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Weight regain after slimming induced by an energy-restricted diet depends on interleukin-6 and peroxisome-proliferator-activated-receptor-γ2 gene polymorphisms

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Weight-loss maintenance after following an energy-restricted diet is a major problem that a number of studies are trying to characterise. The aim of the present study was to investigate the role of IL-6 -174G > C and PPAR-γ2 Pro12Ala variants on weight regulation in obese subjects receiving a low-energy diet and at 1 year after the acute slimming period. Sixty-seven volunteers (age 34·7 (SD 7·0) years; BMI 35·8 (SD 4·8) kg/m^2) were enrolled in a 10-week dietary intervention and were contacted again 1 year after the end of this period. Body composition was measured at three times during the study. Also, PPAR-γ2 Pro12Ala and IL-6 -174G > C polymorphisms were analysed in the participants. No statistical differences were observed depending on the genetic variants at baseline for anthropometric variables, or after the intervention. However, the C allele of the -174G > C IL-6 gene polymorphism was more frequently observed ($P=0·032$) in subjects with successful weight maintenance (< 10 % weight regain). In fact, the C allele partially protected against weight regain (odds ratio 0·24; $P=0·049$), while the conjoint presence of both gene variants (C + and Ala +) further improved the ability for weight maintenance (odds ratio 0·19; $P=0·043$). The present study demonstrates that the C allele of the -174G > C polymorphism gives protection against regain of weight lost. Moreover, the presence of the Ala allele of the PPARγ-2 together with the C allele strengthens this protection. These findings support a role for these polymorphisms on weight regulation and suggest a synergetic effect of both variants on weight maintenance after following a diet to lose weight.

Interleukin-6: Obesity: Weight loss: Gene–nutrient interactions

Obesity is a major health problem associated with multiple morbidities and a higher rate of mortality, being increasingly prevalent (Aronne, 1998; Martinez, 2000). Weight-control methods are mainly focused on dietary changes and increased physical activity approaches (Abeke *et al*. 2006; Greenwald, 2006), which can reduce or eliminate excessive body weight and related disorders. Weight-loss methods often produce successful short-term results, while weight-loss maintenance is often a difficult task to achieve (Wing & Phelan, 2005). The failure to maintain weight loss could be partially explained by genetic factors or by the interaction between genes and environment (Marti *et al.* 2004). In this context, knowledge about genetic predisposition to regain body weight after obesity therapy could offer the possibility to optimise and individualise the treatment in some cases.

Many genes and polymorphisms from different chromosomal regions have been proposed as related to obesity and body-weight control (Rankinen *et al*. 2006). Among these, the IL-6 and PPAR-γ genes could be involved in weight regulation (Goyenechea *et al.* 2005; Sesti *et al.* 2005). IL-6 is an important regulator of the acute-phase response that has been associated with obesity (Trayhurn & Wood, 2005) and with energy balance (Tsigos *et al.* 1997); it is released by adipocytes, as well as by other tissues (Trayhurn & Wood, 2004). Hence, higher circulating levels of this pro-inflammatory cytokine have been reported in obese subjects (Trayhurn & Wood, 2005).

The C-174G functional polymorphism, located in the promoter region of the IL-6 gene, has been found to influence transcriptional regulation and plasma cytokine levels (Fishman *et al.* 1998). Indeed, the polymorphism has been associated with obesity (Berthier *et al.* 2003; Wernstedt *et al.* 2004) and co-morbidities, such as insulin resistance, hypertension and CVD (Fernandez-Real *et al.* 2000*a*; Humphries *et al.* 2001; Hamid *et al.* 2005). Nevertheless, data about the effects of the polymorphism on pathogenesis of these diseases are still contradictory (Dedoussis *et al.* 2004) and long-term intervention programmes have not been carried out.

PPAR-γ is a nuclear receptor affecting the transcriptional process of many target genes to enhance adipocyte differentiation and improve insulin signalling (Auwerx, 1999) which is involved in the regulation of body weight and other metabolic processes (Marti *et al.* 2002). The Pro12Ala polymorphism of the PPAR-γ2 isoform has been shown to decrease PPAR-γ activity (Hara *et al.* 2000; Masugi *et al.* 2000) and to be associated with higher BMI (Deeb *et al.* 1998). In addition, prospective and intervention studies have shown that the Pro12Ala polymorphism could be associated with

Abbreviations: LED, low-energy diet.

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weight regain after a diet-induced weight loss (Ek *et al.* 1999; Nicklas *et al.* 2001).

Because both PPAR-γ2 and IL-6 gene polymorphisms have been related to weight homeostasis, it has been hypothesised that carrying conjunctly both variants could have additive effects on obesity management (Barbieri *et al.* 2005). Based on this, the aim of the present study was to evaluate the effect of IL-6 C-174G and PPAR-γ2 Pro12Ala variants on weight loss and maintenance by means of an intervention trial and by contacting volunteers again 1 year after the slimming period.

Subjects and methods

Subjects

Sixty-seven Caucasian obese subjects (BMI 35·8 (SD 4·8) kg/m^2) were recruited to participate in the study (forty-five women and twenty-two men; age 34·7 (SD 7·0) years). Initial screening evaluations included a medical history, physical examination and fasting blood profile, to exclude subjects with evidence of diabetes, hypertension, liver, renal or haematological disease, or other clinical disorders that could interfere with the weight-loss process. Other exclusion criteria were weight change >3 kg within the 3 months before the start of the study, participation in another scientific study up to 30 d before, drug treatment, pregnancy, surgically or drug-treated obesity, alcohol or drug abuse.

After a clear explanation of the study, all subjects gave written informed consent to participate, which was previously approved by the Ethics Committee of the University of Navarra, in agreement with the Helsinki Declaration.

Study design

All the subjects were enrolled in an initial weight-loss intervention programme with a balanced low-energy diet (LED) designed to contain 55 % energy as carbohydrates, 15 % as proteins and 30 % as fat during the 10-week study. Weight loss was controlled weekly by the dietitian, and the compliance to energy and nutrient intakes by 3 d weighed food records (two weekdays and one weekend day). Food records were performed during the week before the beginning of the intervention (week -1) and during the week before the end of the nutritional trial (week 7). These data provided information about baseline intake and the adherence to the prescribed diets. The energy restriction was 2090 kJ (500 kcal) less than the resting energy expenditure measured by indirect calorimetry (Deltatrac; Datex-Ohmeda, Helsinki, Finland), based on a previously described procedure (Labayen *et al.* 2004). The physical activity pattern was not increased during the weight-loss period, which was assessed and controlled by a trained specialist, based on a validated physical activity questionnaire (Martinez-Gonzalez *et al.* 1999).

Anthropometric determinations, body composition and energy expenditure were measured at baseline and at the end of the LED following standard procedures (Parra *et al.* 2005). A blood sample was taken at the beginning of the study and stored for DNA analysis.

After finishing the dietary intervention, volunteers were given dietary guidelines to maintain the weight loss but without energy restriction or specific follow-up instructions. They were contacted to assess weight maintenance and potential changes in anthropometry measurements and body composition 1 year after the end of the nutritional intervention period (Fig. 1). Successful weight-loss maintenance was considered by using the criterion of <10 % weight regain, according to Weinsier *et al.* (2002).

Anthropometry and body composition

Body-weight measurements were performed using a digital balance accurate to 0·1 kg (Seca 767; Vogel & Halke, Hamburg, Germany) and height using a wall-mounted stadiometer (Seca 220, Vogel & Halke). Measurements were carried out on subjects in underwear, after an overnight fast. BMI was calculated by body weight (kg)/height (m)^2. The waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac crest, with the subject in standing position (Parra *et al.* 2005). Body composition was measured by bioelectric impedance (Quadscan 4000, Bodystat, Isle of Man, UK), based on a previously described procedure (Pérez *et al*. 2005).

Blood sample analysis

Venous blood samples were drawn after an overnight fast of 12 h for genotyping and circulating levels measurements. Volunteers were genotyped for the IL-6 -174 G $>$ C promoter polymorphism and for codon 12 Pro/Ala locus of PPAR-γ2. DNA was extracted from leucocytes using a commercial kit (MasterPure^{TM}; Epicentre, Madison, WI, USA).

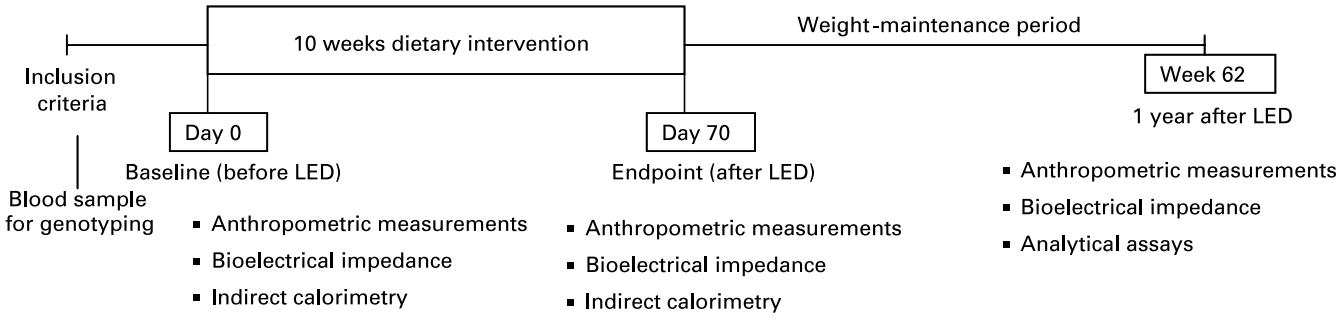

Fig. 1. Study design. Subjects (*n* 67) participated in a low-energy diet (LED) intervention during 10 weeks, and they were assessed for weight maintenance 1 year later.

A 198-bp fragment of the IL-6 gene was generated from genomic DNA by using a PCR procedure with forward primer 5′-TGACTTCAGCTTTACTCTTTGT-3′ and reverse primer 5′-CTGATTGGAAACCTTATTAAG-3′ as reported (Fernandez-Real *et al.* 2000*b*). The PCR products were digested with SfaNI restriction enzyme at 37°C for 90 min and electrophoresed on a 2 % agarose gel. The products after digestion were 140 and 58 bp for G/G homozygotes, 198 bp for C/C homozygotes and 198, 140 and 58 bp for G/C heterozygotes. CC and GC genotypes were grouped and are indicated as C carriers (C +) and the GG genotype was named as C non-carrier (C −).

A 270-bp fragment of the PPAR-γ2 gene was generated from genomic DNA by using PCR with forward primer 5′-GCCAATTCAAGCCCAGTC-3′ and mutagenic reverse primer 5′-GATATGTTTGCAGACAGTGTATCAGTGAAG-GAATCGCTTTC*C*G-3′, the latter of which introduces a *Bst* U-I restriction site when the C → G substitution at nucleotide 34 is present in relation to the Pro12Ala polymorphism (Yen *et al.* 1997). The PCR products were digested with *Bst* U-I restriction enzyme at 60°C for 90 min and electrophoresed on a 2 % agarose gel. The products after digestion were 270 bp for P/P homozygotes, 227 and 43 bp for A/A homozygotes and 270, 227 and 43 bp for P/A heterozygotes. Ala/Ala and Pro/Ala genotypes were grouped and are indicated as Ala carriers (Ala +), while the Pro/Pro genotype was named as Ala non-carrier (Ala −). In order to assure the correct detection of the selected genetic variants, one of three randomised sample and all the homozygotes for the single nucleotide polymorphisms were assessed in duplicate.

Serum levels of leptin, IL-6, TNF-α, and C-reactive protein were analysed at the end of the study (week 62) by Luminex (Linco Researchers, St Charles, MO, USA) using a LINCOplex human kit (Linco Researchers).

Statistical analysis

The Kolmovorov–Smirnov and the Shapiro–Wilk tests were used to determine variable distribution. Changes in weight loss were evaluated and compared between groups with paired *t* tests and with ANOVA, when distribution of variables was parametric. The Kruskal-Wallis, the Wilcoxon for matched pairs, the Mann–Whitney U tests and Friedmann analysis for repeated measurements were performed to analyse non-parametric data between groups at all the times of the study.

Spearman's correlation coefficient was calculated to determine relationships between variables. A χ^2 test was used to evaluate the Hardy–Weinberg equilibrium and a Fisher's exact test to analyse the frequency distribution of the genotypes on weight-maintenance period. Resting energy expenditure was adjusted for fat-free mass and changes in body weight were adjusted for baseline weight by the residuals method. The CV of the weight maintenance was calculated as the quotient between the standard deviation and the mean, and expressed as %. A logistic regression analysis was applied to assess the potential relationship between both variants of the polymorphisms in body-weight regulation, considering the 10 % weight regain as the cut-off.

Results are reported as mean values and SD while the two-sided statistical significance was set at $P<0{\cdot}05$. Statistical analysis was performed using the SPSS 11.0 program for Windows XP (SPSS Inc., Chicago, IL, USA).

Results

Genotype distribution

The frequency distribution of C allele of the IL-6 -174 G > C polymorphism in the participating volunteers was 40·2 %. The frequency of GG, GC and CC genotypes was 31·3, 56·7 and 11·9 %, respectively. Genotype distribution did not differ from that expected by the Hardy–Weinberg equation ($P>0{\cdot}05$). With respect to the PPAR-γ2 gene polymorphism, the frequency of the Ala allele in subjects was 12·1 %. The frequency of Pro/Pro, Pro/Ala and Ala/Ala genotypes was 77·3, 21·1 and 1·5 % respectively. The observed genotypes and allele distribution were also in Hardy–Weinberg equilibrium ($P>0{\cdot}05$) for this gene.

Response to low-energy diet depending on the genetic variants

Volunteers were categorised as subjects with no variants (C − , Ala −), IL-6 variant only (C + , Ala −) and with both IL-6 and PPAR-γ2 variants (C + , Ala +). There was only one subject with the PPAR-γ2 variant only (Ala + , C −), so he was not included in the analysis (Table 1). No statistical differences were observed in initial body weight (Fig. 2), or in other anthropometric and body composition variables (Table 1), depending on the genetic variants at baseline. The energy-restricted intervention induced a statistical reduction ($P<0{\cdot}05$) in these variables in all experimental groups (Table 1). In fact, all volunteers lost weight after the LED (Fig. 2) with no statistical differences between groups (no variants − 8·7 (SD 3·2) kg, IL-6 variant only − 8·4 (SD 3·0) kg, both variants − 9·2 (SD 4·4) kg; $P=0{\cdot}456$). A possible different effect on body composition (fat *v.* fat-free mass) was found depending on the genotype (Table 1). Initial body weight did not affect the response to dietary treatment, with no differences between genetic variant groups ($P=0{\cdot}870$).

Resting energy expenditure adjusted for fat-free mass was no different between groups (no variants 7355 (SD 936) kJ/d, IL-6 variant only 7599 (SD 568) kJ/d, both variants 7658 (SD 669); $P=0{\cdot}406$).

The effect of gene variants on weight maintenance

The evaluation of anthropometric and body composition values was considered as the difference between each variable 1 year after finishing the LED intervention and the measurement at the endpoint (10 weeks) of the nutritional intervention (Table 1). Taking into account all the nutritionally treated volunteers, weight maintenance showed a high variability (0·93 (SD 7·15) kg; CV 770 %). Among these, subjects with no variants tended to regain more weight (+2·06 (SD 6·4) kg) than subjects with the IL-6 variant only (+1·29 (SD 8·3) kg) but without statistical significance ($P=0{\cdot}730$). In contrast, subjects with both variants (−1·49 (SD 5·3) kg) tended to maintain the weight loss ($P=0{\cdot}054$) as compared with subjects carrying no variants (Fig. 2). Indeed, subjects with both variants (Table 1) maintained better the loss of body fat in relation to subjects

Table 1. Anthropometric and body composition changes in response to a low-energy diet and after the weight-maintenance period, depending on genetic variants of interleukin-6 and peroxisome proliferator-activated receptor-γ2: no variants (C −, Ala −), IL-6 variant only (C +, Ala −) and both variants (C +, Ala +)

(Mean values and standard deviations)

	No variants (*n* 19)		IL-6 variant only (*n* 32)		Both variants (*n* 15)		
	Mean	SD	Mean	SD	Mean	SD	Kruskal–Wallis (*P* value)
BMI (kg/m^2)							
Baseline	35·6	5·4	36·0	4·7	35·8	4·8	0·973
Endpoint	32·6*	5·3	33·1*	4·8	32·5*	4·9	0·914
After 1 year	33·4‡	5·8	33·6‡	6·2	32·3‡	4·7	0·911
Waist circumference (cm)							
Baseline	102·2	14·6	104·4	12·1	102·4	10·0	0·783
Endpoint	94·5*	12·2	97·2*	11·9	94·1*	9·5	0·606
After 1 year	95·7‡	9·7	97·7‡	14·0	94·0‡	7·6	0·746
Fat mass (kg)							
Baseline	39·7	12·0	40·8	11·7	38·1	11·6	0·768
Endpoint	32·7*	11·4	34·2*	11·3	31·2*	12·5	0·705
After 1 year	33·6‡	13·4	33·5‡	14·2	29·0†‡	8·8	0·326
Fat-free mass (kg)							
Baseline	56·4	10·3	58·0	12·0	59·8	14·7	0·720
Endpoint	54·6*	9·6	56·2*	12·3	57·6*	14·0	0·763
After 1 year	55·7	9·6	58·2†	13·0	59·1†	16·9	0·877

* Mean value at baseline was significantly different from that at the endpoint of the low-energy diet ($P<0·05$).
† Mean value at endpoint was significantly different from that 1 year after the end of the low-energy diet ($P<0·05$).
‡ Mean value at baseline was significantly different from that 1 year after the low-energy diet ($P<0·05$).

carrying no variants (−2·2 (SD 4·3) *v.* +0·9 (SD 4·1) kg; $P=0·072$).

Involvement of the interleukin-6 and peroxisome-proliferator-activated-receptor-γ2 variants on weight regain

Body-weight maintenance was evaluated by using the criterion of 10 % weight regain 1 year after the end of the diet intervention (Table 2). Analysing anthropometric and body composition indicators depending on weight maintenance, successful subjects who maintained the weight loss had statistically lower ($P<0·05$) values, although fat-free mass did not differ ($P=0·927$) between groups (Table 2). Serum levels of the pro-inflammatory markers IL-6 and C-reactive protein were significantly lower in subjects who maintained the weight loss at the end of the study (Table 2), and were both correlated (r 0·33; $P=0·011$), while no differences were observed in TNF-α serum levels (Table 2). Moreover, successful subjects in weight maintenance had significantly lower serum levels of leptin ($P=0·026$). As expected, leptin correlated with fat mass (r 0·69; $P<0·01$), but also with circulating C-reactive protein (r 0·50; $P<0·01$).

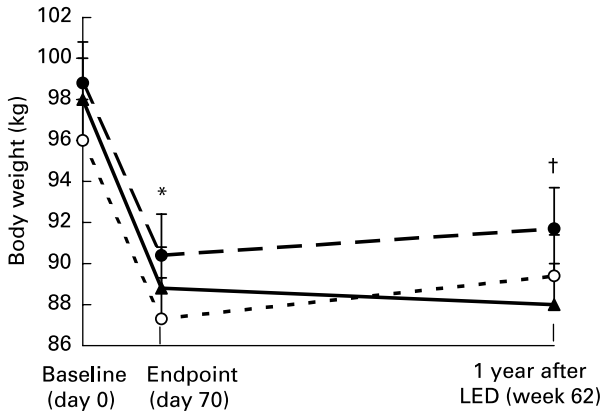

Fig. 2. Evolution of body-weight reduction produced by the low-energy diet (LED) depending on genetic variants of IL-6 and PPAR-γ2: no variants (C–, Ala–; --○--), IL-6 variant only (C +, Ala–; –●–);both variants (C +, Ala +; –▲–). * For the three variant groups, mean values were significantly different between baseline and endpoint of the LED ($P<0·05$). † For the three variant groups, mean values were significantly different between baseline and 1 year after the end of the LED ($P<0·05$). Friedmann analysis indicated statistical significance for the global slimming process in each group ($P<0·001$).

Genotype frequency distributions of IL-6 and PPAR-γ2 polymorphisms were analysed separately according to weight-maintenance criteria (Table 3). With respect to the involvement of the IL-6 polymorphism on weight control, statistical differences ($P=0·032$) were observed in frequency distribution for those individuals carrying the C allele as compared with volunteers without cytosine in this gene region (Table 3). The frequency distribution of the PPAR-γ2 genotypes did not reach statistical significance ($P=0·125$), although subjects with the presence of the Pro allele tended to regain weight. No statistical differences were found in the circulating levels of pro-inflammatory markers between polymorphism genotypes ($P>0·05$).

Taking into account the differences in frequency distribution, a binary logistic regression analysis was carried out to further analyse the effect of the polymorphisms on weight maintenance 1 year after the end of the LED treatment. None of the variables studied (age, sex and baseline body weight), instead of gene polymorphisms, were predictors of the weight maintenance, so they were not included in the logistic model (Table 4).

Interestingly, when the association between the -174G > C polymorphism and the protection against weight regain was

Table 2. Anthropometry, body composition and circulating levels of inflammatory biomarkers depending on the weight-maintenance criterion at 1 year after the end of the low-energy diet (regain less than 10 % body weight)

(Mean values and standard deviations)

Final data at 1 year after the acute slimming period	Subjects with weight regain (*n* 42)		Subjects with weight-loss maintenance (*n* 25)		Mann–Whitney U test (*P* value)
	Mean	SD	Mean	SD	
Weight (kg)	93·7	15·1	84·3	14·1	0·022
BMI (kg/m^2)	35·0	5·7	30·4	4·3	0·001
Waist circumference (cm)	99·5	11·4	90·8	9·7	0·020
Fat mass (kg)	36·0	13·8	25·5	8·5	0·001
Fat-free mass (kg)	57·5	12·6	57·9	13·4	0·927
Leptin (ng/ml)	26·5	18·7	17·7	19·1	0·026
IL-6 (pg/ml)	7·5	6·3	5·1	1·9	0·041
C-reactive protein (μg/ml)	8·2	8·1	3·7	4·3	0·017
TNF-α (pg/ml)	3·3	1·2	3·8	1·7	0·309

considered, the odds ratio was 0·24-fold for weight maintenance of subjects with the presence of the C allele when compared with those within the allele (Table 4). The Pro12Ala polymorphism of the PPAR-γ2 gene itself was not considered as a predictor of the weight maintenance, based on the logistic analysis ($P=0·151$). In contrast, when PPAR-γ2 was added to the IL-6 model, the odds ratio further improved, being 0·19-fold for weight maintenance of those subjects carrying both gene variants (Table 4).

Discussion

Difficulties concerning weight maintenance after weight loss induced by hypoenergetic diets are a major problem that a number of studies are trying to characterise and manage (Weinsier *et al.* 2002; Vogels *et al.* 2005*a*; Wing & Phelan, 2005). Some of the strategies to improve weight maintenance are nowadays focused on considering together the genetic make up and its interaction with dietary intake (Marti *et al.* 2004; Vogels *et al.* 2005*b*). In this context, we evaluated the effect of the -174 > C polymorphism of the IL-6 and the Pro12Ala of PPAR-γ2 genes as well as their potential interaction on weight maintenance in obese patients previously treated with a hypoenergetic diet to produce weight loss.

First, we tested the genotype distribution of the polymorphisms in volunteers, being the frequency of the -174C and 12Ala alleles similar to previously published values in other Caucasian populations (Deeb *et al.* 1998; Fishman *et al.* 1998; Humphries *et al.* 2001). Indeed, other Spanish data revealed a frequency distribution of IL-6 (Vozarova *et al.* 2003) and of PPAR-γ2 (Ochoa *et al.* 2004) polymorphisms in agreement with our observations.

Based on these concordances, we assumed the sample as representative and evaluated the potential effect of both polymorphisms on body composition and on the slimming process. Initial weight, which could be an important factor in the response to the treatment, was not a determinant variable in the present study. This observation could be due to the homogeneous sample studied. Indeed, body weight was uniformly distributed between genetic variants and adiposity was similar in all the included subjects at baseline.

According to previously published reports (Lindi *et al.* 2001; Lieb *et al.* 2004), no differences were observed at baseline on body composition or on the weight-loss process. In contrast, other studies have suggested a role for IL-6 (Kubaszek *et al.* 2003; Sesti *et al.* 2005) and PPAR-γ2 (Deeb *et al.* 1998; Lindi *et al.* 2002) on body-weight homeostasis. For the IL-6 polymorphism, most of the published articles have related the C allele with a higher BMI (Wernstedt *et al.* 2004), lower energy expenditure (Kubaszek *et al.* 2003) and a worse response to weight loss (Sesti *et al.* 2005). On the contrary, the G allele has been related to higher values of some parameters associated with obesity (Dedoussis *et al.* 2004).

Table 3. Differences in frequency distribution of the subjects taking into account the genotypes and the criterion of 10 % weight maintenance 1 year after the end of the nutritional intervention

Gene variants	Subjects with weight-loss maintenance		Subjects with weight regain		Fisher's exact test (*P* value)
	n	%	*n*	%	
IL-6 frequency distribution					
GG (*n* 21)	4	16	17	40	0·032
GC + CC (*n* 46)	21	84	25	60	
PPAR-γ2 frequency distribution					
ProPro (*n* 52)	17	68	35	83	0·125
ProAla + AlaAla (*n* 15)	8	32	7	17	

Table 4. Logistic regression analysis*

	Odds ratio	95 % CI	*P*
Model 1			
IL-6 genotype only	0·24	0·058, 0·995	0·049
Model 2			
IL-6 and PPAR-γ2	0·19	0·037, 0·946	0·043

* The protective effect of the C allele on weight maintenance is shown in model 1. Analysis of model 2 showed an association of C and Ala genotypes in the protection against weight regain. IL-6 model group genotypes were encoded as 0 = C − and 1 = C + ; IL-6 and PPAR-γ2 model group combination genotypes were encoded as 0 = C− Ala− and 1 = C + Ala + . None of the variables studied (age, sex and baseline body weight), instead of polymorphisms, were predictors of the weight maintenance, so they were not included in the logistic model.

In the case of the PPAR-γ2 Ala allele, it has been associated with both higher (Beamer *et al.* 1998) and lower (Deeb *et al.* 1998) BMI. This polymorphism has been mostly associated with insulin resistance, although some contradictory results are found in the literature (Mancini *et al.* 1999).

Reasons for the discrepancies in the effects of both variants may be due to differences in populations, ethnic aspects and applied treatments, as well as the design of the weight-loss intervention programme. Moreover, interactions between genetic heterogeneity and environmental factors may be taken into account to adequately interpret the outcome of these trials (Marti *et al.* 2004).

In order to test the effect of the polymorphisms on weight control, the volunteers were studied depending on success in weight maintenance 1 year after the end of the nutritional intervention. Based on the criterion of Weinsier *et al.* (2002), a success in weight maintenance was considered using the cut-off of $< 10\,\%$ weight regain. This criterion was previously applied to evaluate the relationship between weight maintenance and polymorphisms, such as glucocorticoid receptor, ciliary neurotrophic factor and PPAR-γ2 polymorphisms (Vogels *et al.* 2005*b*). Thus, we found that the frequency of the C allele of the IL-6 polymorphism was evidently higher in subjects with successful weight maintenance, in which the measured pro-inflammatory markers were lower. This fact could be associated with a better response to weight maintenance, considering the recently described association between a high pro-inflammatory status and the risk of obesity and the development of its co-morbidities (Moreno-Aliaga *et al.* 2005).

As mentioned earlier, some studies have found a participation of the -174G > C polymorphism on weight loss (Poitou *et al.* 2005; Sesti *et al.* 2005), although the association of this variant with long-term weight maintenance has apparently not been studied yet. The present study evidenced a protective effect of the IL-6 C allele against weight regain after a weight loss induced by a LED. Functional studies have not fully elucidated the biological role of the -174G > C substitution, but the involvement of IL-6 on weight regulation, and specifically on weight maintenance, could be partially explained by the relationship between IL-6 and the inflammation status of the adipocyte, which could affect weight control (Mohamed-Ali *et al.* 1997). On the other hand, the central effect of IL-6 on energy expenditure regulation could also affect weight control (Kubaszek *et al.* 2003).

Concerning the PPAR-γ2 polymorphism, previous studies have analysed the effect of carrying the variants on weight regulation. Prospective and intervention studies have shown that the AlaAla genotype is associated with weight regain after a diet-induced slimming (Lindi *et al.* 2002), showing an indirect relationship with weight maintenance (Vogels *et al.* 2005*b*), in agreement with the results that we obtained for this gene variant. Functional studies of the Pro12Ala variant have shown that the Ala allele is associated with a reduced capacity to activate transcription and mediate adipogenesis, as a result of a lower binding affinity and a reduced ability to transactivate promotors (Deeb *et al.* 1998; Masugi *et al.* 2000). Thus, it is possible that weight maintenance may result in less efficient stimulation of PPAR-γ2 target genes in subjects with the Pro allele, causing less adipogenesis and protecting against body-weight regain.

Recently, an interaction between IL-6 and PPAR-γ2 gene polymorphisms and an increased risk to develop obesity has been demonstrated, in which subjects carrying both variants (C + , Ala +) have a less favourable profile to obesity (Barbieri *et al.* 2005). In agreement with these authors and with the perspective of a longitudinal trial, the present study suggests a synergetic effect on protection against weight regain for subjects carrying both variants (C + , Ala +). Furthermore, a different change in fat and fat-free mass was noted depending on the studied polymorphisms, which may be related to a specific effect of these two body components since protein deposition is more energetically expensive than fat accumulation.

At the moment, there is not a clear explanation about the mechanisms by which PPAR-γ2 and IL-6 gene polymorphisms interact in reducing the weight regain. However, weight regain could be attributed to the effect that both genes produce on adipose tissue: IL-6, as a pro-inflammatory cytokine (Fantuzzi, 2005) and PPAR-γ as a transcription factor involved in adipogenesis and in the regulation of adipocyte gene expression (Wu *et al.* 1995). Furthermore, these single gene variant mutations could act through convergent pathways to produce synergetic effects on weight regulation (Barbieri *et al.* 2005). In fact, PPAR-γ activators have been shown to directly inhibit the activation of IL-6 and other cytokines by negatively interfering with NF-κB, STAT and AP-1 signalling pathways (Jiang *et al.* 1998; Ricote *et al.* 1998).

One limiting aspect of the present study could be the sample size, which is comparable with other long-term intervention trials (Nicklas *et al.* 2001; Halverstadt *et al.* 2005), but the fact that statistical differences were found suggests that the statistical error type β is not occurring (Lachin, 2005).

In conclusion, the present study demonstrates that the C allele of the IL-6 -174 G > C polymorphism gives protection against weight regain after obesity nutritional therapy, and the presence of the Ala allele of the PPAR-γ2 together with the C allele strengthens the weight-maintenance status. These observations could contribute in providing knowledge for diagnosis and designing specific nutritional treatment of obesity based upon the individual genotype.

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References

Abeke I, Parra MD & Martinez JA (2006) Different dietary strategies for weight less in obesity: role of energy and macronutrient content. *Nutr Res Rev* **19**, 1–14.

Aronne LJ (1998) Obesity. *Med Clin North Am* **82**, 161–181.

Auwerx J (1999) PPARgamma, the ultimate thrifty gene. *Diabetologia* **42**, 1033–1049.

Barbieri M, Rizzo MR, Papa M, Acampora R, De Angelis L, Olivieri F, Marchegiani F, Franceschi C & Paolisso G (2005) Role of interaction between variants in the PPARG and interleukin-6 genes on obesity related metabolic risk factors. *Exp Gerontol* **40**, 599–604.

Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ, Andres R, Roth J & Shuldiner AR (1998) Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-gamma2 gene with obesity in two Caucasian populations. *Diabetes* **47**, 1806–1808.

Berthier MT, Paradis AM, Tchernof A, Bergeron J, Prud'homme D, Despres JP & Vohl MC (2003) The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. *J Hum Genet* **48**, 14–19.

Dedoussis GV, Manios Y, Choumerianou DM, Yiannakouris N, Panagiotakos DB, Skenderi K & Zampelas A (2004) The IL-6 gene G-174C polymorphism related to health indices in Greek primary school children. *Obes Res* **12**, 1037–1041.

Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W & Auwerx J (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* **20**, 284–287.

Ek J, Urhammer SA, Sorensen TI, Andersen T, Auwerx J & Pedersen O (1999) Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* **42**, 892–895.

Fantuzzi G (2005) Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* **115**, 911–919.

Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, Richart C & Ricart W (2000*a*) Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes* **49**, 517–520.

Fernandez-Real JM, Broch M, Vendrell J, Richart C & Ricart W (2000*b*) Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. *J Clin Endocrinol Metab* **85**, 1334–1339.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S & Woo P (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* **102**, 1369–1376.

Goyenechea E, Parra MD & Martinez JA (2005) Role of IL-6 and its -174G > C polymorphism in weight management and in the metabolic comorbidities associated with obesity. *An Sist Sanit Navar* **28**, 357–366.

Greenwald A (2006) Current nutritional treatments of obesity. *Adv Psychosom Med* **27**, 24–41.

Halverstadt A, Phares DA, Roth S, Ferrell RE, Goldberg AP & Hagberg JM (2005) Interleukin-6 genotype is associated with high-density lipoprotein cholesterol responses to exercise training. *Biochim Biophys Acta* **1734**, 143–151.

Hamid YH, Rose CS, Urhammer SA, *et al.* (2005) Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* **48**, 251–260.

Hara K, Kubota N, Tobe K, *et al.* (2000) The role of PPARγ as a thrifty gene both in mice and humans. *Br J Nutr 84,* Suppl. **2**, S235–S239.

Humphries SE, Luong LA, Ogg MS, Hawe E & Miller GJ (2001) The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. *Eur Heart J* **22**, 2243–2252.

Jiang C, Ting AT & Seed B (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* **391**, 82–86.

Kubaszek A, Pihlajamaki J, Punnonen K, Karhapaa P, Vauhkonen I & Laakso M (2003) The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. *Diabetes* **52**, 558–561.

Labayen I, Diez N, Parra D, Gonzalez A & Martinez JA (2004) Basal and postprandial substrate oxidation rates in obese women receiving two test meals with different protein content. *Clin Nutr* **23**, 571–578.

Lachin JM (2005) A review of methods for futility stopping based on conditional power. *Stat Med* **24**, 2747–2764.

Lieb W, Pavlik R, Erdmann J, *et al.* (2004) No association of interleukin-6 gene polymorphism (-174 G/C) with myocardial infarction or traditional cardiovascular risk factors. *Int J Cardiol* **97**, 205–212.

Lindi V, Sivenius K, Niskanen L, Laakso M & Uusitupa MI (2001) Effect of the Pro12Ala polymorphism of the PPAR-gamma2 gene on long-term weight change in Finnish non-diabetic subjects. *Diabetologia* **44**, 925–926.

Lindi VI, Uusitupa MI, Lindstrom J, *et al.* (2002) Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* **51**, 2581–2586.

Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, Riccardi G & Colantuoni V (1999) Pro12Ala substitution in the peroxisome proliferator-activated receptor-gamma2 is not associated with type 2 diabetes. *Diabetes* **48**, 1466–1468.

Marti A, Corbalan MS, Martinez-Gonzalez MA, Forga L & Martinez JA (2002) CHO intake alters obesity risk associated with Pro12Ala polymorphism of PPARgamma gene. *J Physiol Biochem* **58**, 219–220.

Marti A, Moreno-Aliaga MJ, Hebebrand J & Martinez JA (2004) Genes, lifestyles and obesity. *Int J Obes Relat Metab Disord* **28**, Suppl. 3, S29–S36.

Martinez JA (2000) Body-weight regulation: causes of obesity. *Proc Nutr Soc* **59**, 337–345.

Martinez-Gonzalez MA, Martinez JA, Hu FB, Gibney MJ & Kearney (1999) Physical inactivity, sedentary lifestyle and obesity in the European Union. *Int J Obes Relat Metab Disord* **23**, 1192–1201.

Masugi J, Tamori Y, Mori H, Koike T & Kasuga M (2000) Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. *Biochem Biophys Res Commun* **268**, 178–182.

Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S & Coppack SW (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* **82**, 4196–4200.

Moreno-Aliaga MJ, Campion J, Milagro FI, Berjón A & Martinez JA (2005) Adiposity and proinflammatory state: the chicken or the egg. *Adipocytes* **1**, 1–16.

Nicklas BJ, van Rossum EF, Berman DM, Ryan AS, Dennis KE & Shuldiner AR (2001) Genetic variation in the peroxisome proliferator-activated receptor-gamma2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* **50**, 2172–2176.

Ochoa MC, Marti A, Azcona C, *et al.* (2004) Gene-gene interaction between PPAR gamma 2 and ADR beta 3 increases obesity risk

in children and adolescents. *Int J Obes Relat Metab Disord* **28**, Suppl. 3, S37–S41.

Parra MD, Martinez de Morentin BE & Martinez JA (2005) Postprandial insulin response and mitochondrial oxidation in obese men nutritionally treated to lose weight. *Eur J Clin Nutr* **59**, 334–340.

Pérez S, Parra MD, Martínez de Morentin B, Rodríguez MC & Martínez JA (2005) Evaluación de la variabilidad intraindividual de la medida de composición corporal mediante bioimpedancia en voluntarias sanas y su relación con el índice de masa corporal y el pliegue tricipital (Evaluation of intraindividual variability in the measurement of body composition using electrical bioimpedance in healthy volunteers and its relationship with body mass index and triceps skinfold measurement). *Enferm Clin* **15**, 343–347.

Poitou C, Lacorte JM, Coupaye M, *et al.* (2005) Relationship between single nucleotide polymorphisms in leptin, IL6 and adiponectin genes and their circulating product in morbidly obese subjects before and after gastric banding surgery. *Obes Surg* **15**, 11–23.

Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Perusse L & Bouchard C (2006) The human obesity gene map: the 2005 update. *Obesity* **14**, 529–644.

Ricote M, Li AC, Willson TM, Kelly CJ & Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* **391**, 79–82.

Sesti G, Perego L, Cardellini M, *et al.* (2005) Impact of common polymorphisms in candidate genes for insulin resistance and obesity on weight loss of morbidly obese subjects after laparoscopic adjustable gastric banding and hypocaloric diet. *J Clin Endocrinol Metab* **90**, 5064–5069.

Trayhurn P & Wood IS (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* **92**, 347–355.

Trayhurn P & Wood IS (2005) Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans* **33**, 1078–1081.

Tsigos C, Papanicolaou DA, Defensor R, Mitsiadis CS, Kyrou I & Chrousos GP (1997) Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. *Neuroendocrinology* **66**, 54–62.

Vogels N, Diepvens K & Westerterp-Plantenga MS (2005*a*) Predictors of long-term weight maintenance. *Obes Res* **13**, 2162–2168.

Vogels N, Mariman EC, Bouwman FG, Kester AD, Diepvens K & Westerterp-Plantenga MS (2005*b*) Relation of weight maintenance and dietary restraint to peroxisome proliferator-activated receptor gamma2, glucocorticoid receptor, and ciliary neurotrophic factor polymorphisms. *Am J Clin Nutr* **82**, 740–746.

Vozarova B, Fernandez-Real JM, Knowler WC, *et al.* (2003) The interleukin-6 (-174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. *Hum Genet* **112**, 409–413.

Weinsier RL, Hunter GR, Desmond RA, Byrne NM, Zuckerman PA & Darnell BE (2002) Free-living activity energy expenditure in women successful and unsuccessful at maintaining a normal body weight. *Am J Clin Nutr* **75**, 499–504.

Wernstedt I, Eriksson AL, Berndtsson A, Hoffstedt J, Skrtic S, Hedner T, Hulten LM, Wiklund O, Ohlsson C & Jansson JO (2004) A common polymorphism in the interleukin-6 gene promoter is associated with overweight. *Int J Obes Relat Metab Disord* **28**, 1272–1279.

Wing RR & Phelan S (2005) Long-term weight loss maintenance. *Am J Clin Nutr* **82**, Suppl. 1, S222–S225.

Wu Z, Xie Y, Bucher NL & Farmer SR (1995) Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. *Genes Dev* **9**, 2350–2363.

Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J & Shuldiner AR (1997) Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* **241**, 270–274.