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The Mediterranean diet protects against waist circumference enlargement in 12Ala carriers for the PPARγ gene: 2 years' follow-up of 774 subjects at high cardiovascular risk

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The PPARγ gene regulates insulin sensitivity and adipogenesis. The Pro12Ala polymorphism of this gene has been related to fat accumulation. Our aim was to analyse the effects of a 2-year nutritional intervention with Mediterranean-style diets on adiposity in high-cardiovascular risk patients depending on the Pro12Ala polymorphism of the PPARγ gene. The population consisted of a substudy (774 high-risk subjects aged 55–80 years) of the Prevención con Dieta Mediterránea (PREDIMED) randomised trial aimed at assessing the effect of the Mediterranean diet for CVD prevention. There were three nutritional intervention groups: two of them of a Mediterranean-style diet and the third was a control group advised to follow a conventional low-fat diet. All the participants were genotyped by PCR-restriction fragment length polymorphism (RFLP). The results showed that carriers of the 12Ala allele allocated to the control group had a statistically significant higher change in waist circumference (adjusted difference coefficient = 2·37 cm; $P=0·014$) compared with wild-type subjects after 2 years of nutritional intervention. This adverse effect was not observed among 12Ala carriers allocated to both Mediterranean diet groups. In diabetic patients a statistically significant interaction between Mediterranean diet and the 12Ala allele regarding waist circumference change was observed (−5·85 cm; $P=0·003$). In conclusion, the Mediterranean diet seems to be able to reduce waist circumference in a high-cardiovascular risk population, reversing the negative effect that the 12Ala allele carriers of the PPARγ gene appeared to have. The beneficial effect of this dietary pattern seems to be higher among type 2 diabetic subjects.

Genetic epidemiology: Waist circumference: Mediterranean diet: PPARγ

CVD, the leading cause of death in developed countries[1,2], is a good example of complex and multifactorial disease caused by genetic, environmental factors and their interactions[3]. Excess of adiposity is a major risk factor for CVD, especially when this excess is located in visceral depots[4]. The prevention of CVD is currently directed to potential modifiable factors, such as diet and lifestyle factors. In this sense, adiposity indexes (waist circumference and body weight) could be modified by interventions aimed to obtain changes in dietary pattern.

Since the Mediterranean diet was first defined by Keys & Grande in 1957[5], it has been postulated as beneficial against CVD[6,7] and type 2 diabetes[8]. Olive oil consumption, which was the main source of fat (MUFA) in traditional Mediterranean diets, has been widely related to this protective effect[1,7,9,10]. Also, tree nuts (a good source of PUFA) are an integral part of the Mediterranean food pattern and previous studies have found a protective association between nut consumption and reduced risk of CVD[11–13].

One of the most studied genes linked to interactions with dietary compounds is the PPARγ[14–17] that encodes a transcription factor (PPARγ2) that controls the expression of genes involved in adipocyte differentiation, lipid storage and insulin sensitisation. The effects of the 12Ala allele have been studied in functional analysis, revealing that the receptor carrying this allele displays reduced DNA-binding affinity and impaired transcriptional activity in target genes[18]. Therefore the 12Ala *in vivo* would be expected to protect against increased adiposity due to the reduced activity of the receptor. However, previous studies in human subjects showed that the 12Ala allele was associated with increased adiposity[19–21]. These contradictory results *in vitro* and *in vivo* might be

Abbreviation: PREDIMED, Prevención con Dieta Mediterránea.

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explained by a potential enhancing effect of the anti-lipolytic action of insulin, which leads to reduced release of NEFA[18].

The Pro12Ala substitution in this gene has also been widely associated with diabetes[22]. Moreover, a positive association between the 12Ala allele of the PPARγ and obesity-related traits was reported among diabetic patients[23].

Most of the associations between diet, risk factors for chronic diseases and genetics have come from observational epidemiological studies and, as with conventional nutrition research, such observations need to be verified by randomised dietary intervention trials to provide the strongest evidence of causality[24]. As PPARγ is known to be activated by fatty acids[25], we hypothesised that the effects of a high consumption of virgin olive oil or nuts could be partly explained by means of the effects mediated by PPARγ. Thus, our aim was to analyse the effects of a 2-year nutritional intervention with Mediterranean-style diets on adiposity in high-cardiovascular risk patients depending on the Pro12Ala polymorphism of the PPARγ gene.

Research methods and procedures

Study population

The present study has been conducted within the frame of the Prevención con Dieta Mediterránea (PREDIMED) trial. The design of the PREDIMED trial has been reported in detail elsewhere[1,26]. Briefly, the PREDIMED trial is a large, parallel-group, multicentre, randomised and controlled 4-year clinical trial that aims to assess the effects of a Mediterranean-type diet on CVD and in which participants are assigned to one of three different dietary patterns (low-fat diet; Mediterranean diet supplemented with nuts; Mediterranean diet supplemented with virgin olive oil)[1]. The study population is composed of men aged 55–80 years and women aged 60–80 years with no previously documented CVD but at high cardiovascular risk. Inclusion criteria were either diabetes mellitus type 2 or at least three of the following risk factors: current smoking, hypertension, hyperlipidaemia, HDL-cholesterol $<1{\cdot}034$ mM, overweight or obesity, or family history of premature CHD.

We included data from 774 participants enrolled in the AP-UNAV recruitment centre in Pamplona (total number of participants was 1055), in which the retention rate during the second year was greater than 80 %. Of the subjects, 141 were excluded for the present study since we were not able to obtain DNA samples or their adiposity measures at year 2 were not recorded. All participants provided informed consent and the protocol was approved by the institutional review boards of all participating centres according to the Declaration of Helsinki.

Dietary assessment

The dietary habits of the participants, both at baseline and after follow-up for 24 months, were assessed using a semi-quantitative 137-item FFQ previously validated in Spain[27]. More details about the dietary assessment are described elsewhere[1]. We focused the nutritional study on fat intake changes.

Genotyping

Overnight fasting venous blood samples were collected in tubes containing EDTA. 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 Pro12Ala polymorphism of the PPARγ gene (rs1801282) using the PCR-restriction fragment length polymorphism (RFLP) method as described elsewhere[28].

Statistical analysis

The 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 intervention groups were performed using parametric tests (Student's *t* tests; ANOVA followed by Bonferroni *post hoc* tests). Means of changes in body weight and waist circumference were compared among the three randomised groups and among genotypes using general

Table 1. Baseline characteristics of the participants according to intervention group allocation
(Mean values and standard deviations)

	Control (*n* 197)				Virgin olive oil (*n* 318)				Tree nuts (*n* 258)*			
	ProPro (*n* 168)		12Ala allele carriers		ProPro (*n* 272)		12Ala allele carriers		ProPro (*n* 221)		12Ala allele carriers	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sex (% female)	54·8		79·3		52·6		71·7		51·6		45·9	
Age (years)	68·0	6·9	71·6	3·9	67·8	6·7	68·7	5·8	67·5	6·8	67·7	5·6
Waist:height	0·6	0·1	0·6	0·1	0·6	0·1	0·6	0·1	0·6	0·1	0·6	0·1
Waist circumference (cm)	95·2	11·6	93·9	9·9	96·6	10·5	93·1	10·4	95·5	9·6	95·8	10·2
Weight (kg)	74·6	12·7	72·6	10·9	76·1	12·2	73·3	10·1	74·9	10·1	75·0	11·1
BMI (kg/m^2)	29·1	3·5	29·6	3·1	29·3	3·3	28·8	3·4	29·1	3·1	29·1	3·3
Systolic blood pressure (mmHg)	155·5	20·6	159·3	21·7	155·4	20·4	152·9	22·4	155·7	22·8	156·9	14·4
Diastolic blood pressure (mmHg)	86·2	11·0	85·0	10·0	85·9	10·5	84·7	11·5	87·5	11·1	85·8	11·2
Diabetes (%)	32·1		31·0		34·9		45·7		38·0		40·5	
Smoking habit (% current smokers)	17·9		3·4		18·4		6·5		12·7		16·2	

* One subject was not included because of missing data for this analysis.

Table 2. Energy and fat intake after 2 years of nutritional intervention according to intervention group (Mean values and standard deviations)

	Control (*n* 197)		Virgin olive oil (*n* 318)		Tree nuts (*n* 259)	
	Mean	SD	Mean	SD	Mean	SD
Total energy						
kJ/d	8024·5	1940·5	9702·3**	1915·4	9780·9***	1843·1
kcal/d	1917·9	463·8	2318·9**	457·8	2337·7***	440·5
Total fat (% total energy intake)	34·2	9·8	44·2****	9·6	44·2****	9·3
SFA (% total energy intake)	8·9	2·0	9·0	1·7	9·1	1·7
MUFA (% total energy intake)	19·8	4·4	23·4****	3·6	23·1****	3·6
PUFA (% total energy intake)	5·6	1·9	6·7****	1·5	7·1****††	1·0
MUFA:total fat	0·52	0·05	0·55****	0·04	0·54****†	0·04
PUFA:total fat	0·15	0·04	0·16*	0·04	0·17****†††	0·02
SFA:total fat	0·24	0·04	0·21****	0·04	0·21****	0·04

Mean value was significantly different from that of the control group: *$P = 0·009$, **$P = 0·008$, ***$P = 0·004$, ****$P < 0·001$.
Mean value was significantly different from that of the virgin olive oil group: †$P = 0·019$, ††$P = 0·001$, †††$P < 0·001$.

linear models adjusting for age, baseline BMI (continuous), sex, diabetes and smoking habit.

The effects of the polymorphism on changes in anthropometric variables were evaluated with multivariate linear regression models adjusting for relevant variables: age, sex (males as the reference category), baseline BMI, diabetes and smoking habits. For each adiposity index (changes in waist circumference (2nd year waist circumference − baseline waist circumference), body weight (2nd year weight − baseline weight) and BMI (2nd year BMI − baseline BMI)) three regression models were fit: the first model analysed the effects of the nutritional intervention and the polymorphism separately on changes in adiposity indexes. The second model included the interaction product-term between the nutritional intervention (olive oil + nuts *v.* control) and the Pro12Ala polymorphism (12Ala carriers *v.* non-carriers) and the third model included dummy variables to consider each possible combination of the genetic variants and the nutritional intervention group simultaneously.

We repeated all these analyses including only diabetic patients.

Results

Baseline characteristics of the participants, according to the nutritional intervention and the polymorphism, are presented in Table 1. As expected, because of the randomisation, there were no statistically significant differences among the three nutritional groups in any of the biological variables. Most of the PREDIMED subjects, at baseline, were overweight or obese (91 %) and more than one-third had a diagnosis of type 2 diabetes.

The analysis of macronutrient intake at the beginning of the study and after 2 years of nutritional intervention revealed that the intervention brought about an effective change in the dietary pattern of the subjects with significant differences in the expected direction according to the respective allocated group. These changes were remarkable regarding fat consumption (Table 2). A significantly higher consumption of total fat, monounsaturated and polyunsaturated fat (but not saturated fat) was observed in both Mediterranean diet groups compared with the control group (Table 2). Moreover, the control group had a significantly higher ratio of saturated fat:total fat than those subjects allocated to the Mediterranean diet groups (Table 2).

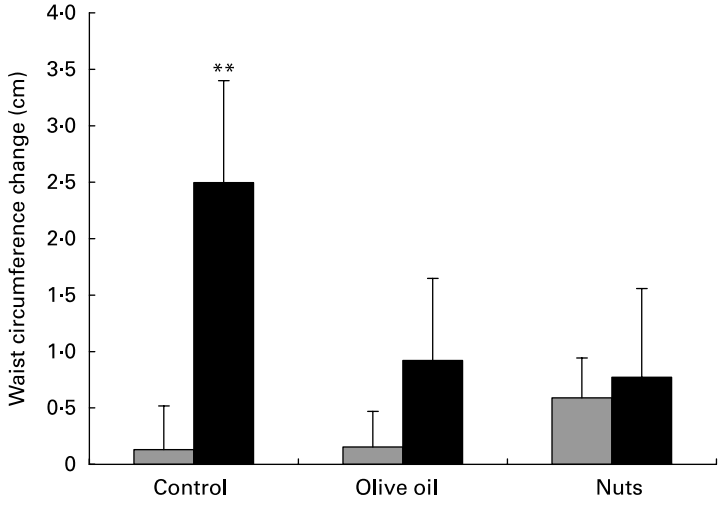

Fig. 1. Waist circumference changes according to the Pro12Ala polymorphism (□, ProPro; ■, Ala) of the PPARγ gene after 2 years of nutritional intervention in the total population. Data are means, with standard deviation represented by vertical bars. **Mean value was significantly different from that of the non-mutated subjects allocated to the control group ($P = 0·011$).

To analyse whether the Mediterranean diet had a beneficial effect on adiposity indexes, we first investigated body weight and waist circumference changes depending on the intervention group. Mean body weight changes for the control, olive oil and nuts groups were similar (0·34 (SEM 0·28), 0·33 (SEM 0·22) and 0·87 (SEM 0·21) kg, respectively). The comparison for mean body weight changes was not statistically significant, neither between control and olive oil ($P=0·992$) nor control and nuts ($P=0·131$). Mean waist circumference changes were also alike in the control, olive oil and nuts groups (0·35 (SEM 0·35), 0·14 (SEM 0·28) and 0·51 (SEM 0·26) cm, respectively). No differences were found when control and olive oil ($P=0·632$) or control and nuts ($P=0·711$) were compared.

The distribution of genotypes for the Pro12Ala of PPARγ gene polymorphism was in Hardy–Weinberg equilibrium in the study groups, being the 12Ala allele frequency 0·07 in each group. Due to the low frequency of 12Ala of the PPARγ gene, only four out of the 774 subjects were homozygous for the polymorphism (Ala12Ala). Therefore we decided to merge in all the analysis the Ala carriers (both heterozygous and homozygous merged together as a single group, representing 14·5 % of the total population) and to compare them with the non-mutated subjects. Therefore this variable had always 1 df.

Adjusted mean body weight changes were 0·56 and 0·92 kg for ProPro and Ala carriers, respectively. Mean waist changes were 0·30 cm in non-mutated (ProPro) and 1·28 cm in mutated subjects (Ala carriers), this difference being statistically significant ($P=0·046$). Moreover, when the three nutritional groups were also considered in the model, we observed that in the control group the 12Ala carriers had a significantly higher ($P=0·011$) waist circumference increase compared with non-carriers after 2 years of nutritional intervention (Fig. 1). The same tendency was observed when weight changes were analysed, although the differences within control subjects carrying the 12Ala allele *v.* non-mutated subjects were not statistically significant (data not shown). The polymorphism was not associated with differences in waist circumference changes within both Mediterranean diet groups.

To go deeper in our analysis, several multiple linear regression models were fitted in order to find out the model that best predicted the effects of the Mediterranean diet on adiposity indexes depending on the Pro12Ala polymorphism of the PPARγ gene. Table 3 shows the model to predict the effects of the diet (considering the three intervention groups) and the two groups of genetic variants on waist circumference changes (2nd year − baseline), adjusting for sex, age, baseline BMI, diabetes and smoking habit. The obtained results supported the finding of the analysis of covariance (ANCOVA) showing that carriers of the 12Ala allele had a statistically significant increase in waist circumference compared with non-mutated subjects (ProPro) ($P=0·046$). Moreover, we found that subjects with higher baseline BMI were those with the highest waist circumference reduction (adjusted changes: −0·20 cm; $P<0·001$) after 2 years of nutritional intervention regardless of nutritional intervention and genotype (Table 3).

When a 1 df product-term to assess effect modification (interaction) between the nutritional intervention (control (0) *v.* Mediterranean diet groups (1)) and the two groups of genotypes (Ala carriers (1) *v.* non-carriers (0)) on waist circumference changes was introduced in the model (data not shown), the *P* value was not statistically significant (B= − 1·89; $P=0·095$).

Thus, to better analyse the effects of the Mediterranean diet and the polymorphism together, we applied a multiple regression model with five dummy variables to include the combinations of the three nutritional groups and the two groups of genotypes as compared with non-mutated subjects (ProPro genotype) allocated to the control group, who were the single reference category (Table 3). This model was adjusted for sex, age, baseline BMI, diabetes and smoking habit and the dependent variable was 2-year waist circumference change (2nd year − baseline). We found that subjects allocated to the control group who also were carriers of the

Table 3. Multiple regression models for the analysis of the effects of the Pro12Ala polymorphism of the PPARγ2 gene on waist circumference change after 2 years of nutritional intervention

(B coefficients and 95 % confidence intervals)

	B*	95 % CI	*P*
Considering the polymorphism and nutritional group main effects			
Intercept	7·889	2·815, 12·962	0·002
Age	−0·042	−0·094, 0·010	0·117
Sex			
Males	0	Reference	
Females	0·312	−0·565, 1·189	0·485
Baseline BMI	−0·199	−0·301, −0·096	<0·001
Diabetes			
No	0	Reference	
Yes	0·187	−0·519, 0·893	0·603
Nutritional intervention			
Control	0	Reference	
Virgin olive oil	−0·208	−1·055, 0·639	0·630
Nuts	0·146	−0·741, 1·032	0·747
Pro12Ala polymorphism			
ProPro	0	Reference	
12Ala allele carriers	0·980	0·017, 1·942	0·046
Smoking habit			
Non-smokers	0	Reference	
Ex-smokers	0·388	−0·609, 1·386	0·322
Current smokers	0·559	−0·547, 1·664	0·217
Introducing five dummy variables to include the combinations of the three nutritional groups and the two genotype groups†			
Intercept	7·965	2·892, 13·038	0·002
Age	−0·044	−0·096, 0·008	0·096
Sex			
Males	0	Reference	
Females	0·303	−0·574, 1·179	0·498
Baseline BMI	−0·201	−0·304, −0·098	<0·001
Diabetes			
No	0	Reference	
Yes	0·173	−0·534, 0·879	0·631
Nutritional intervention and Pro12Ala polymorphism			
Control and ProPro	0	Reference	
Control and Ala‡	2·366	0·478, 4·255	0·014
Olive oil and ProPro	0·025	−0·889, 0·939	0·957
Olive oil and Ala‡	0·790	−0·783, 2·363	0·324
Nuts and ProPro	0·460	−0·497, 1·417	0·346
Nuts and Ala‡	0·643	−1·051, 2·336	0·457
Smoking habit			
Non-smokers	0	Reference	
Ex-smokers	0·422	−0·577, 1·422	0·407
Current smokers	0·607	−0·501, 1·714	0·282

* Dependent variable: waist circumference change (2nd year waist circumference − baseline waist circumference).

† When the interaction was formally tested, the *P* value for interaction (2 df; three diet groups × two genotype groups) was $P = 0·214$.

‡ Homozygous and heterozygous subjects merged together in a single group.

PPARγ polymorphism (12Ala) had the highest 2-year waist circumference increase compared with the reference group (adjusted difference = 2·37 cm; $P=0·014$). In agreement with the ANCOVA results, there were no statistically significant differences within the subjects allocated to the Mediterranean diet groups, independently of the genotype, and the reference category.

Similar models were fit considering as the dependent variables body weight or BMI changes. The results were in line with findings regarding waist circumference, but neither of them reached significance for the product-terms of interaction (data not shown).

Of special interest was the analysis that we performed with the subset of diabetic patients (*n* 276) in order to investigate the effects of the Mediterranean diet on adiposity depending on the genotype for the PPARγ within this group. The general linear model showed that the detrimental effect of the 12Ala allele on waist changes was statistically significant ($P<0·001$) within the subset of diabetic subjects allocated to the control group (Fig. 2), and the magnitude of this effect was higher than in the whole population (Figs. 1 and 2). In diabetic mutated subjects, the Mediterranean diet was able to reduce waist circumference, reversing the negative effect of the 12Ala allele (Fig. 2). As shown in the total population this effect was also observed when analysing body weight changes although it did not reach statistical significance (data not shown).

A statistically significant interaction within diabetic subjects was observed between the nutritional intervention (control (0) *v.* Mediterranean diet groups (1)) and the polymorphism (Ala carriers (1) *v.* non-carriers (0)) in the multiple linear regression model. Ala carriers allocated to the Mediterranean diet groups were predicted to have a statistically significant lower increase in waist circumference compared with control subjects (B = −5·85; $P=0·003$), although the main effect of the polymorphism *per se* predicted a statistically higher waist circumference change after 2 years of nutritional intervention (B = 0·980; $P=0·046$) (Table 4).

The same models fitted among diabetic patients were performed in non-diabetic patients, but they did not reach statistical significance (Table 4).

Regarding these results a three-way interaction assessing genotype (1 df; Ala carriers *v.* non-carriers) × intervention (1 df; control *v.* Mediterranean diet groups) × diabetes (1 df; yes *v.* no) was tested in our regression model for waist circumference change after 2 years of intervention. We obtained a non-statistically significant *P* value (B = −0·66; $P=0·270$). In this context the interaction product diabetes × genotype (1 df) resulted statistically significant within the control group ($P=0·012$) when analysing separately the control and the Mediterranean diet groups (olive oil and nuts merged together).

Discussion

The excess of adiposity is considered as the main cause of the increased morbidity and mortality by CVD, especially when this excess is localised in the abdominal depots[4]. Waist circumference is an excellent marker of abdominal obesity and consequently of CVD risk[4] and mortality[29]. Therefore, the reduction of adiposity indexes, such as waist circumference and body weight, would result in an effective prevention for CVD. We have studied the effect of the Pro12Ala polymorphism of the PPARγ gene on 2-year changes in adiposity indexes in a population assigned to a nutritional intervention trial with the Mediterranean diet (the PREDIMED trial), a randomised and controlled study for the prevention of CVD, which allowed us to control not only for established confounding factors such as sex, age and baseline parameters but also for unknown or unmeasured confounders.

First of all, the present results proved the effectiveness of the Mediterranean diet nutritional intervention since the fat intake distribution in the three nutritional groups changed in the expected direction[26]. After 2 years, both Mediterranean dietary groups had a significantly higher consumption of total fat compared with the control group and the distribution of fatty acid intake was also very different. The Mediterranean diet groups had a significantly higher consumption of MUFA and PUFA and significantly lower saturated fat consumption, which are important characteristics of the Mediterranean dietary pattern[7].

When we analysed the effects of the nutritional intervention, no differences in changes in waist circumference and weight were found according to the nutritional group. This could be explained because the control subjects recruited for the trial already followed a healthier lifestyle before being

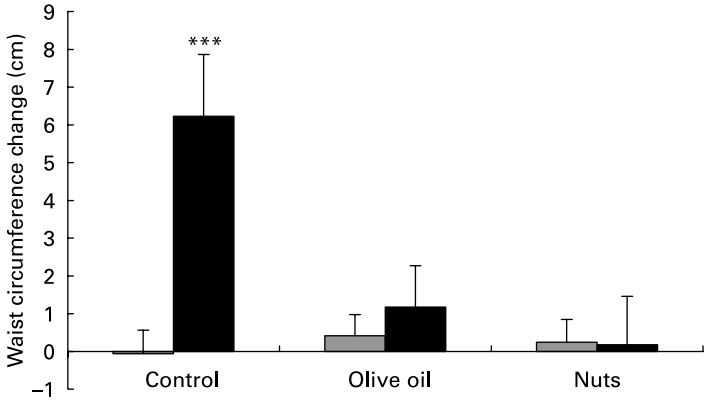

Fig. 2. Waist circumference changes according to the Pro12Ala polymorphism (□, ProPro; ■, Ala) of the PPARγ gene after 2 years of nutritional intervention in diabetic patients. Data are means, with standard deviation represented by vertical bars. *** Mean value was significantly different from that of the non-mutated subjects allocated to the control group ($P<0·001$).

Table 4. Multiple regression models for the analysis of the effects of the Pro12Ala polymorphism of the PPARγ2 gene on waist circumference change in diabetic and non-diabetic patients after 2 years of nutritional intervention

(B coefficients and 95 % confidence intervals)

	B*	95 % CI	P
Diabetic patients			
Main effects of the Pro12Ala polymorphism, adjusted for sex (female), age, baseline BMI, intervention and smoking habit			
Pro12Ala polymorphism			
Non-mutated	0	Reference	
12Ala carriers	0·980	0·017, 1·943	0·046
Considering the interaction product-term nutritional intervention × polymorphism			
Intercept	14·902	5·876, 23·928	0·001
Age	−0·131	−0·228, −0·034	0·008
Sex			
Males	0	Reference	
Females	0·111	−1·379, 1·601	0·884
Baseline BMI	−0·227	−0·389, −0·065	0·006
Nutritional intervention			
Control	0	Reference	
Intervention†	0·396	−1·087, 1·879	0·600
Pro12Ala polymorphism			
ProPro	0	Reference	
12Ala allele carriers	6·272	2·842, 9·702	<0·001
Intervention† × 12Ala allele‡	−5·853	−9·691, −2·016	0·003
Smoking habit			
Non-smokers	0	Reference	
Ex-smokers	−0·013	−1·638, 1·611	0·987
Current smokers	1·126	−0·973, 3·226	0·292
Non-diabetic patients			
Considering the interaction product-term nutritional intervention × polymorphism			
Intercept	5·491	−0·372, 11·353	0·066
Age	−0·005	−0·067, 0·056	0·862
Sex			
Males	0	Reference	
Females	0·462	−0·620, 1·544	0·402
Baseline BMI	−0·199	−0·334, −0·064	0·004
Nutritional intervention			
Control	0	Reference	
Intervention†	0·133	−0·874, 1·140	0·795
Pro12Ala polymorphism			
ProPro	0	Reference	
12Ala allele carriers	0·484	−1·766, 2·737	0·672
Intervention† × 12Ala allele‡	0·244	−2·432, 2·920	0·858
Smoking habit			
Non-smokers	0	Reference	
Ex-smokers	0·660	−0·605, 1·925	0·306
Current smokers	0·310	−0·986, 1·606	0·639

*Dependent variable: waist circumference change (2nd year waist circumference − baseline waist circumference).

†Intervention refers to the comparison between olive oil and nuts groups *v.* control group (1 df). The two intervention groups (olive oil and nuts) were merged together.

‡12Ala allele: comparison between 12Ala carriers (homozygotes and heterozygotes merged together) and non-carriers (ProPro genotype) (1 df).

included in the present study. This fact may justify the small differences in weight and waist changes between the Mediterranean groups and the control group after 2 years of intervention.

Our main objective was to analyse the effects of a Mediterranean dietary pattern depending on the Pro12Ala polymorphism of the PPARγ gene. We found a minor 12Ala allele frequency of 0·07, lower than values reported in Spanish subjects (0·13)[28] but similar to those obtained in European populations[30].

The analysis of the genotype variants on changes on adiposity indexes, adjusted for the nutritional intervention, revealed that the 12Ala allele was associated with statistically significant higher increase in waist circumferences ($P=0·046$). It also showed higher, but not significant, body weight changes and BMI after 2 years of nutritional intervention. The majority of the studies analysing the effects of the polymorphism on adiposity indexes are directed to BMI, but there are few studies analysing the effects of the polymorphism on waist circumference. Similarly to our findings, Pihlajamaki *et al.* reported a significant association of waist circumference and the polymorphism in a healthy Finnish population[31].

When considering the nutritional intervention we observed that the negative effect of the Ala allele on waist changes was only present in the control group ($P=0·011$) and it was highly attenuated in the two Mediterranean diet groups (Fig. 1). It seems that the Mediterranean diet reversed the

detrimental effect that the 12Ala minor allele appears to have on waist circumference. One plausible hypothesis that could partly explained this finding is that PPARγ gene is known to be activated by fatty acids[25] and, as stated before, the Mediterranean diet consists of a high consumption of monounsaturated (olive oil) and polyunsaturated fat (nuts).

The interaction between the Mediterranean diet and the Pro12Ala polymorphism of the PPARγ gene on adiposity presented was not statistically significant ($B = -1{\cdot}863$; $P=0{\cdot}095$) although Robitaille *et al.* reported a Pro12Ala × diet interaction on waist circumference: ProPro subjects had significantly higher waist circumference when higher levels of total fat ($P=0{\cdot}007$) or saturated fat ($P=0{\cdot}002$) were consumed[2].

Due to the potential role that the Pro12Ala polymorphism of the PPARγ gene seemed to have on type 2 diabetes[22,32], we performed a specific analysis on diabetic patients. This analysis revealed that the detrimental effect that the polymorphism appeared to have on waist circumference and weight changes was more relevant among these patients than in the total study population (Fig. 2). Moreover, the reversing effect of the nutritional intervention was also higher in diabetic 12Ala carriers allocated to the two Mediterranean diet groups. The interaction product-term, intervention × 12Ala, was statistically significant, confirming the protective effect of the Mediterranean diet against higher waist circumference, especially in 12Ala carriers. Some authors have reported the beneficial effects of the Mediterranean diet on diabetes[8,33,34] and glycaemic control[35]. Likewise, there are also studies analysing the effects of the polymorphism and dietary compounds on adiposity indexes in diabetic patients. In agreement with our findings, a higher visceral fat deposition on individuals carrying the 12Ala allele and with a low polyunsaturated fat intake was reported[23].

Following the present results, it seems that the negative effect of the 12Ala allele in the total population could be mostly attributed to diabetic patients. Moreover, we observed that this effect was only attenuated when these patients followed a Mediterranean dietary pattern. This attenuation may be partly explained by the fact that fatty acids (high intake of PUFA and MUFA in this dietary pattern) are activating the PPARγ protein and could potentially modify the action of the Pro12Ala substitution on the receptor activity, especially in the presence of diabetes[18,23,25].

The present study has several strengths, such as its design, which is able to provide first-level scientific evidence[36], as we work in real-life conditions (home-prepared foods). But the study also has some limitations. The first was to ensure participants' compliance with the aid of continuous dietary instructions. In fact, we could observe that the dietary pattern (especially fat distribution) was significantly different between the Mediterranean diet groups and the control group after 2 years of intervention (Table 2). Another limitation of the statistical procedure is that multiple comparisons were performed in order to examine the contribution of the three nutritional groups and the different genotypes, but the differences remained statistically significant even after the Bonferroni correction.

In summary, the present study shows that Mediterranean-style diets seemed to be able to reduce 2-year waist circumference enlargement in a high-cardiovascular risk population, reversing the negative effect that carrying the 12Ala allele of the PPARγ gene appeared to have. The beneficial effect of this dietary pattern seemed to be more important among type 2 diabetic subjects.

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C. R. carried out the experimental procedures, the analysis and interpretation of the data, and drafted the manuscript. A. M. participated in the design of the study, contributed to the analysis and interpretation of the data, and helped to draft the manuscript. M. A. M.-G. helped with the statistical analysis and helped to draft the manuscript. J. A. M. helped with the interpretation of the data and helped to draft the manuscript. M. A. M.-G., D. C. and J. M. S. participated in the initiation and design of the study and in the recruitment of the subjects. All authors read and approved the final version of the manuscript.

The authors have no conflicts of interest to declare.

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