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Serum lipid and adiponectin/leptin ratio changes after a Mediterranean dietary pattern in non-g-allele carriers of the genetic variant of adiponectin gene rs822393

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Abstract. – OBJECTIVE: One common genetic variant rs822393 (-4522C/T) is located in the proximal promoter region of the *ADIPOQ* gene. The SNP rs822393 regulates adiponectin promoter activity and is associated with hypoadiponectinemia. The aim of our study was to analyze the effects after a hypocaloric diet with Mediterranean diet pattern on serum lipid and adipokine levels taking to account rs822393 of *ADIPOQ*.

PATIENTS AND METHODS: A population of 270 obese patients was enrolled. Anthropometric parameter and serum parameters (lipid profile, insulin, homeostasis model assessment (HOMA-IR), glucose, C reactive protein, adiponectin, resistin and leptin levels) were measured, at basal time and after 3 months. All patients were genotyped in the rs822393 polymorphism.

RESULTS: The genotype distribution was: 160 patients (59.3%) CC, 96 patients CT (35.6%) and 14 patients CT (5.1%). After dietary intervention, BMI, weight, fat mass, waist circumference, systolic blood pressure, insulin levels, HOMA-IR, total cholesterol and LDL- cholesterol improved significantly in both genotypes. After dietary intervention (CC *vs.* CT+TT), HDL-cholesterol (delta: 5.4±1.4 mg/dl *vs.* -1.8±0.7 mg/dl; *p*=0.03), serum adiponectin (delta: 21.2±4.1 ng/dl *vs.* 3.8±3.3 ng/dl; *p*=0.02) and adiponectin/leptin ratio (delta: 0.53±0.1 *vs.* 0.16±0.3 ng/dl; *p*=0.02) improved only in non-T allele carriers. Basal and post-intervention HDL cholesterol, adiponectin levels and adiponectin/leptin ratio were lower in T-allele carriers than non-T Allele carriers.

CONCLUSIONS: T allele carriers show lower levels of HDL-cholesterol, adiponectin and adiponectin/leptin ratio than non-T allele carriers. During a hypocaloric diet with Mediterranean partner increases HDL Cholesterol, adiponectin levels and ratio adiponectin/leptin in non-T allele carriers.

Key Words:
Adiponectin/leptin ratio, Lipids, Mediterranean diet, Rs822393.

Introduction

Obesity is a public health problem that increased in recent decades, with epidemic proportions worldwide and it is main factor in the development of some diseases such as diabetes mellitus type 2 and dyslipidemia[1]. Adipose tissue is an organ that secretes adipokines, which may provide the link between obesity, diabetes mellitus and dyslipemia[2]. Adiponectin is the most quantitatively abundant adipokine, and its concentrations are reduced in obese subjects[3]. Adiponectin binds to its receptors (AdipoR1 and AdipoR2) and exhorts its actions through the sensitization of the body to the insulin[3].

The polygenic nature of obesity and the interactions of single nucleotide polymorphisms (SNPs) in obesity is a controversial topic area. Adiponectin is encoded by the adiponectin C1Q and collagen domain containing (*ADIPOQ*) gene, which is located on chromosome 3 at q27[4]. In addition, some polymorphisms in *ADIPOQ* gene have related on adiponectin levels and insulin resistance[5], too. One common genetic variant rs822393 (-4522C/T) is located in the proximal promoter region of the *ADIPOQ* gene. The SNP rs822393 regulates adiponectin promoter activity and is associated with hypoadiponectinemia[6,7]. Moreover, this variant has been related with the risk of diabetes mellitus[8] in an Indian population. Despite these previously mentioned relationships, there is no study evaluating the effect of weight loss and this

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genetic variant on metabolic parameters. Contrary to those of most adipokines, adiponectin levels are decreased in obese subjects and are increased after weight reduction[9]. This is a research area with interest, since weight reduction increases serum adiponectin levels[10] and this weight loss could be reach with a Mediterranean dietary pattern[11]. In addition, perhaps, a diet with a Mediterranean pattern could have additional effects secondary to the foods with a direct effect independently of weight change[12]. The Mediterranean dietary pattern has been implied in multiple metabolic improvements such as weight loss, lipid control and glucose levels[13]. Therefore, it seems justified to evaluate the influence of a Mediterranean diet pattern on different parameters taking into account the rs822393.

The aim of our investigation was to analyze the effects after a hypocaloric diet with Mediterranean dietary pattern on lipid profile and adipokines taking to account rs822393 of *ADIPOQ*.

Patients and Methods

Patients and Clinical Investigation

270 obese Caucasian moderately obese subjects were enrolled. Study participants were enrolled from Primary Care Physicians of an urban area of the Northwest of Spain, when these patients were referred to our Department to evaluate their obesity. Inclusion criteria were adult obese subjects body mass index (BMI) >30 kg/m^2 with an age over 18 years. Subjects who had evidence of any history of cardiovascular disease, severe renal or hepatic disorders, alcohol consumption (>20 g/day), active malignant tumor, receiving medications known to influence lipid levels (fibrates, statins, hormonal therapy, glucocorticoids and anti-inflammatory drugs) or glucose levels (sulfonylureas, thiazolidinedione, insulin, Glucagon like peptide (GLP-1) receptor antagonists, S-GLT2 (type 2 sodium-glucose cotransporter), DPP-IV (Dipeptidyl peptidase-4) inhibitors, metformin) were excluded. All participants provided written informed consent. The Ethics Committee (HCUVA Committee PI7/2017) approved the study and was in accordance with the guidelines laid down in the Declaration of Helsinki.

Data of these patients were recorded at the beginning and after 3 months of dietary treatment. Blood pressure and anthropometric data such as weight, height, body mass index (BMI), fat mass by impedance and waist circumference were reported. For biochemical assays and genotyping, 5 ml of venous blood were drawn and aliquoted in ethylenediaminetetraacetic acid EDTA-coated tubes after a 10 hour overnight fast. The next parameters were determined: adipokine levels (leptin, total adiponectin and resistin), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, C-reactive protein and ratio adiponectin/leptin.

Nutritional Treatment

Participants were assigned to consume a calorie-restricted (daily 500-700 calories intake reduction) diet in the form of a Mediterranean diet for 3 months. All participants received personalized dietary counseling. At baseline, the normal dietary habits of subjects were assessed by using 3-day food records. The dietary intervention was a hypocaloric diet with a Mediterranean dietary pattern for 3 months. The balanced distribution of macronutrients in this diet is 54% of the calorie value from carbohydrates, 25% from lipids and 21% from proteins. Percentage of fats was 50.8% from monounsaturated fats, 37.3% from saturated fats and 11.9% from polyunsaturated fats. The diet was designed to include breakfast, lunch, dinner, one snack in the morning and one snack in the afternoon. Food tables were used with a Mediterranean dietary pattern including (legumes, vegetables, poultry, whole grains, fish, fresh fruit, using olive oil and limit unhealthy fats such as margarines, fatty meats, snacks, industrial pastries)[14]. All participants had two individual sessions (60 minutes with diet sheets and example menu plans) with the dietitian at the start of the trail to explain the diet and solve doubts. This dietitian assessed the adherence of this diet each week with a phone call. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Records were analysed with a computer-based data evaluation system (Dietsource®, Geneva, Switzerland). National composition food tables were used as reference[14]. The exercise program consisted of an aerobic exercise at least 3 times per week (60 min each) and the patient with a self-reported questionnaire recorded it.

Genotyping ADIPOQ Gene

Genomic DNA was isolated from peripheral blood leucocytes (300 uL of blood.) by QIAamp® DNA blood kit following the manufacturer's instructions. Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International®, Los Angeles,

CA, USA). The polymerase chain reaction (PCR) was realized with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'-ACGTTGGATGAAAGCATGACACGGAGCTTC-3' and reverse 5'- ACGTTGGATGAACCCTCACCCATGTCAGC-3' in a 2 uL final volume (Termociclador Life Tecnologies, Los Angeles, CA, USA). DNA was denatured at 90°C for 2 min; this was followed by 50 cycles of denaturation at 90°C for 30 s and annealing at 56.1°C for 60 s). The PCR was run in a 30 uL final volume containing 15 uL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase. We used as internal standard for RT-PCR (GAPDH) with a forward sequence‡: ‡GTCTCCTCTGACTTCAA and reverse sequence‡: ‡ACCACCCTGTTGCTGTA. Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed counts. The variant was in Hardy Weinberg equilibrium (p=0.31).

Biochemical Determinations

Lipid profile (total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides), C reactive protein (CRP), fasting glucose and insulin were determined on the same day using a clinical chemistry automated analyzer COBAS INTEGRA 400 analyser (Roche Diagnostic, Montreal, Canada). LDL cholesterol was calculated using Friedewald formula (LDL cholesterol=total cholesterol-HDL cholesterol-triglycerides/5)[15]. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values (glucose x insulin/22.5)[16].

Adipokine was determined by Enzyme-Linked Immunosorbent Assay (ELISA). Leptin was measured with the commercial kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) (DSL1023100) sensitivity of 0.05 ng/ml, a normal range of 10-100 ng/ml and a CV% 3.5%[17]. Adiponectin was realized with other commercial kit (R&D systems, Inc., Minneapolis, MN, USA) (DRP300); sensitivity of 0.246 ng/ml, a normal range of 8.65-21.43 ng/ml and a CV% 3.8%[18] and resistin with the kit (Biovendor Laboratory, Inc., Brno, Czech Republic) (RD191016100); sensitivity of 0.2 ng/ml, a normal range of 4-12 ng/ml[19] and a CV% 3.2%. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl. Adiponectin/leptin ratio was calculated.

Anthropometric Parameters

Anthropometric parameters, like height (cm) and waist circumference (cm), were measured using a non-elastic measuring tape (Omrom, Los Angeles, CA, USA). Body weight was recorded in the morning while the subjects were minimally unclothed and not wearing shoes, using digital scales (Omrom, Los Angeles, CA, USA) and recorded to the nearest 50 g. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Fat mass was measured by impedance with an accuracy of 5 g[20] (EFG BIA 101 Anniversary, Akern, Florence, Italy). This equation was used (0.756Height2/Resistance) + (0.110 Body mass) ± (0.107 Reactance) – 5.463. Mean systolic and diastolic blood pressures were calculated by averaging three measurements (Omrom, Los Angeles, CA, USA), after the subjects sat for 10 minutes.

Statistical Analysis

Sample size was calculated to detect differences over 4 mg/dl of HDL-cholesterol after dietary intervention with 90% power and 5% significance. The Kolmogorov-Smirnov test was used to analyze variable distribution. The results were showed as average+/- standard deviation. Numerical variables with normal distribution were analyzed with a two-tailed Student's *t*-test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. Bonferroni test was applied for multiple testing to reduce Type I error in association analysis. The statistical analysis to evaluate the interaction between the gene and the dietary intervention was performed using ANCOVA (covariance analysis) adjusted by age, sex, and BMI modeling the dependent variable with the starting values. *p*-values in Tables I, II and III are as follow: first *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs.* CC + CT baseline values, third *p*, significance of dietary intervention after 3 months in CC + CT genotype, fourth *p*, significance between TT genotypes *vs.* CC + CT post-treatment values. Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed data. Hardy Weinberg equilibrium in genotype frequencies was confirmed (p=0.31). All analysis were performed under a dominant genetic model with rs822393 T-allele as the risk allele (CC+CT *vs.* TT). All the data were analyzed using SPSS for Windows, version 23.0

Table I. Association of circ_001680 expression with clinicopathologic characteristics of glioma.

	CC (n = 160)		CT ± TT (n = 110)		
Parameters	Basal	3 months	Basal	3 months	*p*-values -Time CC - Basal genotype - Time CT ± TT - 3 months genotype
BMI	36.6 ± 1.1	35.4 ± 1.2*	36.4 ± 1.3	35.2 ± 1.9*	$p = 0.03$ $p = 0.31$ $p = 0.02$ $p = 0.34$
Weight (kg)	96.9 ± 1.2	93.7 ± 2.1$^{\$}$	96.1+2.1	92.4 ± 2.3$^{\$}$	$p = 0.03$ $p = 0.41$ $p = 0.02$ $p = 0.55$
Fat mass (kg)	41.3 ± 1.2	38.5 ± 2.1$^{\#}$	40.9 ± 1.1	38.3 ± 2.0$^{\#}$	$p = 0.03$ $p = 0.46$ $p = 0.03$ $p = 0.47$
WC (cm)	109.6 ± 4.1	106.3 ± 3.1$^{\&}$	108.9 ± 6.1	105.1 ± 4.1$^{\&}$	$p = 0.01$ $p = 0.38$ $p = 0.02$ $p = 0.48$
SBP (mmHg)	140.6 ± 4.2	125.6 ± 5.9**	137.1 ± 7.2	126.6 ± 5.2**	$p = 0.02$ $p = 0.31$ $p = 0.03$ $p = 0.40$
DBP (mmHg)	84.3 ± 7.0	82.1 ± 4.2	83.9 ± 6.1	81.2 ± 6.0	$p = 0.41$ $p = 0.69$ $p = 0.62$ $p = 0.67$

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; Statistical differences $p < 0.05$, in each genotype group (*BMI, $^{\$}$Weight, $^{\#}$fat mass, $^{\&}$WC, **SBP). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs*. CC + CT baseline values, third *p*, significance of dietary intervention after 12 weeks in CC + CT genotype, fourth *p*, significance between TT genotypes vs. CC + CT post-treatment values.

software package (SPSS Inc. Chicago, IL, USA). A *p*-value <0.05 was considered significant.

Results

We investigated the effects of this SNP rs822393 on the change of anthropometric, serum adipokines and biochemical parameters in 270 obese Caucasian subjects. The mean age of the population was 46.1±3.0 years (range: 21-61) and the average body mass index (BMI) was 36.5±1.9 kg/m^2 (range: 31.4-39.1). Sex distribution was 188 females (69.5%) and 82 males (30.6%). The genotype distribution was as follow: 160 patients (59.3%) CC, 96 patients CT (35.6%) and 14 patients CT (5.1%). The variant was in Hardy Weinberg equilibrium (p=0.31). Sex distribution was similar in all genotype groups (CC; 31.2% males *vs*. 68.8% females, CT; 31.2% males *vs*. 68.8% females and TT; 26.4% males *vs*. 73.6% females: p=1.18). The mean age was similar in these groups (CC; 46.3±1.1 years *vs*. CT; 46.1±2.2 years *vs*. TT; 46.1±1.9 years: p=0.26). All analysis of the following parameters were performed under a dominant genetic model with rs822393 T- allele as the risk allele (CC+CT *vs*. TT).

Patients reached the dietary recommendations as prescribed in method section, with a total caloric amount of 1431.1±112.9 calories, the percentage of macronutrients was: 53.7% from carbohydrates, 25.2% from lipids and 21.1% from proteins. Percentage of fats was 52.1% from monounsaturated fats, 36.8% from saturated fats and 12.1% from polyunsaturated fats, without statistical differences between genotype groups. Basal physical activity was similar in both gen-

Table II. Biochemical parameters (mean + SD).

Parameters	CC (n = 160)		CT ± TT (n = 110)		
	Basal	3 months	Basal	3 months	*p*-values -Time CC - Basal genotype - Time CT ± TT - 3 months genotype
Glucose (mg/dl)	99.8 ± 8.1	96.5 ± 7.0	99.7 ± 8.0	94.9 ± 7.3	$p = 0.11$ $p = 0.54$ $p = 0.12$ $p = 0.43$
Total cholesterol (mg/dl)	205.9 ± 4.7	189.6 ± 4.2$^{\$}$	211.2 ± 4.1	190.4 ± 5.2$^{\$}$	$p = 0.03$ $p = 0.51$ $p = 0.04$ $p = 0.31$
LDL-cholesterol (mg/dl)	126.9 ± 9.1	108.8 ± 8.2$^{\#}$	129.5 ± 6.1	114.2 ± 5.2$^{\#}$	$p = 0.01$ $p = 0.44$ $p = 0.02$ $p = 0.39$
HDL-cholesterol (mg/dl)	50.8 ± 1.6	56.2 ± 1.9 *	44.9 ± 2.0^{+}	46.7 ± 1.3^{+}	$p = 0.03$ $p = 0.04$ $p = 0.59$ $p = 0.03$
Triglycerides (mg/dl)	126.2 ± 17.0	121.9 ± 12.2	130.1 ± 16.2	127.8 ± 12.1+	$p = 0.12$ $p = 0.51$ $p = 0.20$ $p = 0.47$
Insulin (mUI/l)	18.1 ± 2.1	13.6 ± 2.2$^{\&}$	18.7 ± 2.2	14.8 ± 2.0$^{\&}$	$p = 0.03$ $p = 0.40$ $p = 0.03$ $p = 0.43$
HOMA-IR	5.6 ± 1.1	3.2 ± 1.0**	5.9 ± 0.9	3.3 ± 1.1**	$p = 0.01$ $p = 0.37$ $p = 0.02$ $p = 0.41$
CRP	4.3 ± 1.1	4.1 ± 1.2	4.6 ± 2.0	4.5 ± 2.9	$p = 0.21$ $p = 0.34$ $p = 0.33$ $p = 0.42$

HOMA-IR (homeostasis model assessment). CRP (C reactive protein) Statistical differences $p < 0.05$, in each genotype group (total cholesterol $^{\$}$LDL cholesterol $^{\#}$HDL Cholesterol *insulin $^{\&}$HOMA IR **) (HDL Cholesterol between genotypes +). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs*. CC + CT baseline values, third p, significance of dietary intervention after 12 weeks in CC + CT genotype, fourth *p*, significance between TT genotypes *vs*. CC + CT post-treatment values.

otype groups (CC *vs.* CT+TT) (103.9±21.4 min/week *vs.* 101.1±29.9 min/week; p=0.51). Moreover, after 3 months of the study, this physical activity was similar that basal without differences in deltas (104.2±14.8 min/week *vs.* 103.2±11.8 min/week; p=0.28).

Anthropometric Results

The anthropometric and clinical characteristics of the study participants are presented in Table I. For rs822393 T- allele as the risk allele, there were no differences in adiposity parameters and blood pressure with dominant model (CC *vs.* CT+TT) (Table I). After the caloric restriction in both genotypes (CC *vs.* CT+TT), the following parameters decreased: BMI (delta: -1.2±0.3 kg/m^2 *vs.* -1.2±0.1 kg/m^2; p=0.13), weight (delta: -3.2±2.0 kg *vs.* -3.7±1.9 kg; p=0.23), fat mass (delta: -2.8±1.1 kg *vs.* -2.6±1.2 kg; p=0.43), waist circumference (delta: -2.3±0.8 cm *vs.* -1.6±0.9 cm; p=0.41) and systolic blood pressure (delta: -15.6±2.1 mmHg *vs.* -11.2±2.9 mmHg; p=0.31). These changes were similar in both genotype groups.

Table III. Serum Adipokine levels (mean + SD).

Parameters	CC (n = 160)		CT ± TT (n = 110)		
	Basal	3 months	Basal	3 months	*p*-values -Time CC - Basal genotype - Time CT ± TT - 3 months genotype
Resistin (ng/dl)	3.9 ± 1.9	3.8 ± 2.0	3.9 ± 2.1	4.0 ± 1.9	$p = 0.51$ $p = 0.69$ $p = 0.34$ $p = 0.48$
Adiponectin (ug/dl)	26.7 ± 5.0	47.9 ± 4.0$	15.1 ± 4.1^{+}	18.9 ± 4.2^{+}	$p = 0.02$ $p = 0.03$ $p = 0.32$ $p = 0.01$
Leptin (ng/dl)	83.3 ± 11.6	56.2 ± 9.5*	79.1 ± 8.1	53.8 ± 9.1*	$p = 0.02$ $p = 0.28$ $p = 0.03$ $p = 0.33$
Ratio adiponectin/leptin	0.32 ± 0.2	0.85 ± 0.1$^{\#}$	0.19 ± 0.1^{++}	0.35 ± 0.3^{++}	$p = 0.02$ $p = 0.02$ $p = 0.13$ $p = 0.03$

Statistical differences $p < 0.05$, in each genotype group ($^{\$}$Adiponectin, *Leptin, $^{\#}$Ratio adiponectin/leptin). ($^{+}$adiponectin, $^{++}$adiponectin/leptin ratio between genotypes). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs*. CC + CT baseline values, third *p*, significance of dietary intervention after 12 weeks in CC + CT genotype, fourth p, significance between TT genotypes *vs*. CC + CT post-treatment values.

Biochemical Results

In Table II we showed the effects of dietary intervention on biochemical parameters, while lipid profile and adipokine levels were reported in Table III. In glucose metabolism and after dietary intervention (CC *vs*. CT+TT), insulin levels (delta: -4.5±0.9 UI/L *vs* 4.1±0.7 UI/L; *p*=0.43) and HOMA-IR (delta: -2.4±0.2 units *vs*. -2.6±0.3 units; *p*=0.42) improved significantly in both genotypes. After dietary intervention (CC *vs*. CT+TT), total cholesterol (delta: -16.7±4.9 mg/dl *vs*. -21.0±3.8 mg/dl; *p*=0.12) and LDL cholesterol (delta: -18.3±3.4 mg/dl *vs*. -15.7±2.7 mg/dl; *p*=0.31) decreased in both genotypes, too. Moreover, HDL- cholesterol (delta: 5.4±1.4 mg/dl *vs*. -1.8±0.7 mg/dl; *p*=0.03) also improved in non-T allele carriers. Basal and post-intervention levels of HDL-Cholesterol were lower in T-allele carriers than non-T Allele carriers.

Adipokine Results

Table III shows changes of serum adipokines and ratio adiponectin/leptin. After weight loss and in non-T-allele carriers (CC *vs*. CT+TT), serum adiponectin (delta:21.2±4.1 ng/dl *vs*. 3.8±3.3 ng/dl; *p*=0.02) increased in a significative way. In addition, patients with both genotypes showed a significant decrease on leptin levels (delta: -27.2±5.1 ng/dl *vs*. -26.4±5.9 ng/dl; *p*=0.28). Serum resistin levels remained unchanged during the intervention trial in both genotype groups. Finally, adiponectin/leptin ratio increased in non-T allele carriers (delta: 0.53±0.1 *vs*. 0.16±0.3 ng/dl; *p*=0.02). Basal and post-intervention adiponectin levels and adiponectin/leptin ratio were lower in T-allele carriers than non-T Allele carriers.

Discussion

In the present study, we have reported that at a 3-month follow-up, a calorie-restricted dietary treatment with a Mediterranean partner in obese Caucasian subjects increased HDL-Cholesterol, adiponectin levels and ratio adiponectin/leptin in non-T allele carriers of

the genetic variant rs822393. T allele carriers show lower levels of HDL-cholesterol, adiponectin and adiponectin/leptin ratio than non-T allele carriers.

To our knowledge, this is the first study investigating the association between this *ADIPOQ* gene polymorphism and metabolic response secondary to a hypocaloric Mediterranean diet in obese subjects. The data in the literature on this genetic variant of the *ADIPOQ* gene are scarce and contradictory. In a cross-sectional study[18], a lack of significant associations between this SNP and neither adiponectin levels nor metabolic parameters has been reported. In contrast, in the *Healthy Lifestyle in Europe by Nutrition in Adolescente Study*[7], it has been showed an important association between rs822393 polymorphism and HDL-cholesterol levels, as our present results. These findings are interesting due to the strong evidence of the inverse association between HDL-cholesterol levels and cardiovascular risk[21,22]. This means that this genetic variant can be used in clinical practice as a potential marker of cardiovascular risk.

Our study also shows lower levels of adiponectin in patients with the T allele. Rayma et al[8] had already corroborated these data in an Indian population; this may be a link with low levels in HDL-cholesterol levels. Adiponectin increases serum HDL cholesterol[23] through two physiological mechanisms: *via* the increase of the hepatic production of ApoA1, the main apolipoprotein of HDL-cholesterol[24] and *via* the activation of lipoprotein lipase and ATP-binding cassette transporter A1 and inhibition of hepatic lipase[25]. The reported decrease in adiponectin levels could be explained by the fact that rs822393 is an intronic variant with the potential capacity to modify the alternative-splicing pattern[6], and these low levels of adiponectin could produce low levels of HDL-cholesterol. In addition, one study[27] has shown a relationship with insulin resistance in a Caucasian population, without association with diabetes mellitus or metabolic syndrome[8]. Moreover, the reported beneficial effects may be secondary to weight loss and to the Mediterranean diet. The presence of nutrients such as olive oil, fish and lean meats can also explain the improvement in the profile lipid[11].

Moreover, there is a lack of information about the influence of caloric restriction and weight loss on these metabolic relationships in interventional designs. Our study shows that this genetic variant of *ADIPOQ* is associated with a differential regulation of adiponectin synthesis after weight loss and the above-mentioned metabolic pathways could explain this relationship. Despite the absence of studies in the literature, there are designs[11] that have reported the association of other polymorphisms of the *ADIPOQ* rs266729 G allele with higher weight after 4-year follow-up. In our work, there is no relationship between rs822393 and the modification in parameters related to adiposity. Moreover, one cross sectional study[27] reported that this SNP was associated with body mass index, and subjects with T allele showed higher BMI than non-T allele carriers. Perhaps other polymorphisms, not only in the *ADIPOQ* gene, but also in the receptors, may be modulating the findings found because actions of adinopectin is realized by two receptors, ADIPOR1 (located on chromosome 1q32) and ADIPOR2 (located in chromosome 12p13.33)[3,4].

Finally, the CARDIA study (Coronary Artery Development in Young Adults) demonstrated a relationship between this polymorphism and low levels of adiponectin[28], as our data. A novelty finding in our work was the association of this SNP with the adiponectin/leptin ratio and its secondary modifications to the diet. The adiponectin/leptin ratio is a marker of a subacute inflammation status[29], which associated leptin as an adipokine related with the degree of adiposity[30]. For example, an adiponectin/leptin ratio higher than 1 could be a normal value whereas a ratio below or near to 0.5 may warm an increase in the metabolic risk[31], as shown by the carriers of the T allele, before and after dietary intervention.

This study has limitations that should be acknowledged. Firstly, we only analysed one SNP of *ADIPOQ* and it could be that other genes exist, working in concert, with ADIPOQ -related genes, which would explain our results. Secondly, the lack of a control group without diet might be a bias. Finally, the self-reported dietary intake and physical activity are not reliable, and it might include bias of under- or over-reporting.

Conclusions

In brief, a calorie-restricted dietary treatment with a Mediterranean partner increases

HDL-Cholesterol, adiponectin levels and ratio adiponectin/leptin in non-T allele carriers. T allele carriers show lower levels of HDL-cholesterol, adiponectin and adiponectin/leptin ratio than non-T allele carriers. Further designs are needed to confirm this association, especially because the minor allele can be a marker of high cardiovascular risk.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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 and with the 1964 Helsinki Declaration and its later amendments or comparable Ethical standards. And it was approved by our Local Committee (Hospital Clinic Universitary of Valladolid CommitteePI7/2017).

Informed Consent
Informed consent was obtained from all individual participants included in the study..

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Authors' Contribution
Daniel de Luis designed the study, realized statistical analysis and wrote the article. He contributed to the analysis of data for the work and revised it critically for important intellectual content. He approved the version to be published and accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Olatz Izaola, realized anthropometric evaluation and control of dietary intake. She contributed to the acquisition of data for the work and revised it critically for important intellectual content. She approved the version to be published and accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. David Primo realized biochemical evaluation, genotype and wrote the article. He contributed to the analysis of data for the work and revised it critically for important intellectual content. He approved the version to be published and accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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