5.44 ± 1.17	5.44 ± 0.76	5.31 ± 0.63	0.19
Insulin, μU/mL	10.4 ± 5.7	10.6 ± 7.2	9.7 ± 5.8	0.29
HOMA-IR	2.59 ± 1.70	2.68 ± 2.23	2.38 ± 1.60	0.22
HOMA-B	108.9 ± 53.6	110.2 ± 67.0	107.2 ± 61.9	0.87
Total cholesterol, mmol/L	4.89 ± 0.98	4.97 ± 0.84	4.96 ± 0.90	0.59
LDL, mmol/L	3.28 ± 0.90	3.33 ± 0.77	3.36 ± 0.80	0.58
HDL, mmol/L	1.12 ± 0.29	1.14 ± 0.31	1.13 ± 0.32	0.80
Triacylglycerides, mmol/L	1.09 ± 0.61	1.11 ± 0.70	1.08 (0.59)	0.88

[1] Data were calculated with the use of the chi-square test for categorical variables and an ANOVA for continuous variables. HOMA-B, homeostasis model assessment of β cell function; *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K; WC, waist circumference.
[2] Mean ± SD (all such values).

The dietary intervention produced a mean ± SD weight loss of 6.7 ± 3.5 kg in the high-fat diet group and of 6.9 ± 3.3 kg in the low-fat diet group with no significant differences between groups ($P = 0.30$). The *PPM1K* rs1440581 genetic variant was not associated with weight loss after adjustment for age, sex, diet group, and baseline BMI ($P = 0.19$). In addition, the *PPM1K* genotype did not interact with dietary fat intake regarding the changes in weight after being adjusted for covariates (P-interaction = 0.94).

Genotype effects on glucose-metabolism traits

The *PPM1K* rs1440581 effects on changes in glucose-metabolism traits after the 10-wk dietary intervention in the whole study population were analyzed (**Table 3**). After adjustment for age, sex, diet group, the respective baseline variable, and baseline BMI, the T allele was associated with a smaller reduction in fasting glucose ($\beta = 0.06$ mmol/L per each risk allele; $P = 0.02$). Further adjustment for body weight loss slightly attenuated the association although the P value remained significant ($\beta = 0.05$ mmol/L per each risk allele; $P = 0.03$). No significant associations were shown between the polymorphism and changes in insulin, HOMA-IR, or HOMA-B.

Genotype-diet interactions on glucose-metabolism traits

We tested whether the genotype effect of *PPM1K* rs1440581 on glucose-metabolism traits differed by diet composition (high-fat diet compared with low-fat diet) regarding the additive model (**Table 4**). Significant interactions between the *PPM1K* genotype

TABLE 2
Dietary intake by the *PPM1K* rs1440581 genetic variant and diet group during the intervention[1]

	All population	CC	CT	TT	*P*
Low-fat diet[2]					
Energy, kcal	1560 ± 372	1564 ± 339	1597 ± 415	1487 ± 313	0.15
Protein, %	18.0 ± 2.9	18.1 ± 2.7	17.9 ± 2.8	18.4 ± 3.1	0.47
Fat, %	24.7 ± 4.8	24.9 ± 4.8	24.7 ± 4.7	24.6 ± 5.1	0.94
Carbohydrate, %	56.9 ± 5.7	56.8 ± 5.2	57.0 ± 5.6	56.7 ± 6.3	0.83
High-fat diet[3]					
Energy, kcal	1622 ± 328[4]	1625 ± 277	1608 ± 358	1655 ± 310	0.71
Protein, %	17.0 ± 2.5[4]	17.2 ± 2.1	17.0 ± 2.8	16.9 ± 2.2	0.77
Fat, %	40.3 ± 5.2[4]	40.8 ± 4.4	40.4 ± 5.8	40.0 ± 4.5	0.63
Carbohydrate, %	42.4 ± 5.4[4]	42.0 ± 4.8	42.5 ± 5.6	42.8 ± 5.2	0.53

[1] All values are means ± SDs. Data were calculated with the use of an ANCOVA after adjustment for age and sex. *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K.
[2] Data were available for 361 individuals (CC: n = 86; CT: n = 175; TT: n = 100).
[3] Data were available for 345 individuals (CC: n = 84; CT: n = 182; TT: n = 79).
[4] $P < 0.05$ compared with low-fat diet.

and dietary fat and carbohydrate on changes in insulin and HOMA-IR after adjustment for age, sex, the respective baseline variable, and BMI at baseline were observed (*P*-interaction = 0.01 and 0.02, respectively). In the low-fat and high-carbohydrate diet group, the T allele was marginally associated with a smaller improvement in insulin markers (β = 0.69 μU/mL per risk allele; P = 0.07 for fasting insulin, and β = 0.21 per risk allele; P = 0.04 for HOMA-IR). Conversely, in response to the high-fat and low-carbohydrate diet, carrying the T allele was positively associated with larger changes in these outcomes. Consistent with the effect of gene-diet interaction on changes in insulin concentrations and HOMA-IR, a significant interaction between the *PPM1K* genetic variant and the low-fat and high-fat diets was shown for changes in HOMA-B (*P*-interaction = 0.003). In response to the high-fat and low-carbohydrate diets, a significant association was shown between the T allele and changes in HOMA-B; per each risk allele, HOMA-B decreased 12.5 U (P = 0.006). Additional adjustment for changes in body weight did not appreciably change the *P* values for interactions between the genotype and the low-fat or high-fat diet in changes of insulin, HOMA-IR, and HOMA-B (*P*-interaction = 0.006, 0.01, and 0.002, respectively).

Moreover, an analysis of gene-diet interactions with the use of a codominant model was performed (**Figure 1**). Consistent with the results from the additive model, significant gene-diet interactions were shown for changes in insulin, HOMA-IR, and HOMA-B (*P*-interaction = 0.03, 0.049, and 0.01, respectively).

Because differences were shown between diets for energy intake (P = 0.005), gene-diet interaction analyses were further adjusted for total energy intake during the intervention. However, the results were similar (data not shown).

DISCUSSION

The present study aimed to investigate the relation between the *PPM1K* genetic variant and changes in glucose-metabolism traits during diet-induced weight loss and to assess potential interactions between the genetic variant and the fat intake compared with carbohydrate intake of the diet. In a large sample of obese European subjects, we showed a significant association of the *PPM1K* rs1440581 polymorphism with changes in glucose concentrations and significant interactions of dietary fat and carbohydrate intakes with *PPM1K* genotypes for changes in insulin resistance and β cell function markers.

The *PPM1K* gene encodes mitochondrial protein phosphatase 1K, which is an activator of the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex that is a major determinant of the rate of BCAA catabolism (23, 24). From one point of view, the *PPM1K* rs1440581 has been associated with high concentrations of BCAAs as well as the ratio of BCAAs and AAAs (6, 9, 11). Moreover, the presence of such a polymorphism has also been linked to increased risk of T2D with an OR of 1.04 per risk allele (95% CI: 1.02, 1.07 per risk allele) (6). In contrast, subjects with mutations of *PPM1K* (25) and the knock-out Ppm1k mice (26) presented impaired BCKD activity and high concentrations of BCAAs and branched-chain α ketoacids. Moreover, a fat transplant led to a reduction in plasma BCAAs in Ppm1k knock-out mice (27). In this context, Lotta et al. (6) observed an association between the metabolites that accumulated upstream of BCKD action and the incidence of T2D, whereas the associations of metabolites that accumulated downstream of BCKD action were inconsistent. The authors suggested that reduced BCKD activity could be one of the mechanistic links between BCAA metabolism and risk of T2D.

TABLE 3
Effect of the *PPM1K* rs1440581 genetic variant on changes in glucose-metabolism traits after a 10-wk diet intervention (n = 637)[1]

	Model 1		Model 2	
	β ± SE	*P*	β ± SE	*P*
ΔGlucose, mmol/L	0.06 ± 0.02	0.02	0.05 ± 0.02	0.03
ΔInsulin, μU/mL	0.03 ± 0.27	0.93	−0.07 ± 0.26	0.76
ΔHOMA-IR	0.05 ± 0.08	0.52	0.02 ± 0.08	0.99
ΔHOMA-B	−3.28 ± 2.73	0.43	−4.06 ± 2.68	0.26

[1] Data were calculated with the use of linear regression (an additive genetic model was used in the analyses). β represents changes in outcomes for the increasing number of T allele of the rs1440581 *PPM1K* genetic variant. Model 1 was adjusted for age, sex, diet group, the respective baseline variable, and baseline BMI. Model 2 was adjusted as for model 1 and for weight loss. *P* values were log transformed. HOMA-B, homeostasis model assessment of β cell function; *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K; Δ, change.

TABLE 4
Effect of the *PPM1K* rs1440581 genetic variant on changes in glucose-metabolism traits in response to low- and high-fat diet at 10 wk of a diet intervention (n = 637)

	Low-fat diet		High-fat diet		
	$\beta \pm$ SE	P	$\beta \pm$ SE	P	P-interaction
Model 1					
ΔGlucose, mmol/L	0.06 ± 0.03	0.08	0.08 ± 0.03	0.05	0.72
ΔInsulin, μU/mL	0.69 ± 0.34	0.07	−0.64 ± 0.42	0.09	0.01
ΔHOMA-IR	0.21 ± 0.12	0.04	−0.09 ± 0.12	0.20	0.02
ΔHOMA-B	4.30 ± 3.79	0.16	−12.5 ± 3.87	0.006	0.003
Model 2					
ΔGlucose, mmol/L	0.05 ± 0.03	0.14	0.07 ± 0.03	0.07	0.69
ΔInsulin, μU/mL	0.60 ± 0.32	0.10	−0.77 ± 0.40	0.04	0.006
ΔHOMA-IR	0.17 ± 0.11	0.08	−0.12 ± 0.11	0.08	0.01
ΔHOMA-B	3.36 ± 3.70	0.24	−13.2 ± 3.81	0.003	0.002

[1] Data were calculated with the use of linear regression models (an additive genetic model was used in the analyses). The interaction term was included in the models to test gene-diet interactions. β represents changes in outcomes for the increasing number of T allele of the rs1440581 *PPM1K* genetic variant. Model 1 was adjusted for age, sex, the respective baseline variable, and baseline BMI. Model 2 was adjusted as for model 1 and for weight loss. P values were log transformed. HOMA-B, homeostasis model assessment of β cell function; *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K; Δ, change.

However, it should be highlighted that *PPM1K* could have pleiotropic effects, thus limiting the interpretation that the association with glucose-metabolism traits is mediated through BCAA metabolism and suggesting that other metabolic pathways could be involved. To our knowledge, despite the evidence about the involvement of *PPM1K* on BCAAs metabolism and T2D, there has been a paucity of information on the functional effect of the rs1440581 on *PPM1K* gene expression and consequently on BCKD activity. However, because the common *PPM1K* genetic variant is located in an intronic region, the splicing process would be altered and, with it, the structure and function of the protein.

The effect of the *PPM1K* rs1440581 genetic variant on changes in insulin resistance and β cell function markers was significantly modified by dietary fat and carbohydrate intakes. Carriers of the T allele had a greater decrease in insulin, HOMA-IR, and HOMA-B when consuming a high-fat diet. Our results are in accordance with the study by Xu et al. (16), who observed that carriers of the T allele responded better to a high-fat diet than to a low-fat diet in terms of body weight, insulin concentrations, and the insulin-resistance index. Although, to our knowledge, the mechanisms that underlie the effects of the modulation of dietary fat and carbohydrate intakes on the *PPM1K* rs1440581 are unknown, a synergistic interference of BCAAs and lipids with the development of insulin resistance has been proposed (28, 29). This hypothesis is based on the results in animal studies that have shown that a high-fat diet strengthened the effect of BCAA supplementation on insulin resistance (29). Although the concentrations of plasma BCAAs were not altered in rats that were fed a high-fat diet, Kadota et al. (30) showed that the hepatic BCKD complex was

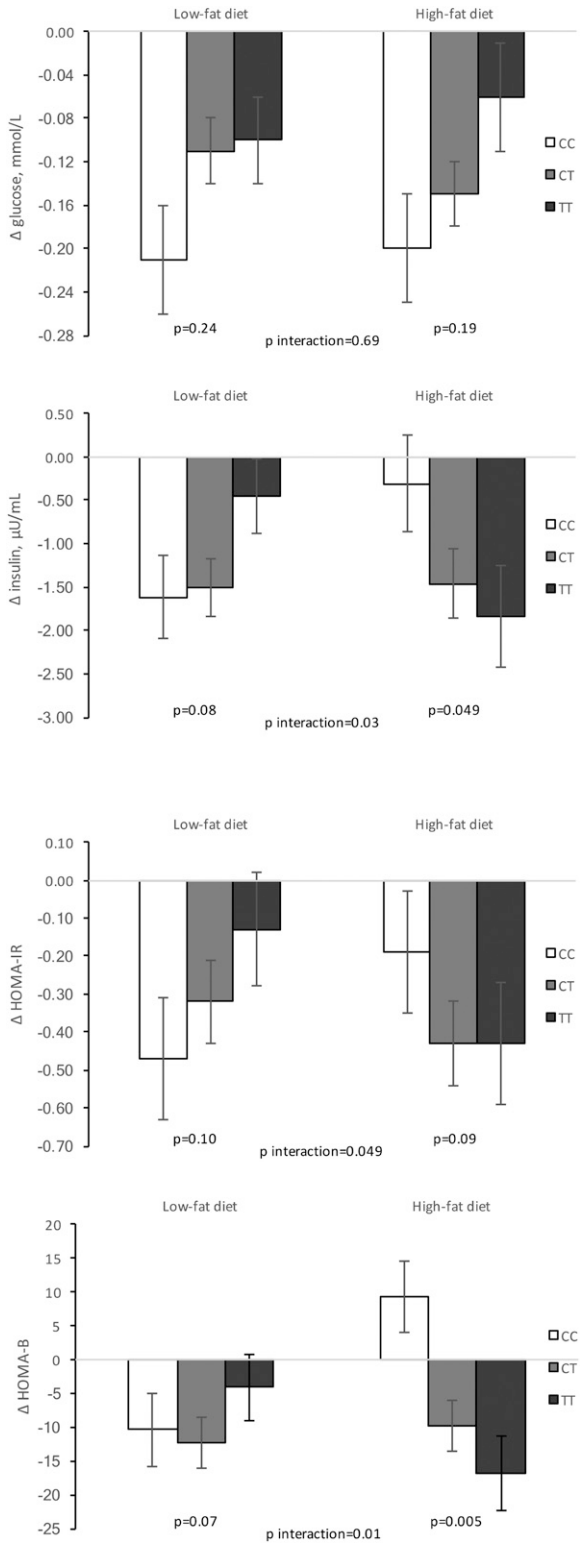

FIGURE 1 Mean ± SE effects of the *PPM1K* rs1440581 genetic variant on changes in glucose-metabolism traits in response to a low- or high-fat diet at 10 wk of a diet intervention. Data were calculated with the use of linear regression models (codominant genetic model was used in the analyses). The interaction term was included in the models to test gene-diet interactions. Data are means ± SE values after adjustment for age, sex, the respective baseline variable, baseline BMI, and weight loss (model 2). P values for glucose, insulin, HOMA-IR, HOMA-B log transformed (n = 637). HOMA-B, homeostasis model assessment of β cell function; *PPM1K*, protein phosphatase Mg2+/Mn2+ dependent 1K; Δ, change.

upregulated in such a model. In addition, it has been reported that a glucose challenge resulted in a reduction of circulating BCAA concentrations and increased expression of *PPM1K* in muscle biopsies in normoglycemic subjects in agreement with results in animals (31–33). In the present study, we showed that the genetic effects on changes in glucose-metabolism traits depend on dietary fat and carbohydrate intakes. This opposite nutrigenetic effect could be partly explained by the "differential susceptibility hypothesis," which proposes that genes could be considered as plasticity factors because genetic risk can be modified by environmental exposures such as dietary habits (34–37). Thus, although some individuals proved highly susceptible to environmental conditions, for better or worse, depending on the genotype, other individuals appeared to be hardly affected (35). Further experimental studies are needed to clarify the mechanisms underlying our results.

In the present study, adjustment for body weight loss did not notably alter the association between the *PPM1K* polymorphism and glucose concentrations, and the observed effects of gene-diet interaction on changes in insulin resistance and β cell function markers remained significant. Thus far, the genotype effect on changes in glucose-metabolism traits may be independent of the degree of weight loss. However, the experiment was not designed to compare the results of the dietary interventions with a control group without weight loss. Weight loss has been associated with improvements in glucose-metabolism markers and BCAAs and AAAs in previous studies (5, 38, 39). Moreover, in our study, body weight loss was positively associated with changes in glucose, insulin, HOMA-IR, and HOMA-B. The positive effect of weight loss on changes in glucose-metabolism traits can be explained by different mechanisms of increasing peripheral insulin sensitivity and insulin secretion and decreasing hepatic glucose production as well as circulating free fatty acids (38). These findings suggest that the biological mechanisms underlying the relation of weight loss and *PPM1K* gene with BCAAs and glucose-metabolism traits might be different.

The major strength of the study is the relatively large sample, which allows for the replication of the results that were previously reported by Xu et al. (16) from the POUNDS LOST trial. The consistent findings of gene-diet interactions in 2 randomized trials minimize the potential false-positive errors and highlight the robustness of the results. Moreover, considering the findings of both studies, it may be concluded that the effect of the *PPM1K* rs1440581, depending on dietary fat intake, on glucose-metabolism traits could be generalized to different ethnic groups because the participants in the NUGENOB study were of European ancestry whereas the population in the POUNDS LOST trial encompassed predominantly European Americans and African Americans. Nevertheless, several limitations of this study need to be considered. Circulating concentrations of BCAAs were not measured, but several studies have shown the relation between the *PPM1K* polymorphism and BCAA concentrations (6, 9, 11). In addition, a genetic variant could be a better marker than biochemical biomarkers are because it is less likely to be affected by confounding and reverse causation according to the Mendelian randomization principle (40). Finally, because low-fat diets are characterized by high-carbohydrate intake and vice versa, to preserve an energy balance, it is difficult to define which macronutrient would best explain the observed gene-diet interactions.

In conclusion, our findings indicate that the rs1440581 *PPM1K* polymorphism affects changes in glucose concentrations in response to weight loss interventions. Furthermore, dietary fat and carbohydrate might modify the genetic effects of the *PPM1K* genetic variant on changes in insulin resistance and β cell function markers. Individuals with the *PPM1K* rs1440581 CC genotype might be more responsive to a low-fat and high-carbohydrate diet in lowering insulin, HOMA-IR, and HOMA-B than are individuals without this genotype. However, it can also be interpreted that, for improving glucose-metabolism markers, T-allele carriers may benefit more from a high-fat and low-carbohydrate diet. Ultimately, the results of the present study could lead toward the development of more precise dietary strategies that are based on the genotype (41).

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REFERENCES

1. Rauschert S, Uhl O, Koletzko B, Hellmuth C. Metabolomic biomarkers for obesity in humans: a short review. Ann Nutr Metab 2014;64:314–24.
2. Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J, Hu FB. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 2016;39:833–46.
3. Yoon M-S. The emerging role of branched-chain amino acids in insulin resistance and metabolism. Nutrients 2016;8:E405.
4. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 2014;10:723–36.
5. Zheng Y, Ceglarek U, Huang T, Li L, Rood J, Ryan DH, Bray GA, Sacks FM, Schwarzfuchs D, Thiery J, et al. Weight-loss diets and 2-y changes in circulating amino acids in 2 randomized intervention trials. Am J Clin Nutr 2016;103:505–11.
6. Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, Schmidt AF, Imamura F, Stewart ID, Perry JRB, et al. Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of type 2 diabetes: a Mendelian randomisation analysis. PLoS Med 2016; 13: e1002179.
7. Mahendran Y, Jonsson A, Have CT, Allin KH, Witte DR, Jørgensen ME, Grarup N, Pedersen O, Kilpeläinen TO, Hansen T. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. Diabetologia 2017;60:873–8.
8. Gieger C, Geistlinger L, Altmaier E, De Angelis MH, Kronenberg F, Meitinger T, Mewes HW, Wichmann HE, Weinberger KM, Adamski J, et al. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. PLoS Genet 2008;4:e1000282.
9. Kettunen J, Tukiainen T, Sarin A-P, Ortega-Alonso A, Tikkanen E, Lyytikäinen L-P, Kangas AJ, Soininen P, Würtz P, Silander K, et al. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat Genet 2012;44:269–76.
10. Rhee EP, Ho JE, Chen MH, Shen D, Cheng S, Larson MG, Ghorbani A, Shi X, Helenius IT, O'Donnell CJ, et al. A genome-wide association study of the human metabolome in a community-based cohort. Cell Metab2013;18:130–43.
11. Shin S-Y, Fauman EB, Petersen A-K, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang T-P, et al. An atlas of genetic influences on human blood metabolites. Nat Genet 2014;46:543–50.
12. Draisma HHM, Pool R, Kobl M, Jansen R, Petersen A-K, Vaarhorst AAM, Yet I, Haller T, Demirkan A, Esko T, et al. Genome-wide association study identifies novel genetic variants contributing to variation in blood metabolite levels. Nat Commun 2015;6:7208.

13. Kettunen J, Demirkan A, Würtz P, Draisma HHMM, Haller T, Rawal R, Vaarhorst A, Kangas AJ, Lyytikäinen L-P, Pirinen M, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun 2016;7:11122.
14. Soeters P, Fischer J. Insulin, glucagon, aminoacid imbalance, and hepatic encephalopathy. Lancet 1976;2:880–2.
15. Taneera J, Lang S, Sharma A, Fadista J, Zhou Y, Ahlqvist E, Jonsson A, Lyssenko V, Vikman P, Hansson O, et al. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab 2012;16:122–34.
16. Xu M, Qi Q, Liang J, Bray GA, Hu FB, Sacks FM, Qi L. Genetic determinant for amino acid metabolites and changes in body weight and insulin resistance in response to weight-loss diets: the preventing overweight using novel dietary strategies (POUNDS LOST) trial. Circulation 2013;127:1283–9.
17. Petersen M, Taylor MA, Saris WH, Verdich C, Toubro S, Macdonald I, Rössner S, Stich V, Guy-Grand B, Langin D, et al. Randomized, multicenter trial of two hypo-energetic diets in obese subjects: high- versus low-fat content. Int J Obes (Lond) 2006;30:552–60.
18. Sørensen TIA, Boutin P, Taylor MA, Larsen LH, Verdich C, Petersen L, Holst C, Echwald SM, Dina C, Toubro S, et al. Genetic polymorphisms and weight loss in obesity: a randomised trial of hypo-energetic high- versus low-fat diets. PLoS Clin Trials 2006;1:e12.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
20. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191–2.
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
22. Borai A, Livingstone C, Ferns GAA. The biochemical assessment of insulin resistance. Ann Clin Biochem 2007;44:324–42.
23. Joshi M, Jeoung NH, Popov KM, Harris RA. Identification of a novel PP2C-type mitochondrial phosphatase. Biochem Biophys Res Commun 2007;356:38–44.
24. Lu G, Sun H, She P, Youn J, Warburton S, Ping P, Vondriska TM, Cai H, Lynch CJ, Wang Y. Protein phosphatase 2Cm is a critical regulator of branched-chain amino acid catabolism in mice and cultured cells. J Clin Invest 2009;119:1678–87.
25. Oyarzabal A, Martínez-Pardo M, Merinero B, Navarrete R, Desviat LR, Ugarte M, Rodríguez-Pombo P. A novel regulatory defect in the branched-chain alpha-keto acid dehydrogenase complex due to a mutation in the PPM1K gene causes a mild variant phenotype of maple syrup urine disease. Hum Mutat 2013;34:355–62.
26. Loh P-R, Bhatia G, Gusev A, Finucane HK, Bulik-Sullivan BK, Pollack SJ, Schizophrenia Working Group of Psychiatric Genomics Consortium, de Candia TR, Lee SH, Wray NR, et al. Contrasting regional architectures of schizophrenia and other complex diseases using fast variance components analysis. Nat Genet 2015;47:1385–92.
27. Zimmerman HA, Olson KC, Chen G, Lynch CJ. Adipose transplant for inborn errors of branched chain amino acid metabolism in mice. Mol Genet Metab 2013;109:345–53.
28. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9:311–26.
29. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 2012;15:606–14.
30. Kadota Y, Toyoda T, Kitaura Y, Adams SH, Shimomura Y. Regulation of hepatic branched-chain α-ketoacid dehydrogenase complex in rats fed a high-fat diet. Obes Res Clin Pract 2013;7:e439–44.
31. Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE, Mootha VK. Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol 2008;4: 214.
32. Lian K, Du C, Liu Y, Zhu D, Yan W, Zhang H, Hong Z, Liu P, Zhang L, Pei H, et al. Impaired adiponectin signaling contributes to disturbed catabolism of branched-chain amino acids in diabetic mice. Diabetes 2015;64:49–59.
33. Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, Florez JC, Clish CB, Gerszten RE, Wang TJ. Metabolite profiles during oral glucose challenge. Diabetes 2013;62:2689–98.
34. Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? Mol Psychiatry 2009;14:746–54.
35. Belsky J, Hartman S. Gene-environment interaction in evolutionary perspective: differential susceptibility to environmental influences. World Psychiatry 2014;13:87–9.
36. Hartman S, Belsky J. An evolutionary perspective on family studies: differential susceptibility to environmental influences. Fam Process 2016;55:700–12.
37. Dalle Molle R, Fatemi H, Dagher A, Levitan RD, Silveira PP, Dubé L. Gene and environment interaction: is the differential susceptibility hypothesis relevant for obesity? Neurosci Biobehav Rev 2017;73:326–39.
38. Grams J, Garvey WT. Weight loss and the prevention and treatment of type 2 diabetes using lifestyle therapy, pharmacotherapy, and bariatric surgery: mechanisms of action. Curr Obes Rep 2015;4:287–302.
39. Perez-Cornago A, Brennan L, Ibero-Baraibar I, Hermsdorff HHM, O'Gorman A, Zulet MA, Alfredo Martínez J. Metabolomics identifies changes in fatty acid and amino acid profiles in serum of overweight older adults following a weight loss intervention. J Physiol Biochem 2014;70:593–602.
40. Qi L. Mendelian randomization in nutritional epidemiology. Nutr Rev 2009;67:439–50.
41. Goni L, Cuervo M, Milagro FI, Mart JA. Future perspectives of personalized weight loss interventions based on nutrigenetic, epigenetic, and metagenomic data. J Nutr 2016;146:905–12.