

A 3-year Mediterranean-style dietary intervention may modulate the association between adiponectin gene variants and body weight change

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Abstract

Purpose Adiponectin gene variations have been associated with obesity. There are few interventional studies analyzing this association. The aim of this study was to analyze the effects of a nutritional intervention with Mediterranean-style diet and three (−4034A/C, +45T/G, and +276 G/T) adiponectin gene variants on 3-year body weight changes in high cardiovascular risk patients.

Subjects and methods A total of 737 participants, aged 55–80 at high cardiovascular risk were assigned to a low-fat diet or to a Mediterranean-style diet (MD) groups, one with high intake of virgin olive oil (VOO) and the other with high intake of nuts. Anthropometric parameters were taken at baseline and after 3-year follow-up, and the genotyping of the −4034A/C, +45T/G, and +276 G/T polymorphisms was done.

Results GG genotype of the +45T/G polymorphism was associated with 3-year higher body weight gain

($B = 1.399$; $B = 0.043$). TT genotype of the +276G/T polymorphism was linked to the highest 3-year body weight gain in men. Both Mediterranean diets appeared to reverse this effect (p for interaction = 0.053).

Conclusion Adiponectin gene variation appeared to be associated with 3-year body weight changes in a high cardiovascular risk population. This association may be modulated by a nutritional intervention with a Mediterranean-style diet.

Keywords Adiponectin SNPs · Body weight change · Mediterranean diet · Nutritional intervention · PREDIMED

Introduction

Adipose tissue, traditionally considered as an energy storage depot, has demonstrated to be an active endocrine organ secreting numerous proteins that play important metabolic roles [1]. Adiponectin is abundantly expressed in adipose tissue [2], and can have antiatherogenic, anti-inflammatory, and insulin-sensitizing properties [3]. Low-plasma adiponectin levels have been observed in patients with obesity [4–6], coronary artery disease [7–9], and type 2 diabetes [10].

A synonymous SNP in exon 2 (45T/G) has been significantly associated with obesity and metabolic syndrome as an independent SNP [11], or as a haplotype when combined with 276G/T, located in intron 2 [12]. Likewise the promoter region gene variants have been implicated in obesity and type 2 diabetes features [13, 14].

Moreover, modifiable factors, such as diet, which could induce increases in adiponectin, might be useful for improving the outcomes of such disease. Since the Mediterranean diet was first defined by Keys and Grande [15],

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it has been postulated as beneficial against obesity [16] and cardiovascular disease [17, 18]. Olive oil consumption, which was the main source of dietary lipids (monounsaturated fatty acids) in traditional Mediterranean diets, has been widely related with this protective effect [18–21]. Also, tree nuts (a good source of polyunsaturated fatty acids) are an integral part of the Mediterranean food pattern and previous studies have found nut consumption to be associated with reduced risk of cardiovascular disease [22–24]. In this context the PREDIMED project is a clinical trial aimed at assessing the effects of the Mediterranean diet on the primary prevention of cardiovascular disease (<http://www.predimed.org>).

We hypothesized that a Mediterranean-style diet might be able to outweigh the tendency to higher weight gain associated with these variants of the adiponectin gene.

The aim of this study was to analyze the effects of a nutritional intervention with a Mediterranean-style diet and three (−4034A/C, +45T/G, and +276 G/T) adiponectin gene variants on 3-year body weight changes in high cardiovascular risk patients.

Materials and methods

Study population

The present study has been conducted within the frame of the PREDIMED project. The PREDIMED project is a clinical trial aimed at assessing the effects of the Mediterranean diet on the primary prevention of cardiovascular disease (<http://www.predimed.org>). It could be thought that there is already enough available evidence to support that a Mediterranean-style diet is able to prevent cardiovascular disease. However, the rationale of the PREDIMED trial is that there are no available randomized trials of primary prevention and this evidence is based only in observational studies of MD and cardiovascular disease [25–27]. Furthermore, in the meta-analysis by Sofi et al. [27] most observational information came from studies conducted in non-Mediterranean countries. The single available trial of MD in cardiovascular prevention [28] was a secondary prevention trial (i.e., all participants had had a previous myocardial infarction before entering the trial) and it has been criticized because of methodological limitations [17]. The PREDIMED trial was launched to overcome these limitations and to provide the best possible evidence to support the hypothesis that a MD can prevent heart disease. The design of the PREDIMED trial has been reported in detail elsewhere [19, 29]. Briefly, the PREDIMED trial is a large, parallel-group, multicenter, randomized, and controlled clinical trial that aims to assess the effects of a Mediterranean-type diet on CVD. Eligible participants

were community-dwelling men, 55 to 80 years of age, and women, 60 to 80 years of age, who fulfilled at least 1 out of 2 criteria: type 2 diabetes or 3 or more CVD risk factors [19]. Type 2 diabetes mellitus was defined according to the American Diabetic Association criteria. The duration of diabetes at enrollment was higher than 5 years for 49% of diabetics included, 38% had the diagnosis between 1 and 5 years, and only 13% were diagnosed in the previous year. As much as 13% of diabetics included in the trial in our center were treated with insulin and 54% were using oral antidiabetic agents.

Participants are assigned to one of three different dietary patterns (low-fat diet, Mediterranean diet [MD] supplemented with nuts [MD + nuts], and Mediterranean diet supplemented with virgin olive oil [MD + VOO]) [19].

In the current analysis, data from one of the center of this trial were analyzed as an observational cohort study because the genetic analysis was not a part of the interventional project. In the present analyses we included data from 774 participants enrolled in the AP-UNAV recruitment center in Pamplona, in which the retention rate during the third year was greater than 80%. As much as 137 subjects were excluded from the trial because we did not obtain DNA samples from them or because their adiposity measures were not recorded. All participants provided informed consent and the protocol was approved by the institutional review boards of the participating center according to the Declaration of Helsinki.

Dietary assessment

The dietary habits of participants, both at baseline and after follow-up for 36 months, were assessed using a semi-quantitative 137-item FFQ previously validated in Spain [30]. Furthermore, details about the dietary assessment are described elsewhere [19].

After the screening visit, and based on a baseline short (14-item) questionnaire specifically targeted to assess adherence to the Mediterranean diet [21, 29, 31], each participant was given personalized dietary advice by the dietician during a 30-min session. Participants allocated to a low-fat diet were advised to reduce all types of fat and were given written recommendations according to American Heart Association guidelines [32]. The intensity of the intervention in the two MD groups was higher than in the control group [29]. The MD participants received instructions directed to upscale the MD 14-item score, including (1) the use of olive oil for cooking and dressing; (2) increased consumption of vegetables, nuts, and fish products; (3) consumption of white meat instead of red or processed meat; (4) preparation of homemade sauce by simmering tomato, garlic, onion, and aromatic herbs with olive oil to dress vegetables, pasta, rice, and other dishes;

and (5) for alcohol drinkers, to follow a moderate pattern of red wine consumption. No energy restrictions were suggested for the MD groups. Participants in the MD groups were given free VOO (15 L for 3 months) or sachets of walnuts, hazelnuts, and almonds (1,350 g of walnuts [15 g/day], 675 g of hazelnuts [7.5 g/day], and 675 g of almonds [7.5 g/day], for 3 months). To improve compliance and account for family needs, participants in the corresponding MD groups were given excess VOO or additional packs of nuts. One week after a participant's inclusion, 1-h group session (up to 20 participants) for each MD group, was held by the dietician. Each session consisted of an informative talk and written material with elaborated descriptions of typical Mediterranean foods, seasonal shopping lists, meal plans, and recipes. All participants had free and continuous access to their dietician throughout the study [29, 33, 34].

Genotyping

Overnight fasting venous blood samples were collected in EDTA added tubes. DNA was extracted from the buffy coat fraction using a commercial kit (Master Pure™; Epicentre, Madison, WI, USA). All the subjects were genotyped for the −4034 A/C (rs 822395), +45 T/G (rs 2241766), +276 G/T (rs 1501299) SNPs of the adiponectin gene using Taqman real time PCR followed by an allelic discrimination by means of an ABI PRISM 7000. The probes and the primers for this assay were designed by Applied Biosystems (Madrid, Spain). Replicate quality control samples were included in every genotyping plate with more than 99% of concordance.

Statistical analysis

A χ^2 test was used to evaluate the Hardy–Weinberg equilibrium. The Kolmogorov–Smirnov test was used to determine variable distribution. Descriptive analyses of variables between the three interventional groups were performed using parametric tests (Student's *t*-tests, ANOVA followed by Bonferroni post hoc tests).

Regarding the statistical power, assuming a two-tailed alpha error of 0.05 and two groups of 196 and 302 subjects, we would be able to detect a difference in body weight changes ≥ 1 kg, with a statistical power of 80%.

The effects of the adiponectin gene variants on body weight changes were evaluated with multivariate linear regression models adjusting for relevant variables: age, sex, baseline BMI, and diabetes. Baseline leisure time physical activity was initially included in the model, but did not have any significant effect so it was removed.

To analyze potential interactions between the nutritional intervention and the polymorphisms, the interaction

product-term (MD $\times$ [polymorphism model]) was included in each regression model. *F* partial tests were performed to assess the statistical significance of the interaction product-terms were included.

Results

Prevalence of overweight and obesity in the study population was 51 and 38%, respectively. Baseline characteristics of the participants according to the allocation to the three nutritional groups are shown in Table 1. As expected, no statistically significant differences were found among the three groups.

We observed that the distribution of macronutrient intake was significantly different among the three groups after 3 years of intervention (Table 2). The control group had the highest protein and carbohydrate intake, whereas MD subjects had the highest intake of mono and polyunsaturated fat but not of saturated fat. Moreover, we confirmed that the highest intake of virgin olive oil was present in the MD + VOO group ($p < 0.001$), and that the nuts group had also significantly higher intake of virgin olive oil than the control group ($p = 0.008$). Likewise, the highest intake of nuts was observed in the MD + nuts group ($p < 0.001$), having the MD + VOO group significantly higher intake of nuts compared to the control group ($p = 0.001$).

The genotype distribution for the three studied polymorphisms did not deviate from Hardy–Weinberg equilibrium. The genotype frequencies for the adiponectin polymorphisms were: for the −4034A/C SNP: AA 43%, AC 47%, and CC 10%; for the +45T/G SNP: TT 63%, TG 32%, and GG 5%; for the +276G/T SNP: GG 52%, GT 41%, and TT 7%. Table 3 shows the baseline characteristics according to the studied polymorphisms: −4034A/C, +45T/G, and +276G/T of the adiponectin gene. GG subjects for the +45T/G gene variant presented higher systolic blood pressure at the beginning of the study ($p = 0.004$). The other studied variables were similar among the genotypes for the different polymorphisms.

To analyze the effects of the nutritional intervention and the polymorphism on 3-year body weight changes, we first investigated the influence of the intervention. Mean body weight changes in the three groups were similar: 0.04 ± 3.9 kg, -0.17 ± 4.2 kg, and 0.03 ± 3.8 kg in the control, MD + VOO, and MD + Nuts groups, respectively. The analysis regarding the three adiponectin gene variants was performed. The −4034A/C appeared to follow a dominant model when mean body weight changes were analyzed: for AA subjects: 0.05 ± 3.8 kg and for AT + TT subjects: -0.12 ± 4.2 kg. The +45T/G and +276G/T polymorphisms of the adiponectin gene seem to follow a recessive model being body weight changes for the

Table 1 Baseline and 3-year characteristics of the participants according to the nutritional group

	Control (<i>n</i> = 196)	Virgin olive oil (<i>n</i> = 302)	Tree nuts (<i>n</i> = 239)
Sex (% female)	57	56	52
Age (years)	68.3 ± 6.0	67.7 ± 6.7	67.6 ± 6.7
Waist/Height	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Waist circumference (cm)			
Baseline	94.9 ± 11.0	96.0 ± 10.4	95.1 ± 9.6
3-Year	95.1 ± 10.9	96.3 ± 10.0	95.0 ± 9.5
Weight (kg)			
Baseline	74.5 ± 11.8	75.6 ± 11.9	74.6 ± 10.3
3-Year	74.4 ± 12.4	75.6 ± 11.8	74.6 ± 10.4
BMI (kg/m ²)			
Baseline	29.2 ± 3.5	29.2 ± 3.3	29.1 ± 3.1
3-Year	29.1 ± 3.5	29.2 ± 3.3	29.1 ± 3.2
Systolic blood pressure (mm Hg)	155.4 ± 21.3	154.7 ± 21.0	155.1 ± 21.1
Diastolic blood pressure (mm Hg)	86.4 ± 10.5	85.8 ± 10.6	87.1 ± 10.6
Diabetes (%)	68	64	63
Smoking habit (% current smokers)	18	16	13
3-Year body weight changes (kg)	0.04 ± 3.9	−0.17 ± 4.2	0.02 ± 3.8

Table 2 Distribution of macronutrients and Mediterranean diet specific nutrients after 3 years of nutritional intervention according to the nutritional group

	Control (<i>n</i> = 196) ^a	MD + Virgin olive oil (<i>n</i> = 302) ^a	MD + Tree nuts (<i>n</i> = 239) ^a	<i>p</i> Values for the between groups differences		
				TMD + VOO versus control	TMD + Nuts versus control	TMD + NUTS versus TMD + VOO
Total energy intake (Kcal/day)	2,287.8 ± 676.8	2,532.8 ± 551.1	2,574.0 ± 595.3	<0.001	<0.001	1.000
Carbohydrates (%total energy intake)	43.9 ± 6.8	39.2 ± 5.6	38.6 ± 5.8	<0.001	<0.001	0.772
Proteins (%total energy intake)	16.4 ± 2.9	15.3 ± 2.2	15.4 ± 2.0	<0.001	<0.001	1.000
Total fat (%total energy intake)	37.2 ± 6.2	42.5 ± 5.3	42.8 ± 5.4	<0.001	<0.001	1.000
Saturated fat (%total energy intake)	9.0 ± 2.1	9.2 ± 1.6	9.3 ± 1.6	0.756	0.281	1.000
MUFA (%total energy intake)	19.1 ± 4.1	23.1 ± 3.4	22.8 ± 3.7	<0.001	<0.001	1.000
PUFA (%total energy intake)	5.6 ± 1.9	6.7 ± 1.5	7.3 ± 1.3	<0.001	<0.001	<0.001
Virgin olive oil (10 g) (servings/day)	3.0 ± 2.4	6.3 ± 1.1	4.5 ± 2.8	<0.001	<0.001	<0.001
Nuts (25 g) (servings/ day)	2.5 ± 5.2	6.1 ± 8.2	13.4 ± 6.5	<0.001	<0.001	<0.001

MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid, TMD traditional Mediterranean diet, VOO virgin olive oil

^a Mean ± standard deviation

+45T/G: TT + TG subjects: -0.11 ± 4.0 kg and GG subjects: 1.13 ± 4.3 kg; and for the +276G/T: GG + GT subjects: -0.10 ± 4.0 kg and TT subjects 0.83 ± 4.2 kg. Although there were no statistically significant differences,

both recessive models appeared to be associated with higher body weight gain.

To better characterize the effects of the three polymorphisms and the nutritional intervention on 3-year body

Table 3 Baseline and 3-year characteristics of the participants according to the genetic variants of the adiponectin gene

	−4034 A/C (rs 822395)		+45 T/G (rs 2241766)		+276 G/T (rs 1501299) ^a	
	AA (<i>n</i> = 316)	AC+CC (<i>n</i> = 421)	TT+TG (<i>n</i> = 702)	GG (<i>n</i> = 35)	GG+GT (<i>n</i> = 685)	TT (<i>n</i> = 50)
Sex (% female)	55	56	55	60	54	64
Age (years)	68.0 ± 6.2	67.7 ± 6.7	67.8 ± 6.6	68.1 ± 5.6	67.8 ± 6.6	67.5 ± 6.1
Waist/height	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Waist circumference (cm)						
Baseline	95.2 ± 10.5	95.5 ± 10.2	95.4 ± 10.4	95.4 ± 9.3	95.4 ± 10.2	94.9 ± 11.3
3-Year	95.5 ± 10.6	95.6 ± 9.7	95.5 ± 10.1	97.3 ± 9.4	95.5 ± 10.0	95.9 ± 11.0
Weight (kg)						
Baseline	75.3 ± 11.5	74.7 ± 11.3	75.0 ± 11.4	75.3 ± 10.9	75.1 ± 11.3	74.5 ± 11.9
3-Year	75.3 ± 12.0	74.7 ± 11.1	74.8 ± 11.5	76.7 ± 12.2	75.0 ± 11.4	75.3 ± 13.2
BMI (kg/m ²)						
Baseline	29.1 ± 3.1	29.2 ± 3.3	29.1 ± 3.3	29.6 ± 2.9	29.2 ± 3.3	29.2 ± 2.9
3-Year	29.1 ± 3.3	29.2 ± 3.3	29.1 ± 3.3	30.1 ± 3.4	29.1 ± 3.3	29.6 ± 3.0
Systolic blood pressure (mm Hg)	156.3 ± 20.5	154.1 ± 21.5	154.6 ± 20.9	165.2 ± 22.6 ^b	155.1 ± 21.1	154.0 ± 22.0
Diastolic blood pressure (mm Hg)	87.0 ± 11.0	86.0 ± 11.0	86.3 ± 10.6	89.0 ± 11.0	86.1 ± 0.6	86.5 ± 11.1
Diabetes (%)	32	37	35	26	35	34
Smoking habit (% current smokers)	16	15	16	17	16	14
3-Year body weight change (kg)	0.05 ± 3.82	−0.12 ± 4.17	−0.11 ± 4.01	1.13 ± 4.33	−0.10 ± 4.00	0.83 ± 4.20

^a Two subjects were not included because of missing data for this analysis^b The differences between TT + TG and GG subjects were statistically significant (*p* = 0.004)

weight changes, multiple linear regression models adjusted for age, sex, diabetes status, baseline BMI were fitted. The first model considered the dominant model for the −4034A/C polymorphism. Neither the polymorphism nor the intervention presented a significant effect on body weight changes (data not shown). The second regression model included the recessive model of the +45T/G adiponectin gene variant (Table 4). GG subjects had the highest body weight gain (*B* = 1.399; *B* = 0.043). The nutritional intervention, per se, had no significant effect. When in a third regression model the +276G/T polymorphism was included as the recessive model, the TT subjects presented a tendency to have higher body weight gain (*B* = 1.033; *p* = 0.079). This effect was statistically significant among men (*B* = 0.038; *p* = 0.038) having TT subjects the highest 3-year body weight gain (data not shown). A study merging both recessive models together (+45T/G and +276G/T) is not possible due to the low frequency of homozygous subjects for the mutations.

To analyze the combined effects of the polymorphisms, multiple regression models were adjusted for age, sex, diabetes, and baseline BMI. Each model considered the intervention, control versus MD (MD + VOO and MD + Nuts merged together), the studied polymorphism, and the interaction product-term between the intervention (Control vs. MD) and the polymorphism. The first included the dominant model for the −4034 A/C adiponectin gene

Table 4 Multiple regression model assessing the effects of the +45 T/G (rs 2241766) polymorphism of the adiponectin gene on body weight changes after three years of a nutritional intervention

	<i>B</i> (95% CI) ^a	<i>p</i> value
Age	−0.028 (−0.074 to 0.018)	0.235
Sex		
Males	0 (ref.)	
Females	−0.369 (−0.973 to 0.234)	0.230
Baseline BMI	−0.276 (−0.365 to −0.187)	<0.001
Diabetes		
No	0 (ref.)	
Yes	−0.618 (−1.228 to −0.009)	0.047
Nutritional intervention		
Control	0 (ref.)	
MD + VOO	−0.141 (−0.865 to 0.584)	0.703
MD + Nuts	0.005 (−0.756 to 0.766)	0.989
+45 T/G polymorphism (recessive model)		
TT + TG	0 (ref.)	
GG	1.399 (0.045 to 2.754)	0.043

^a 3-Year body weight change (3-year body weight-baseline body weight)

variant, and the interaction product-term (AA vs. AC + CC) × intervention. No significant result was observed (data not shown). The second model included the +45T/G as the recessive model and the interaction

Table 5 Multiple regression model assessing the effects of the interaction between the +276 G/T polymorphism (recessive model) of adiponectin gene and the nutritional intervention on 3-year body weight changes

	<i>B</i> (95% CI) ^a	<i>p</i> value
Age	−0.027 (−0.074 to 0.019)	0.242
Sex		
Males	0 (ref.)	
Females	−0.374 (−0.977 to 0.229)	0.223
Baseline BMI	−0.274 (−0.363 to −0.186)	<0.001
Diabetes		
No	0 (ref.)	
Yes	−0.637 (−1.246 to −0.029)	0.040
Nutritional intervention		
Control	0 (ref.)	
MD	0.193 (−0.496 to 0.882)	0.583
+276 G/T polymorphism		
GG + GT	0 (ref.)	
TT	2.586 (0.632 to 4.540)	0.010
Nutritional intervention × polymorphism		
Control × TT	0 (ref.)	
MD ^b × TT	−2.381 (−4.798 to 0.035)	0.053

^a 3-Year body weight change (3-year body weight-baseline body weight)

^b MD refers to MD + VOO and MD + nuts merged together

product-term (TT + TG vs. GG) × intervention. The interaction was not statistically significant (data not shown). The third model included the recessive model of the +276G/T and the interaction (GG + GT vs. TT) × intervention. The interaction was marginally significant ($B = -2.381$; $p = 0.053$) showing that TT subjects allocated to a MD diet group appeared to have a reduction in body weight gain (Table 5). The F partial test revealed that the model tended to improve when the interaction product-term was included in the model ($F = 3.74$; $p = 0.053$).

Moreover, in every regression model, we observed that the baseline BMI ($p < 0.001$) was inversely associated body weight gain. Meanwhile, the presence of diabetes was linked to a lower 3-year body weight gain in this intervention study ($p < 0.05$).

Discussion

Our data suggest an implication of adiponectin gene variants (+45T/G and +276G/T) in body weight changes after 3 years of nutritional intervention. Moreover, a potential interaction between the TT genotype of the +276T/G SNP and the MD + VOO was found.

This study analyzes the combined effects of a nutritional intervention with a Mediterranean diet and genetic variation in adiponectin gene on 3-year body weight changes. On one hand, our results proved the effectiveness of the Mediterranean diet intervention since the fat intake distribution in the three nutritional groups changed in the expected direction [29, 31]. We observed that the MD did not lead to a higher 3-year body weight change. Furthermore, it seemed that both MD, but specially the diet rich in virgin olive oil, tended to lead to a lower body weight gain. The finding that the MD adherence did not lead to a higher BMI was previously reported [35].

On the other hand, we analyzed three adiponectin gene variants: −4034A/C, +45T/G, and +276G/T. The first SNP is located in the promoter region of the gene and we did not find any effect on body weight changes. There are few studies analyzing this polymorphism and no association with serum adiponectin levels [36] but a slight relationship with diabetes risk [37] was reported. We observed that the +45T/G (located in exon 2) was associated with body weight change, having GG subjects greater 3-year body weight gain independently of the nutritional group. The G-allele was previously reported to be associated with obesity, usually linked to diabetes [11, 38]. Regarding the +276G/T polymorphism, located in the second intron of the gene, we observed a tendency within TT subjects to have higher body weight gain than subjects with the other genotypes. This tendency was statistically significant in men showing that TT subjects had higher 3-year body weight gain. It was found that T-allele of the +276G/T polymorphism was associated with higher body fat in Swedish population [39] and with severe obesity in a French Caucasian population [40].

In our study, a potential interaction between the MD (VOO + Nuts) intervention and +276TT genotype in the whole population was found, showing that TT subjects allocated to MD had lower body weight gain. Thus, it appeared that although the TT genotype was predicted to have higher body weight gain, this effect was attenuated when these subjects followed a Mediterranean-style diet. Although there are studies analyzing the effects of interactions between dietary components and this polymorphism on serum adiponectin levels [41], there are no reports assessing the interaction between the Mediterranean diet and adiponectin gene variants on adiposity. It has been shown that the MD was able to reduce the risk of obesity [42] and metabolic syndrome [43]. There are studies showing that a greater adherence to MD is associated with increased adiponectin levels in diabetic [44] and healthy women [45]. We hypothesized that the +276T/G adiponectin gene variant may be interacting in this association and consequently modifying body weight regulation. In a previous study, Goyenechea et al. [14] suggested

an association between an energy restricted dietary intervention and a promoter polymorphism (−11391 G/A) of the adiponectin gene protecting against weight gain.

In this substudy, we previously found that MD diet may be modifying the effects of other gene variants and body weight changes, such as Pro12Ala polymorphism of PPARG gene or rs9939609 T/A of FTO gene [31, 46]. Moreover, in the literature we found interactions between SNPs and other lifestyle changes, such as FTO rs9939609 and physical activity on BMI [47].

Moreover, we observed that the highest the baseline BMI the lowest was the body weight gain after 3 years of intervention. This result agrees with previous findings that showed a positive and independent relationship between weight loss and pre-treatment body weight [48, 49].

A potential drawback of the design of our study is that a real control group (not Mediterranean, not low fat) that would possibly show a body weight increase associated with the gene variants is missing. We acknowledge this limitation that should be taken into account for the interpretation of our findings. However, the intensity of the nutritional intervention in the low-fat diet group was lower than that in the two MD groups. Moreover, the absolute magnitude of the achieved reduction in total fat intake in the low-fat group was not impressive [29]. Therefore, the low-fat group was in fact playing the role of a control group.

Our data reinforce the idea that MD-style diet by lowering the risk of overweight/obesity may reduce obesity comorbidities. Moreover, the study of interactions between MD and adiponectin gene variants will provide evidence in the field of personalized nutrition.

In conclusion, adiponectin gene variation appeared to be associated with 3-year body weight changes in a high cardiovascular risk population. This association may be modulated by a nutritional intervention with a Mediterranean-style diet.

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References

- Ronti T, Lupattelli G, Mannarino E (2006) The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf)* 64:355–365
- Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose most abundant gene transcript 1). *Biochem Biophys Res Commun* 221:286–289
- Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96:939–949
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83
- Bilgili S, Celebiler AC, Dogan A, Karaca B (2008) Inverse relationship between adiponectin and plasminogen activator inhibitor-1 in metabolic syndrome patients. *Endocr Regul* 42:63–68
- Valle M, Martos R, Gascon F, Canete R, Zafra MA, Morales R (2005) Low-grade systemic inflammation, hypo adiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome. *Diabetes Metab* 31:55–62
- Dzielinska Z, Januszewicz A, Wiecek A, Demkow M, Makowiecka-Ciesla M, Prejbisz A, Kadziela J, Mielniczuk R, Florczak E, Janas J, Januszewicz M, Ruzyllo W (2003) Decreased plasma concentration of a novel anti-inflammatory protein—adiponectin—in hypertensive men with coronary artery disease. *Thromb Res* 110:365–369
- Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y (2003) Association of hypo adiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 23:85–89
- Rothenbacher D, Brenner H, Marz W, Koenig W (2005) Adiponectin, risk of coronary heart disease and correlations with cardiovascular risk markers. *Eur Heart J* 26:1640–1646
- Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G (2004) Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 53:2473–2478
- Stumvoll M, Tschrötter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Haring H (2002) Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 51:37–41
- Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, Trischitta V, Doria A (2002) A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 51:2306–2312
- Tanko LB, Siddiq A, Lecoeur C, Larsen PJ, Christiansen C, Walley A, Froguel P (2005) ACDC/adiponectin and PPAR- γ gene polymorphisms: implications for features of obesity. *Obes Res* 13:2113–2121
- Goyenechea E, Collins LJ, Parra D, Abete I, Crujeiras AB, O'Dell SD, Martinez JA (2009) The - 11391 G/A polymorphism of the adiponectin gene promoter is associated with metabolic syndrome traits and the outcome of an energy-restricted diet in obese subjects. *Horm Metab Res* 41:55–61

15. Keys A, Grande F (1957) Role of dietary fat in human nutrition. III. Diet and the epidemiology of coronary heart disease. *Am J Public Health Nations Health* 47:1520–1530
16. Schroder H, Marrugat J, Vila J, Covas MI, Elosua R (2004) Adherence to the traditional mediterranean diet is inversely associated with body mass index and obesity in a spanish population. *J Nutr* 134:3355–3361
17. Martinez-Gonzalez MA, Sanchez-Villegas A (2004) The emerging role of Mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? *Eur J Epidemiol* 19:9–13
18. Trichopoulou A (2004) Traditional Mediterranean diet and longevity in the elderly: a review. *Public Health Nutr* 7:943–947
19. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, Fiol M, Gomez-Gracia E, Lopez-Sabater MC, Vinyoles E, Aros F, Conde M, Lahoz C, Lapetra J, Saez G, Ros E (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 145:1–11
20. Fernandez-Jarne E, Martinez-Losa E, Prado-Santamaria M, Brugarolas-Brufau C, Serrano-Martinez M, Martinez-Gonzalez MA (2002) Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case-control study in Spain. *Int J Epidemiol* 31:474–480
21. Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, Wright M, Gomez-Gracia E (2004) Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr* 58:1550–1552
22. Albert CM, Gaziano JM, Willett WC, Manson JE (2002) Nut consumption and decreased risk of sudden cardiac death in the Physicians' Health Study. *Arch Intern Med* 162:1382–1387
23. Hu FB, Stampfer MJ, Manson JE, Rimm EB, Colditz GA, Rosner BA, Speizer FE, Hennekens CH, Willett WC (1998) Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. *BMJ* 317:1341–1345
24. Sabate J, Fraser GE (1994) Nuts: a new protective food against coronary heart disease. *Curr Opin Lipidol* 5:11–16
25. Buckland G, Bach A, Serra-Majem L (2008) Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. *Obes Rev* 9:582–593
26. Fung TT, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB (2009) Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* 119:1093–1100
27. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A (2008) Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* 337:a1344
28. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 99:779–785
29. Zazpe I, Sanchez-Tainta A, Estruch R, Lamuela-Raventos RM, Schroder H, Salas-Salvado J, Corella D, Fiol M, Gomez-Gracia E, Aros F, Ros E, Ruiz-Gutierrez V, Iglesias P, Conde-Herrera M, Martinez-Gonzalez MA (2008) A large randomized individual and group intervention conducted by registered dietitians increased adherence to Mediterranean-type diets: the PREDIMED study. *J Am Diet Assoc* 108:1134–1144 discussion 1145
30. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, Willett WC (1993) Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol* 22:512–519
31. Razquin C, Martinez JA, Martinez-Gonzalez MA, Corella D, Santos JM, Marti A (2009) The Mediterranean diet protects against waist circumference enlargement in 12Ala carriers for the PPARGgamma gene: 2 years' follow-up of 774 subjects at high cardiovascular risk. *Br J Nutr* 102:672–679
32. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL, Bazzarre TL (2000) AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation* 102:2284–2299
33. Corella D, Gonzalez JI, Bullo M, Carrasco P, Portoles O, Diez-Espino J, Covas MI, Ruiz-Gutierrez V, Gomez-Gracia E, Aros F, Fiol M, Herrera MC, Santos JM, Saez G, Lamuela R, Lahoz C, Vinyoles E, Ros E, Estruch R (2009) Polymorphisms cyclooxygenase-2 –765G>C and interleukin-6 –174G>C are associated with serum inflammation markers in a high cardiovascular risk population and do not modify the response to a Mediterranean diet supplemented with virgin olive oil or nuts. *J Nutr* 139:128–134
34. Fito M, Guxens M, Corella D, Saez G, Estruch R, de la Torre R, Frances F, Cabezas C, Lopez-Sabater Mdel C, Marrugat J, Garcia-Arellano A, Aros F, Ruiz-Gutierrez V, Ros E, Salas-Salvado J, Fiol M, Sola R, Covas MI (2007) Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch Intern Med* 167:1195–1203
35. Bes-Rastrollo M, Sanchez-Villegas A, de la Fuente C, de Irala J, Martinez JA, Martinez-Gonzalez MA (2006) Olive oil consumption and weight change: the SUN prospective cohort study. *Lipids* 41:249–256
36. Menzaghi C, Ercolino T, Salvemini L, Coco A, Kim SH, Fini G, Doria A, Trischitta V (2004) Multigenic control of serum adiponectin levels: evidence for a role of the APM1 gene and a locus on 14q13. *Physiol Genomics* 19:170–174
37. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE (2004) Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes* 53:209–213
38. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T (2002) Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51:536–540
39. Fredriksson J, Carlsson E, Orho-Melander M, Groop L, Riddervall M (2006) A polymorphism in the adiponectin gene influences adiponectin expression levels in visceral fat in obese subjects. *Int J Obes (Lond)* 30:226–232
40. Bouatia-Naji N, Meyre D, Lobbens S, Seron K, Fumeron F, Balkau B, Heude B, Jouret B, Scherer PE, Dina C, Weill J, Froguel P (2006) ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. *Diabetes* 55:545–550
41. Ntalla I, Dedoussis G, Yannakoulia M, Smart MC, Louizou E, Sakka SD, Papoutsakis C, Talmud PJ (2009) ADIPOQ gene polymorphism rs1501299 interacts with fibre intake to affect adiponectin concentration in children: the GENe-Diet Attica Investigation on childhood obesity. *Eur J Nutr* 48:493–497
42. Mendez MA, Popkin BM, Jakszyn P, Berenguer A, Tormo MJ, Sanchez MJ, Quiros JR, Pera G, Navarro C, Martinez C, Larranaga N, Dorronsoro M, Chirlaque MD, Barricarte A, Ardanaz E, Amiano P, Agudo A, Gonzalez CA (2006) Adherence to a Mediterranean diet is associated with reduced 3-year incidence of obesity. *J Nutr* 136:2934–2938
43. Salas-Salvado J, Fernandez-Ballart J, Ros E, Martinez-Gonzalez MA, Fito M, Estruch R, Corella D, Fiol M, Gomez-Gracia E, Aros F, Flores G, Lapetra J, Lamuela-Raventos R, Ruiz-Gutierrez V, Bullo M, Basora J, Covas MI (2008) Effect of a Mediterranean

- diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. *Arch Intern Med* 168:2449–2458
44. Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FB (2006) Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. *Am J Clin Nutr* 84:328–335
45. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS (2008) A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. *Metabolism* 57:824–830
46. Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernandez-Crehuet J, Marti A (2009) A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. *Int J Obes (Lond)* (in press)
47. Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, Clausen JO, Sandbaek A, Lauritzen T, Hansen L, Jorgensen T, Pedersen O, Hansen T (2008) Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 57:95–101
48. Astrup A, Ryan L, Grunwald GK, Storgaard M, Saris W, Melanson E, Hill JO (2000) The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. *Br J Nutr* 83(Suppl 1):S25–S32
49. Goyenechea E, Parra D, Crujeiras AB, Abete I, Martinez JA (2009) A nutrigenomic inflammation-related PBMC-based approach to predict the weight-loss regain in obese subjects. *Ann Nutr Metab* 54:43–51