RESEARCH ARTICLE

A Mediterranean diet rich in virgin olive oil may reverse the effects of the -174G/C IL6 gene variant on 3-year body weight change

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Only a few studies have analyzed the effects of the potential interaction between the -174G/C polymorphism of IL6 gene and the adherence to the Mediterranean diet (MD) on adiposity indexes. Our aim was to investigate the interplay between the -174G/C polymorphism of the IL6 gene and a Mediterranean-style diet on body weight changes after 3 years of nutritional intervention in a high cardiovascular risk population. A total of 737 participants, aged 55–80 years were assigned to a low-fat diet or to a Mediterranean-style diet group with high intake of virgin olive oil (VOO) or nuts. Anthropometric measurements were taken at baseline and after 3-year follow-up. The -174G/C polymorphism of the IL6 gene was genotyped. Minor allele frequency (C) was 0.39. At baseline, the CC genotype was associated with higher measures of adiposity. After 3 years, a significant interaction ($p = 0.028$) was found between the polymorphism (GG+GC *versus* CC) and the nutritional intervention: CC subjects following the MD+VOO had the lowest body weight gain. In conclusion, at baseline, CC subjects for the -174G/C polymorphism of IL6 had the highest body weight and BMI. However, after 3 years of nutritional intervention with MD+VOO, these subjects were predicted to have the greatest reduction in body weight.

Keywords:
Body weight change / IL6 polymorphism / Mediterranean diet / Nutritional intervention / PREDIMED

1 Introduction

In recent years, evidence has been accumulating for the excessive growth of adipose tissue is accompanied by an underlying low-grade inflammation [1]. IL6 is a pleiotropic cytokine ubiquitously expressed in many tissues involved in inflammatory processes [2, 3]. Thus, increased adiposity is associated with higher circulating levels of IL6 [4].

The -174G/C polymorphism, located in the IL6 promoter region, has been found to influence transcriptional regulation and plasma IL6 levels [5, 6]. Although functional analyses suggest that the -174C allele may reduce IL6 gene expression [5], epidemiologic studies have produced conflicting results [7–9], indicating that the regulation of IL6 expression *in vivo* is more complex.

This polymorphism has been previously associated with a higher risk of hypertension [10], coronary heart disease [11],

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Abbreviations: MD, Mediterranean diet; **PREDIMED,** PREvención con Dleta MEDiterranea; **SNP,** single nucleotide polymorphism; **VOO,** virgin olive oil

and diabetes [12, 13]. The relationship between this polymorphism and BMI is not clear. Several studies have reported that the C allele is associated with an increase in BMI [14, 15], with long-term body weight gain and obesity [16], whereas others have found that the C allele was associated with lower adiposity [17] and weight gain [18]. In a recent meta-analysis, no association has been found between the -174G/C polymorphism and adiposity. However, this polymorphism was in strong linkage disequilibrium with a single nucleotide polymorphism (SNP) in the promoter region (rs 2069827) consistently associated with adiposity [19].

Most research concerning the relationship between diet, risk factors for chronic diseases, and genetics was derived from observational epidemiological studies. However, dietary intervention studies represent the most consistent design that is able to provide stronger evidence of causality [20]. Consequently, this study was aimed to analyze the role of -174G/C polymorphism of IL6 on body weight changes within the framework of a nutritional intervention study with a Mediterranean-style diet for a large population sample.

Since the Mediterranean diet (MD) was first defined by Keys & Grande (1957), it is postulated to be beneficial against cardiovascular disease [21, 22]. Olive oil consumption, the main source of fat in traditional MDs, has an important cardio-protective effect [22–25]. Also, tree nuts, rich in polyunsaturated fatty acids, are an integral part of the Mediterranean food pattern. Previous studies found a protective association between nut consumption and risk of cardiovascular disease [26–29], diabetes [30], or weight gain [31]. However, data on the benefits of a fat-rich diet (as would result from the recommendation to consume high levels of olive oil and nuts) in the prevention of cardiovascular disease are contradictories, because it is believed that such dietary pattern might increase body weight [32].

The aim of our study was to analyze the effects of the potential interaction between the -174G/C polymorphism of the IL6 gene and a Mediterranean-style diet on body weight changes after 3 years of nutritional intervention in a high cardiovascular risk population (PREvención con DIeta MEDiterranea (PREDIMED) study).

2 Materials and methods

2.1 Subjects

The PREDIMED study is a large, parallel-group, multicenter, controlled and randomized, clinical trial aimed at assessing the effects of the MD on the primary prevention of cardiovascular disease (http://www.predimed.org) [33]. Seven hundred and thirty-seven participants who had enrolled in the AP-UNAV recruitment center in Pamplona [34] were analyzed in this study. All the participants gave informed consent to a protocol approved by the local review boards.

2.2 Study design

The present study was conducted within the framework of the PREDIMED trial (International Standard Randomized Controlled Trial Number (ISRCTN): 35739639). The design of this trial has been reported in detail elsewhere [33, 35]. Briefly, the PREDIMED trial is a large, parallel-group, multicenter, randomized and controlled 4-year clinical trial that aims to assess the effects of a Mediterranean-type diet on the prevention of cardiovascular disease. Inclusion criteria were the presence of diabetes mellitus, or the presence of three or more major cardiovascular risk factors (BMI $\geq 25\,kg/m^2$, hypertension (blood pressure $\geq 140/90\,mmHg$ or treatment with antihypertensive drugs), LDL cholesterol $\geq 160\,mg/dL$ or treatment with lipid-lowering drugs, HDL cholesterol $\leq 40\,mg/dL$, and current smoking or family history of premature coronary disease). Exclusion criteria were: previous history of cardiovascular disease, any severe chronic illness, drug or alcohol addiction, history of allergy or intolerance to olive oil or nuts, and a low predicted likelihood of changing dietary habits [34].

Each participant was assigned to one of the three different dietary patterns: low-fat diet; MD supplemented with virgin olive oil (MD+VOO); and MD supplemented with nuts (MD+Nuts) [33, 36]. The initial evaluation consisted of the administration of a 47-item questionnaire about educational level, lifestyle, history of illnesses, and medication use [34]. Anthropometric and blood pressure measurements were taken using conventional procedures.

2.3 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 PureTM; Epicentre, Madison, WI, USA). All the subjects were genotyped for the IL6 -174G/C promoter polymorphism (rs 1800795) using Taqman SNP allelic discrimination (ABI PRISM 7000). The probes and the primers for this assay were designed by Applied Biosystems (Madrid, Spain). We obtained an average genotyping success rate of more than 95% and an average genotyping accuracy of more than 98% by regenotyping 11% of the samples.

2.4 Statistical analysis

A χ^2-test was used to evaluate the Hardy–Weinberg equilibrium. The Kolmogorov–Smirnov test was used to determine the variable distribution. Descriptive analyses of variables, including food intake, between the three interventional groups were performed using parametric tests (Student's *t*-tests, analysis of variance followed by Bonferroni *post hoc* tests). The geometric means of body weight changes were compared among the three randomized

groups and genotypes using general linear models after adjustment for age, sex, baseline BMI (continuous), and diabetes.

Regarding statistical power, assuming that a two-tailed α-error of 0.05 will be able to detect a difference of <1 kg, two groups of 196 and 302 subjects would achieve a statistical power of 80%.

The values are presented as mean $\pm$ SEM. The effects of the IL6 polymorphism on body weight changes were evaluated with multivariate linear regression models adjusting for relevant variables: age, sex, baseline BMI, and diabetes. To analyze potential interactions (effect modification) between the nutritional intervention and the polymorphisms, the interaction product terms (MD+VOO) × (IL6 recessive model) and (MD+Nuts) × (IL6 recessive model) were included in the model. An *F*-partial test was performed to study the improvement of the models when the interaction product terms were included.

The level of probability was set at $p<0.05$ as statistically significant.

The false discovery rate method from Benjamini and Hochberg was used to control for multiple testing as appropriate in general linear regression models [37].

All analyses were performed using the SPSS software (Chicago, IL, USA).

3 Results

First, we confirmed that the -174G/C polymorphism was in Hardy–Weinberg equilibrium in this population. Genotype frequencies were 37.6, 46.8 and 15.6% for GG, GC and CC, respectively.

As expected, due to the randomization, there were no differences in baseline biological parameters in the population according to the nutritional group (Table 1). However, when baseline characteristics were analyzed according to the -174G/C polymorphism of IL6 gene, we observed that the CC carriers had significantly higher baseline body weight ($p=0.024$), BMI ($p=0.020$), and also tended to have higher baseline waist circumference ($p=0.059$) suggesting a recessive model for the effect of the polymorphism (Table 2).

The analysis of macronutrient intake at the beginning of the study and after 3 years of nutritional intervention revealed an effective change in the dietary pattern of the subjects with significant differences in the expected direction (data not shown). These changes were particularly marked for fat consumption. A significantly higher consumption of total fat, monounsaturated and polyunsaturated fat (but not saturated fat), was observed in both MD groups compared with the control group ($p<0.001$). Moreover, the control group had a significantly higher ratio of saturated fat/total fat than those subjects allocated to the MD groups ($p<0.001$).

To analyze whether the MD and the -174G/C polymorphism had any effect on body weight changes, we first investigated the potential effects of the nutritional intervention. The mean adjusted weight changes in the control, MD+VOO, and MD+nuts groups were -0.10 ± 0.3, -0.21 ± 0.2, and -0.07 ± 3.8 kg, respectively (Fig. 1A). We observed that although the three groups had similar body weight changes, it seems that subjects allocated to MD+VOO group were those with the most important body weight reduction.

The mean adjusted weight changes after 3 years of the intervention for the three genotypes were GG subjects, -0.19 ± 0.2 kg; GC subjects, 0.02 ± 0.2 kg; and CC subjects, -0.41 ± 0.4 kg (Fig. 1B). We observed that the polymorphism appeared to follow a recessive model (GG+GC *versus* CC) to explain body weight changes, and that the CC subjects seemed to have a greater body weight reduction (not statistically significant).

To better study of the effects of the nutritional intervention and genotype, a linear multiple regression model was fitted. Neither the nutritional intervention nor the polymorphism had a significant effect on body weight changes (data not shown), but both the MD+VOO and the CC genotypes for the -174G/C polymorphism suggested a nonsignificant protective effect.

To study the combined effects of nutritional intervention and genotype on body weight changes, an adjusted general linear model was fitted (Fig. 2). We observed that the CC

Table 1. Baseline characteristics of the participants according to the nutritional group

	Control ($n=196$)	Virgin olive oil ($n=302$)	Tree nuts ($n=239$)
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)	94.9±11.0	96.0±10.4	95.1±9.6
Weight (kg)	74.5±11.8	75.6±11.9	74.6±10.3
BMI (kg/m^2)	29.2±3.5	29.2±3.3	29.1±3.1
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 (%)	69	64	63
Smoking habit (% Current smokers)	18	16	13

Table 2. Baseline characteristics according to the genotype for the -174G/C polymorphism of IL6 gene

	GG+GC ($n = 622$)	CC ($n = 115$)
Sex (% Female)	55	54
Age (years)	67.7±6.3	68.3±7.4
Waist/height	0.6±0.1	0.6±0.1
Waist circumference (cm)	95.1±10.1	97.1±11.4
Weight (kg)	74.6±11.3[a)]	77.2±11.4
BMI (kg/m^2)	29.1±3.2[b)]	29.8±3.5
Systolic blood pressure (mm Hg)	155.0±21.2	155.5±20.5
Diastolic blood pressure (mm Hg)	86.4±10.4	86.2±11.4
Diabetes (%)	65	65
Smoking habit (% Current smokers)	15	18

a) The differences between GG+GC and CC subjects were statistically significant ($p = 0.024$).
b) The differences between GG+GC and CC subjects were statistically significant ($p = 0.020$).

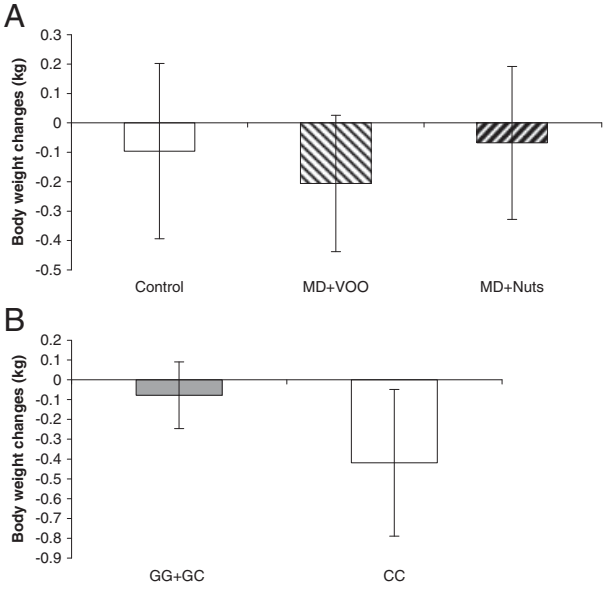

Figure 1. (A) General linear model, adjusted for age, sex, baseline BMI and diabetes, to assess mean body weight changes according to the nutritional group. (B) General linear model, adjusted for age, sex, baseline BMI and diabetes, to assess mean body weight changes according to genotype for the -174G/C polymorphism of the IL6 gene.

subjects had a significantly higher mean adjusted body weight reduction as compared with GG+GC subjects only in the MD+VOO group. To corroborate this finding, a multiple regression model adjusted for the same confounding factors was fitted for each nutritional group. In subjects allocated to the MD+VOO group (Table 3), we observed that the CC subjects had a significantly lower body weight gain ($B = -2.061$; $p = 0.003$) compared with the GG+GC subjects. The results from the control and MD+Nuts groups were not statistically significant (data not shown), suggesting a potential MD+VOO–genotype interaction. Thus, an adjusted multiple linear regression model including the nutritional intervention, the polymorphism (recessive model), and the interaction product term was fitted (Table 4 and Fig. 3). This model showed a statistically significant interaction between MD+VOO and CC genotype ($p = 0.028$). The *F*-partial test revealed that the model significantly improved when the interaction product term was included ($F = 5.581$; 2 df; $p = 0.004$).Moreover, in the multiple regression models for the total population (Table 1–4) and for the MD+VOO group (Table 3) a significant effect of diabetes on body weight changes was observed. Diabetic subjects were predicted to have a lower body weight gain after 3 years of intervention independent of nutritional group. The regression model that included the interaction (genotype × nutritional intervention group) was built separately in diabetic ($n = 257$) and nondiabetic subjects ($n = 480$). We observed that the protective effect against body weight gain that the CC genotype–MD+VOO appeared to confer within the whole population was only observed among nondiabetics ($n = 28$; p for interaction = 0.007).

4 Discussion

We have studied the -174G/C polymorphism on the IL6 gene in a population allocated to a nutritional intervention trial: the PREDIMED project, a randomized controlled trial of primary cardiovascular prevention. The randomization of our design allowed us to control not only for established confounding factors (sex, age, and baseline parameters) but also for unknown or unmeasured confounders (such as a general healthier lifestyle or health consciousness or different medical stories). We have found that the participants with the CC genotype exhibited at baseline higher

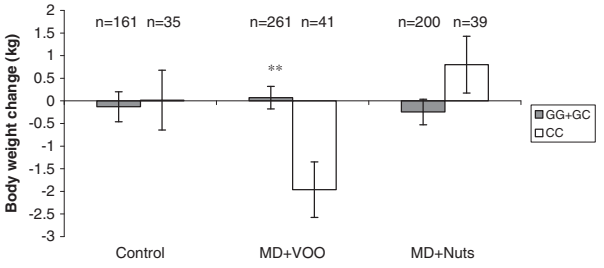

Figure 2. General linear model, adjusted for age, sex, baseline BMI and diabetes, to assess mean body weight changes according to genotype for the -174G/C polymorphism of IL6 gene. **The differences between GG+GC and CC subjects within MD+VOO group statistically significant ($p = 0.002$). The interaction (CC genotype × MD+VOO) was also statistically significant ($p = 0.028$). The differences between GG+GC and CC subjects within MD+VOO group were not statistically significant after false discovery rate correction ($p = 0.075$) [37].

measures of adiposity (body weight, BMI, and waist circumference). After 3 years of intervention with a Mediterranean-style diet rich in VOO, the CC subjects were those with the lowest body weight gain.

Our results confirmed the effectiveness of the MD intervention since the fat intake distribution in the three nutritional groups was modified [35, 38]. Both groups allocated to a Mediterranean dietary pattern achieved a significantly higher total fat, mono- and polyunsaturated fatty acid intake, and lower saturated fat intake as compared with the control groups [25].

As expected, we found that the genotype distribution of the -174G/C polymorphism of IL6 gene was similar in the three groups. The −174C allele frequencies did not differ from those previously found in other Spanish populations [8, 18, 39] but were slightly lower than the prevalence in other Caucasian populations [2, 40].

Due to the fact that obesity is a major risk factor of cardiovascular disease, controlling BMI is an important target in cardiovascular prevention. Since most of the PREDIMED subjects were overweight or obese (90%), our interest was to study the response (weight change) to the nutritional intervention depending on the genotype for the −174G/C polymorphism of the IL6 gene. We observed no significant differences in body weight changes across the three nutritional groups, although the MD+VOO subjects tended to have a lower body weight gain. The finding that a higher consumption of olive oil does not lead to a higher weight gain was also reported in a large Spanish cohort study by Bes-Rastrollo *et al.* (2006). These authors found that higher olive oil consumption was associated with a lower (albeit non significant) likelihood of weight gain in the SUN prospective cohort study [41].

To the best of our knowledge this is the first study that analyzed the effects of the possible interaction between the -174G/C polymorphism of IL6 and a Mediterranean-style dietary intervention on body weight changes. Before the study was begun, we observed that CC subjects had a significantly higher body weight and BMI. This finding agreed with other cross-sectional studies that showed a significant association between the C allele and higher measures of adiposity [14–16]. Moreover, a recent study, found an association between the CC genotype and BMI in a 1334 subject cohort [42]. Two cross-sectional joint analyses

Table 3. Effects of the -174G/C polymorphism on 3-year body weight changes among the MD+VOO group

		B (95% CI)[a]	*p*-Value
Age		0.038 (−0.033 to 0.109)	0.295
Sex	Males	0 (ref.)	
	Females	−0.582 (−1.539 to 0.375)	0.232
Baseline BMI		−0.354 (−0.495 to −0.214)	**<0.001**
Diabetes	No	0 (ref.)	
	Yes	−1.095 (−2.056 to −0.133)	**0.026**
-174G/C polymorphism	GG+GC	0 (ref.)	
	CC	−2.061 (−3.394 to −0.728)	**0.003**

a) Multiple linear regression model. Dependent variable: 3-year body weight change (third year weight-baseline weight).

Table 4. Effects of the interaction between the -174G/C polymorphism of the IL6 gene and the nutritional intervention on 3-year body weight changes

		B (95% CI)[a]	*p*-Value
Age		−0.024 (−0.070 to 0.022)	0.301
Sex	Males	0 (ref.)	
	Females	−0.400 (−1.004 to 0.204)	0.194
Baseline BMI		−0.271 (−0.360 to −0.182)	**<0.001**
Diabetes	No	0 (ref.)	
	Yes	−0.680 (−1.289 to −0.072)	**0.028**
	Control group	0 (ref.)	
	MD+VOO	0.202 (−0.593 to 0.997)	0.618
	MD+Nuts	−0.114 (−0.954 to 0.725)	0.789
-174G/C polymorphism (recessive model)	GG+GC	0 (ref.)	
	CC	0.147 (−1.299 to 1.593)	0.842
-174G/C polymorphism × nutritional intervention	CC × control group	0 (ref.)	
	CC × MD+VOO group	−2.180 (−4.119 to −0.241)	**0.028**
	CC × MD+Nuts group	0.898 (−1.082 to 2.878)	0.374

a) Multiple linear regression model. Dependent variable: 3-year body weight change (third year weight-baseline weight).

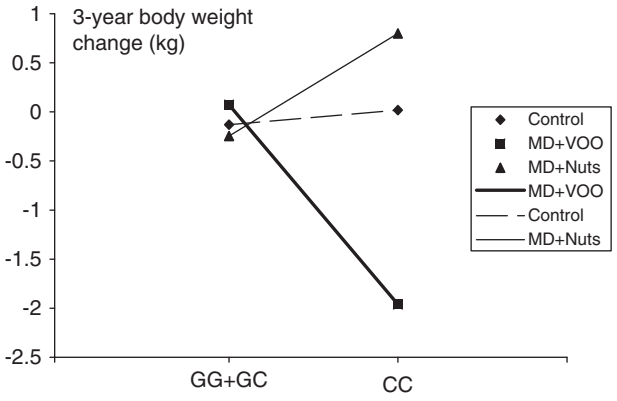

Figure 3. Interaction plots showing the effects of the interaction between the nutritional group and the genotype for the -174G/C polymorphism of IL6 gene on 3-year body weight changes. The model was adjusted for age, sex, baseline BMI, and diabetes.

reported no association between the C allele and BMI [12, 13]. However, a meta-analysis with 26 944 subjects pointed out that this SNP was not associated with BMI, but the -174G/C SNP was in strong linkage disequilibrium with a SNP in the promoter region (rs 2069827) consistently associated with adiposity [19].

Interestingly, when the effect of the polymorphism was analyzed independently of the nutritional intervention, it seemed that the CC subjects had a lower body weight gain than the GG+GC subjects. However, when both nutritional intervention and genotype were analyzed together, this effect was only present in the MD+VOO group. This fact suggested a potential diet by genotype interaction, which was confirmed by the multiple regression models in our population showing that CC subjects allocated to MD+VOO had the lowest body weight gain after 3 years in the nutritional intervention trial. As stated earlier, there is a strong relationship between a chronic low inflammatory state and excess body weight. In this sense, it is possible that the association between the -174C allele of IL6 gene and higher plasma levels of inflammatory markers such as IL6 and C-reactive protein [15, 42, 43] might be involved in low-grade inflammation related to adiposity. Moreover, the anti-inflammatory effect of VOO is well-documented [44] since it is able to decrease IL6 and C-reactive protein levels [33, 45]. Thus, the potential interaction between the CC genotype and the intervention with MD +VOO that we found may suggest that the CC subjects allocated to this diet benefit more from the anti-inflammatory properties of VOO and this beneficial effect could help them to achieve body weight reduction.

On the other hand, we observed that the significant interaction (MD+VOO × CC) observed in the whole population was not found in the diabetic subjects. This may be explained by the reduced sample size of the diabetic subsample compared with nondiabetics or by the hypothesis that genetic factors for diabetes or some specific metabolic features of diabetics may be masking this effect.

The present study has several strengths, such as its randomized design, which is able to provide first-level scientific evidence [46]. In addition, the nutritional intervention was conducted in real-life conditions (home-prepared foods). But the study also has some limitations. The first one was to ensure participants' compliance. To minimize this problem, all the participants have received continuous dietary instructions. Another limitation is related to the possibility of false-positive findings due to multiple comparisons. Further studies are necessary to confirm our results in other MD settings.

In summary, in our high cardiovascular risk population CC homozygous subjects for the -174G/C polymorphism of IL6 had the highest baseline body weight and BMI. However, after 3 years of a nutritional intervention with a Mediterranean-style diet rich in VOO these CC subjects were observed to have the highest reduction in body weight.

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The authors have declared no conflict of interest.

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