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ORIGINAL ARTICLE

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Role of the *rs10401670* variant in the resistin gene on the metabolic response after weight loss secondary to a high-fat hypocaloric diet with a Mediterranean pattern

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Abstract

Background: The single nucleotide polymorphism (SNP) (*rs10401670*) of the *RETN* gene has been associated with metabolic disorder in obese subjects and has scarcely been evaluated after dietary interventions. The present study aimed to analyse the effects of the *rs10401670 RETN* gene polymorphism on metabolic changes secondary to weight loss and secondary to a high-fat hypocaloric diet with a Mediterranean dietary pattern.

Methods: A Caucasian population comprising 284 obese patients without diabetes mellitus was analysed. Before and after 3 months of a high-fat hypocaloric diet with a Mediterranean pattern, an anthropometric evaluation, an assessment of nutritional intake and a biochemical analysis were performed. A statistical analysis was conducted for the combined *CT* and *TT* as a group and for wild-type *CC* as a second group.

Results: Decreases in weight, body mass index (BMI), fat mass, systolic blood pressure and waist circumference were similar in both genotypes groups. In *T* allele carriers, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), triglycerides and C-reactive protein levels were decreased. The decrease in these parameters was statistically significant for triglycerides (-22.3 ± 9.3 mg dl^{-1}: $p = 0.03$), C-reactive protein (-2.8 ± 0.5 mg dl^{-1}: $p = 0.03$), insulin (-7.4 ± 2.9 mUI L^{-1}: $p = 0.03$) and HOMA-IR (-2.4 ± 1.0: $p = 0.02$). Leptin levels were decreased in both genotypes groups after the hypocaloric diet, as well as the anthropometric parameters BMI, weight, waist circumference and fat mass. Resistin and adiponectin levels remained unchanged in both groups.

Conclusions: In the present study, we have detected a significant association between the *T* allele of this SNP and a better response of insulin resistance, triglycerides and C-reactive protein compared to non *T* allele carriers after weight loss with a high-fat hypocaloric diet and a Mediterranean diet.

KEYWORDS

high-fat hypocaloric diet, insulin resistance, resistin, *rs10401670* gene variant

Key points

- In *T* allele carriers of the *rs10401670* variant, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), triglycerides and C-reactive protein levels were decreased.

- Leptin levels were decreased in both genotypes groups (carriers and non *T* allele carriers) after the hypocaloric diet, as well as the anthropometric parameters body mass index, weight, waist circumference and fat mass.
- Resistin and adiponectin levels remained unchanged in both groups.

INTRODUCTION

Resistin is an adipokine that produces insulin resistance in rodent models,[1] and it is secreted by adipocytes and macrophages in adipose tissue and liver. In humans, its role in insulin resistance remains controversial. Resistin was identified as a gene for which the expression is induced by adipocyte differentiation and inhibited by peroxisome proliferator-activated receptor ligands in 3T3-L1 cells.[2] Serum resistin levels are associated with increased obesity, visceral fat,[3] metabolic syndrome[4] and type 2 diabetes mellitus,[5] whereas other studies have failed to observe such metabolic associations.[6,7]

The gene encoding resistin (*RETN*) is located on chromosome 19p13 and many studies have reported genetic variants in *RETN*.[8–12] In Caucasians, up to 70% of the variations in serum resistin can be explained by genetic factors.[8] For example, some single nucleotide polymorphisms (SNPs) in *RETN* have been associated with indices of insulin resistance.[9,10] The SNP 3′UTR C/T (*rs10401670*) is a genetic variant that has been associated with diabetes mellitus in the Framingham Offspring Study.[11] Ortega et al.[12] have described an association between *rs10401670* and low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-C levels. Despite the obvious relationships with metabolic parameters of this polymorphism in obese subjects, few studies have evaluated the effect of weight reduction in these variables and related it to the *rs10401670* variant of *RETN*. One study has shown,[13] after a bariatric intervention with biliopancreatic diversion, an average weight loss of 41 kg in 1 year, with resistin levels changing depending on genotypes and also on the improvement of insulin and homeostatic model assessment for insulin resistance (HOMA-IR). Moreover, after a dietary intervention with a standard hypocaloric diet for 3 months, there were no differences in resistin levels but, again, there were differences in the modifications of insulin and HOMA-IR levels as a function of genotype after an average weight loss of 3.5 kg.[14]

The present study aimed to analyse the effects of the *rs10401670 RETN* gene polymorphism on metabolic changes secondary to weight loss and secondary to a high-fat hypocaloric diet with a Mediterranean dietary pattern.

METHODS

Subjects

We enrolled, in a prospective way, a sample of 284 adults comprising obese, non-diabetic Caucasian outpatients. Adult obesity was defined by a body mass index (BMI) $\geq 30\ kg\ m^{-2}$. The recruitment of subjects was carried out with a consecutive method of sampling among patients with obesity who were sent from primary care physicians. All participants provided their written informed consent to a protocol that had been approved by the local ethical review board. The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki. The local ethics committee approved all of the procedures involving patients.

Inclusion criteria were age >18 years, BMI $> 30\ kg\ m^{-2}$ and the absence of dieting during the 6 months previous to the study. Exclusion criteria included: history of cardiovascular disease or stroke during the previous 24 months, total cholesterol $> 200\ mg\ dl^{-1}$, triglycerides $> 250\ mg\ dl^{-1}$, blood pressure > 140/90 mmHg and fasting plasma glucose $> 126\ mg\ dl^{-1}$, as well as the use of metformin, sulphonilurea, dypeptidil type IV inhibitor drugs, thiazolidinedions, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs.

Biochemical and anthropometric procedures

Ragarding biochemical parameters, fasting (12 h) venous blood samples were obtained by venipuncture and collected in Vacutainer tubes (Becton Dickinson). Basal fasting glucose, C-reactive protein (CRP), insulin, insulin resistance (HOMA-IR), and the lipid profile composed of total cholesterol, LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration and basal serum adipokines levels (leptin, adiponectin and resistin) were measured within the basal time of the trial and repeated after 3 months of follow-up. The *rs10401670* variant of *RETN* gene was evaluated.

Regarding anthropometric parameters, a bioimpedance analysis was conducted to measure fat mass. Weight, height and blood pressure measures were measured at the start of the trail and repeated after 3 months of intervention. These measurements were carried out at

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same time of the day (morning). Systolic and diastolic blood pressure were also measured.

Genotyping of the *rs10401670 RETN* gene polymorphism

Genomic DNA was obtained from peripheral mononuclear blood cells. A real-time-polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 µl of 100 µM of each oligonucleotide primer (primer forward: 5′-ACGTTGGATGGCTGTTGACGTGCTA ATGAG-3′ and reverse 5′-ACGTTGGATGAGCCA CCCTCAGCGATCTAA-3′), 0.25 µl of 10 µM probes (wild probe: 5′-Fam-TAT ACA CAC GGG CTG ACC TGA-Tamra-3′ and mutant probe: 5′-Hex-CTT ATA CAC ACA GGC TGA CCT GA- Tamra-3′), 0.5 µl of iScript reverse transcriptase, 6.25 µl of nuclease free water and 5 µl of nucleic acid extract. The oligonucleotide primers and probes were designed with Beacon Designer 5.0 (Premier Biosoft International). During the PCR, DNA was denaturated at 95°C for 3 min; this was followed by 45 cycles at 95°C for 15 s, and annealing at 59.3°C for 45 s, with an extension step of 60°C for 5 min with hot start Taq DNA polymerase. Thermocycler software (CFX Opus Real-time PCR System [Biorad, CA, LA, USA]) was used to classify each patient as wild-type homozygous (*CC*), heterozygous (*CT*) and risk homozygous (*TT*).

Biochemical parameters

Regarding lipid profile, serum total cholesterol and triglyceride concentrations were determined by an enzymatic colorimetric assay (Technicon Instruments, Ltd), whereas HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using the Friedewald formula.[15]

Fasting plasma glucose levels were determined using an automated glucose oxidase method (Glucose Analyser 2; Beckman Instruments). Insulin was measured using a radioimmunoassay (RIA Diagnostic Corporation) with a sensitivity of 0.5 mUI L^{-1} (normal range 0.5–30 mUI L^{-1})[16] and the homeostasis model assessment for insulin sensitivity (HOMA-R) was calculated using these values[17] and the equation: fasting plasma insulin (mU L^{-1}) × glucose (mmol L^{-1})/22.5.

Regarding adipokine determination, resistin was measured using an enzyme-linked immunosorbent assay (ELISA) (Biovendor Laboratory, Inc.) with a sensitivity of 0.2 ng ml^{-1} and a normal range of 4–12 ng ml^{-1}.[18] Leptin was measured using an ELISA (Diagnostic Systems Laboratories, Inc.) with a sensitivity of 0.05 ng ml^{-1} and a normal range of 10–100 ng ml^{-1}.[19] Adiponectin was measured using an ELISA (R&D Systems, Inc.) with a sensitivity of 0.246 ng ml^{-1} and a normal range of 8.65–21.43 ng ml^{-1}.[20]

Adiposity parameters and blood pressure

For body composition, body weight was measured to an accuracy of 0.1 kg and BMI was computed as body weight in kg/height in m^2 (kg m^{-2}). Waist circumference was determined at the narrowest diameter between the xiphoid process and iliac crest. Bioimpedance was used to measure body composition with an accuracy of 50 g[21] (BIA 101 EFG; Akern). Blood pressure was determined three times after a 10-min rest with a random zero mercury sphygmomanometer (Omrom) and then averaged.

Nutritional intervention

The present study was designed to achieve a calorie reduction of 500 calories daily compatred to the usual intake. The subjects during this interventional study (12 weeks) received individualised counselling on a high-fat hypocaloric diet with a Mediterranean profile and physical exercise. At baseline, the dietary habits of subjects were determined using 7-day food records. Tables and images comprising color photographs of food were used to illustrate the intervention with a Mediterranean dietary pattern, including legumes, vegetables, poultry, whole grains, fish, fresh fruit and olive oil, and limiting unhealthy fats such as margarines, fatty meats, snacks and industrially made pastries.[22] The prescribed percentage of macronutrients was 35% carbohydrates, 41% fats and 24% proteins. Percentage of fats was 60.0% monounsaturated fats, 25.0% saturated fats and 15.0% polyunsaturated fats. All participants had three educational sessions (90 min with diet sheets and example menu plans) at the start of the trial to explain the dietary intervention and solve doubts. The same dietitian assessed the completion of the diet each 10 days via a phone call. All enrolled subjects received instruction to record their daily dietary intake for 7 days including a weekend day, before the dietary intervention and after 3 months. Records were analysed with a computer-based data evaluation system (Dietosource®) with national composition food tables being used as a reference.[22] The recommended physical exercise program consisted of an aerobic exercise at least three times per week (60 min each, reaching a total of 180 min each week) and the patient recorded this using a self-reported questionnaire

Statistical analysis

Sample size was assesed to detect differences over 4 kg in body weight after diets with 90% power and 5% significance ($n = 280$). The Kolmogorov–Smirnov test was used to determine variable distribution. The data are reported as the mean ± SD. Numerical variables with a

normal distribution were analysed with a two-tailed Student's *t* test. Categorical variables were evaluated with a chi-squared test, with Yates correction as necessary. Non-parametric variables were analysed with the Mann–Whitney *U* test. The differences in anthropometric and biochemical variables between the genotype groups were tested with analysis of the covariance, adjusting for age and sex. The statistical analysis was performed for the combined C*T* and *TT* genotypes as a group (risk genotype) and the *CC* genotype as a second group (wild genotype) in a dominant model. $p < 0.05$ was considered statistically significant. All analysis was conducted using SPSS, version 23.0 (IBM Corp.).

RESULTS

Two hundred and eighty-four patients provided their informed consent and were included in the study. All patients completed the 3-month follow-up period without dropping out. The mean ± SD age was 52.9 ± 8.3 years and the mean ± SD BMI was 36.5 ± 4.2, with 76 males (26.8%) and 208 females (73.2%). Ninety-nine patients (34.9%) had the genotype *CC* (major allele group) and 185 (65.1%) patients had the other genotypes: *CT* (148 patients, 52.1%) or *TT* (37 patients, 13.0%) (minor allele group). Hardy–Weinberg equilibrium was assessed with a chi-squared test to compare our expected and observed counts. This genetic variant was in Hardy–Weinberg equilibrium ($p = 0.21$).

Average ages were similar in both genotypes groups (major allele group: 53.1 ± 9.0 years vs. minor allele group: 52.7 ± 8.1 years; not significant). The sex distribution was similar in both groups, males (21.2% vs. 29.7%) and females (78.7% vs. 70.3%).

Following the food recommendations and sessions of the dietitian, both groups (as indicated in the Methods) reached the dietary recommendations. The total caloric amount was similar in both genotypes groups (*CC* vs. *CT* + *TT*) (1550.2 ± 191.9 calories vs. 1497.8 ± 220.2; not significant). The distribution of macronutrients in both groups (*CC* vs. *CT* + *TT*) was also similar for carbohydrates (35.9 ± 3.3% vs. 36.0 ± 2.5%; $p = 0.28$), fats (41.1 ± 3.3% vs. 40.9 ± 2.9%; $p = 0.41$) and proteins (23.0 ± 2.6% vs. 22.1 ± 2.3%; $p = 0.13$). Finally, the distribution of dietary fats in both groups (*CC* vs. *CT* + *TT*) was similar for monounsaturated fats (60.0 ± 4.1% vs. 59.5 ± 4.9%; $p = 0.41$), saturated fats (24.9 ± 3.1% vs. 25.3 ± 2.9%; $p = 0.31$) and polyunsaturated fats (16.1 ± 1.3% vs. 15.2 ± 1.9%; $p = 0.43$).

The modifications in anthropometric parameters and blood pressure are shown in Table 1. After a high-fat hypocaloric diet with a Mediterranean pattern, weight, BMI, fat mass, systolic blood pressure and waist circumference decreases were similar in both genotypes groups, without any statistical differences. In the *CC* group, the decrease in weight was −3.1 ± 1.2 kg (decrease in *T* allele carriers −3.5 ± 1.5 kg; $p = 0.49$), the decrease in BMI was −2 to 0 ± 0.5 kg m^{-2} (decrease in *T* allele carriers −1.9 ± 0.6 kg m^{-2}; $p = 0.39$), the decrease in fat mass was −3.0 ± 1.1 kg (decrease in *T* allele carriers −2.9 ± 1.2 kg; $p = 0.31$) and the decrease in waist circumference was −5.6 ± 2.4 cm (decrease in *T* allele carriers −5.9 ± 2.1 cm; $p = 0.39$). In non *T* allele carriers, the decrease in systolic blood pressure was −6.6 ± 3.9 mmHg (decrease in non *T* allele carriers −6.1 ± 2.9 mmHg; $p = 0.24$). No differences were detected in diastolic blood pressure after dietary intervention. Finally, no differences were detected among basal and post-treatment values of anthropometric parameters between both genotypes groups *CC* vs. *CT*/*TT*.

We report the biochemical parameters in Table 2. The decrease in biochemical variables was not significant in patients with the *CC* genotype. In *T* allele carriers, insulin, HOMA-IR, triglycerides and CRP levels decreased. The decrease of these parameters was statistically significant for triglycerides (−22.3 ± 9.3 mg dl^{-1}; $p = 0.03$), CRP (−2.8 ± 0.5 mg dl^{-1}; $p = 0.03$), insulin −7.4 ± 2.9 mUI L^{-1}; $p = 0.03$) and HOMA-IR (−2.4 ± 1.0; $p = 0.02$). Finally, no statistical differences were detected among the basal and post-treatment values of variables between major allele genotype *CC* and minor allele genotype (*CT* + *TT*).

Table 3 shows the levels of serum adipokines. Leptin levels decreases in both genotypes groups after the hypocaloric diet (−23.3 ± 9.5 ng dl^{-1} in non T allele carriers vs. −19.0 ± 8.2 ng dl^{-1} in T allele carriers; $p > 0.05$). Resistin and adiponectin levels remained unchanged in both groups. No differences were detected among the basal and post-treatment values of adipokines between both genotypes groups *CC* vs. *CT*/*TT*.

DISCUSSION

Despite the importance of adipose tissue, as a result of its secretion of adipocytokines with multiple biological actions, and especially resistin,[1,2] studies that evaluate the effects of weight loss in obese patients and different polymorphisms of the *RETN* gene are rare. In our design analysing the *rs10401670* variant of the *RETN* gene, we detected a significant association between the *T* allele of this SNP and a better response of insulin resistance, triglycerides and CRP compared to non-carriers after weight loss with a high-fat hypocaloric diet and a Mediterranean diet.

The previous investigations into the role of resistin on cardiovascular parameters are contradictory. Some studies have reported that resistin levels were related to obesity and insulin resistance[2,23] and other studies did not detect associations between resistin levels and metabolic parameters.[24,25] Furthermore, the effect of weight loss on resistin levels is also an area with conflicting results. Santoro et al.[26] reported a reduction in resistin levels after weight loss secondary to an omentectomy

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TABLE 1 Basal and post-intervention antropometric parameters of obesity and blood pressure measurement (mean ± SD)

Parameters	*CC* (*n* = 99)		*CT* + *TT* (*n* = 148)		*p* values
	Basal	3 months	Basal	3 months	− Time *CC* − Basal genotype − Time *CT* + *TT* − 3 months genotype
BMI	36.6 ± 6.0	34.6 ± 5.2[a]	36.5 ± 5.4	34.6 ± 5.2[a]	*p* = 0.01 *p* = 0.36 *p* = 0.02 *p* = 0.39
Weight (kg)	92.2 ± 11.6	88.1 ± 9.9[b]	93.9 ± 6.2	90.4 ± 6.3[b]	*p* = 0.02 *p* = 0.40 *p* = 0.03 *p* = 0.49
Fat mass (kg)	40.8 ± 8.2	37.8 ± 7.1[c]	39.8 ± 6.2	36.9 ± 5.9[c]	*p* = 0.02 *p* = 0.28 *p* = 0.02 *p* = 0.31
WC (cm)	108.4 ± 12.1	102.8 ± 10.1[d]	109.2 ± 9.9	103.3 ± 7.1[d]	*p* = 0.03 *p* = 0.41 *p* = 0.04 *p* = 0.49
SBP (mmHg)	127.9 ± 9.2	121.3 ± 8.1[e]	128.7 ± 5.2	122.6 ± 5.8[e]	*p* = 0.01 *p* = 0.37 *p* = 0.01 *p* = 0.42
DBP (mmHg)	83.3 ± 7.1	81.0 ± 4.1	81.9 ± 6.1	80.4 ± 5.2	*p* = 0.51 *p* = 0.60 *p* = 0.61 *p* = 0.52

Note: First *p*, significance of dietary intervention after 12 weeks in *CC* genotype, second *p*, significance between *CC* genotypes vs. *CT* + *TT* baseline values, third *p*, significance of dietary intervention after 12 weeks in *CT* + *TT* genotype, fourth *p*, significance between *CC* genotypes vs. *CT* + *TT* post-treatment values. Statistical differences: $p < 0.05$, in each genotype group ([a]BMI; [b]weight; [c]fat mass; [d]WC; [e]SBP).

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.

plus enterectomy. Similar results were reported after a biliopancreatic diversion[13] and gastric bypass.[27] Whithson et al.[28] reported an increase of resistin levels after weight loss with bariatric techniques. In addition, Moschen et al.[29] demonstrated a biphasic response after adjustable gastric banding, with a decrease in resistin levels initially and an increase at 12 months of follow-up. Lastly, studies have been conducted with caloric restrictions[14] in which, after a weight loss that was not as high as that previously achieved with bariatric surgery, the levels of resistin were not changed. Some dietary intervention studies have demonstrated a decrease of resistin levels after weight loss[30] or a contradictory increase of serum levels after weight loss.[31] Moreover, Cabrera et al.[32] showed that, in the general population, the resistin level is positively associated with saturated fat intake and inversely associated with monounsaturated fat intake.

To the best of our knowledge, this is the second study to analyse the effects of a caloric restriction and the *RETN* gene variant *rs10401670* on body weight loss and subsequent changes of metabolic parameters. In a previous study[14] with a standard low-calorie diet of 1500 calories and a macronutrient distribution of 52% of calories in the form of carbohydrates, 25% in the form of lipids (50% monounsaturated fats) and 23% in the form of proteins, the presence of the *T* allele was shown to produce a better response in insulin and HOMA-IR levels. These results are similar to those obtained in our present study with a diet that reaches the same caloric restriction (approximately 1500 calories), but with a fat percentage of 41% with 60% monounsaturated fats. Moreover, in the present study, we also show a greater decrease in triglyceride and CRP levels in *T* allele carriers. Our study did not reveal a relationship between this polymorphism and resistin levels. Moreover, a previous study that analysed the effect of the *rs10401670*[11] polymorphism reported a strong association between the minor allele and higher resistin levels. These contradictory results regarding the relationship of resistin levels

TABLE 2 Basal and post-intervention levels biochemcial parameters (mean ± SD)

Parameters	*CC* (*n* = 99)		*CT* + *TT* (*n* = 148)		*p* values
	Basal	3 months	Basal	3 months	− Time *CC* − Basal genotype − Time *CT* + *TT* − 3 months genotype
Glucose (mg dl^{-1})	100.2 ± 9.1	97.1 ± 8.1	100.7 ± 8.2	96.1 ± 9.3	$p = 0.12$ $p = 0.31$ $p = 0.19$ $p = 0.49$
Total cholesterol (mg dl^{-1})	197.1 ± 20.7	189.2 ± 13.2	205.2 ± 23.1	190.4 ± 16.2	$p = 0.13$ $p = 0.50$ $p = 0.01$ $p = 0.36$
LDL-cholesterol (mg dl^{-1})	123.6 ± 18.3	112.1 ± 12.1	121.2 ± 9.1	110.1 ± 8.0	$p = 0.12$ $p = 0.49$ $p = 0.11$ $p = 0.16$
HDL-cholesterol (mg dl^{-1})	53.7 ± 4.1	52.1 ± 6.2	54.6 ± 5.0	53.8 ± 3.1	$p = 0.22$ $p = 0.45$ $p = 0.53$ $p = 0.44$
Triglycerides (mg dl^{-1})	110.6 ± 21.9	109.7 ± 16.4	119.1 ± 13.2	98.8 ± 10.2[a]	$p = 0.12$ $p = 0.61$ $p = 0.03$ $p = 0.45$
Insulin (mUI L^{-1})	14.2 ± 6.1	13.5 ± 4.1	18.4 ± 3.2	11.0 ± 4.7[b]	$p = 0.22$ $p = 0.31$ $p = 0.03$ $p = 0.41$
HOMA-IR	3.6 ± 1.1	3.4 ± 1.0	5.4 ± 1.0	3.0 ± 0.9[c]	$p = 0.33$ $p = 0.35$ $p = 0.02$ $p = 0.49$
CRP	6.5 ± 2.1	6.9 ± 1.1	6.6 ± 1.8	3.8 ± 1.1[d]	$p = 0.22$ $p = 0.39$ $p = 0.03$ $p = 0.05$

Note: First *p*, significance of dietary intervention after 12 weeks in *CC* genotype, second *p*, significance between *CC* genotypes vs. *CT* + *TT* baseline values, third *p*, significance of dietary intervention after 12 weeks in *CT* + *TT* genotype, fourth *p*, significance between *CC* genotypes vs. *CT* + *TT* post-treatment values. Statistical differences: $p < 0.05$, in each genotype group ([a]triglicerides; [b]insulin; [c]HOMA-IR; [d]CRP).

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment; LDL, low-density lipoprotein.

with this polymorphism may be a result of different genetic disequilibrium linkages or other unknown genetic and environmental variables.

An interesting result of our work is the best response for HOMA-IR and insulin levels in *T* allele carriers. These results were already observed in a previous study,[14] although, in the present study, the improvement is still greater. This may be a result of the greater amount of monounsaturated fats in our diet and the effect of olive oil consumption on these markers.[33] This better metabolic response in *T* allele carriers could be secondary to a potential interaction of transcriptors of the *rs10401670* variant in the *RETN* gene and glucose pathways because this genetic variant is located in an intron of *MCEMP1* (mast cell expressed membrane protein 1). This gene encode a protein with a single transmembrane domain expressed mainly by mast cells lines and monocytes.[34] This could also explain our findings for inflammatory markers such as CRP. Because resistin is mainly expressed by macrophages that evolve from monocytes in adipose tissue, it would be interesting to determine whether MCEMP1 and its product are functionally influenced by *rs10401670* or the SNPs in its 5_region and whether this protein is

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TABLE 3 Basal and post-intervention levels of serum adipokines (mean ± SD)

Parameters	*CC* (*n* = 99)		*CT* + *TT* (*n* = 148)		*p* values
	Basal	3 months	Basal	3 months	− Time *CC* − Basal genotype − Time *CT* + *TT* − 3 months genotype
Resistin (ng dl^{-1})	3.6 ± 1.5	3.7 ± 1.3	3.9 ± 1.1	2.8 ± 0.9	$p = 0.41$ $p = 0.69$ $p = 0.16$ $p = 0.49$
Adiponectin (ng dl^{-1})	33.1 ± 9.1	34.2 ± 8.1	30.1 ± 7.9	32.3 ± 3.2	$p = 0.21$ $p = 0.52$ $p = 0.21$ $p = 0.51$
Leptin (ng dl^{-1})	93.5 ± 11.6	70.2 ± 12.5[a]	93.8 ± 10.1	64.8 ± 9.1[a]	$p = 0.02$ $p = 0.41$ $p = 0.02$ $p = 0.46$

Note: First *p*, significance of dietary intervention after 12 weeks in *CC* genotype; second *p*, significance between *CC* genotypes vs. *CT* + *TT* baseline values; third *p*, significance of dietary intervention after 12 weeks in *CT* + *TT* genotype; fourth *p*, significance between *CC* genotypes vs. *CT* + *TT* post-treatment values. Statistical differences: $p < 0.05$ in each genotype group ([a]leptin).

involved in glucose metabolism and the inflammatory processes. For example, there are indications that resistin is involved in the pathogenesis of other inflammatory states such as rheumatoid arthritis. Resistin has been found in the plasma and synovial fluid of rheumatoid arthritis patients.[35] Qi et al.[36] reported an association of resistin with inflammatory markers and fibrinolytic markers such as fibrinogen, CRP and plasminogen activator inhibitor.

The observed improvement in triglyceride levels may also be related to the improvement in the inflammatory status of patients observed after weight loss in *T* allele carriers.[37] Previously, Ortega et al.[12] reported an association between *rs10401670* and LDL-cholesterol levels in 12–16-year-old boys, as well as between the polymorphism and HDL-cholesterol levels in girls. The relationship with both types of lipids has different pathophysiological bases.

There are some limitations to the present study. First, only one SNP in the *RETN* gene has been evaluated, whereas several others could be related to the metabolic parameters. Second, there is the lack of a control group without dietary intervention with which to compare the effect of weight loss. Finally, the short duration of the clinical trial does not allow us to observe what would happen to the resistance levels over a longer period.

In conclusion, we describe an association of the *rs10401670T* allele with a better metabolic response (insulin, HOMA-IR, trygliceride and CRP) secondary to weight loss after a high-fat hypocaloric diet with a Mediterranean pattern. However, further studies are necessary to confirm our results and to explore the effect of new dietary interventions,[37] taking into account a possible functional variant of the *RETN* gene.[38,39]

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (HVUVA committee 2/2018) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS

Daniel Antonio de Luis designed the study and wrote article. Rocío Aller and Olatz Izaola conducted the nutritional intervention. David Primo conducted the laboratory analysis.

TRANSPARENCY DECLARATION

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted.

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