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The effect of single-nucleotide polymorphisms at the *ADIPOQ* gene locus rs1501299 on metabolic parameters after 9 mo of a high-protein/low-carbohydrate versus a standard hypocaloric diet

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Objective: Some adiponectin gene (*ADIPOQ*) single-nucleotide polymorphisms (SNPs) have been related to basal and adiponectin levels and metabolic parameters. The aim of this study was to evaluate the effect of the genetic variant rs1501299 *ADIPOQ* gene on biochemical changes after weight loss secondary to a high-protein and low-carbohydrate diet versus a standard severe hypocaloric diet over 9 mo as the primary endpoint.
Methods: A white population of 270 obese patients was enrolled in a randomized clinical trial with two hypocaloric diets (high-protein and low carbohydrate diet [HP] versus standard diet [S]) over 9 mo of intervention. The statistical analysis was performed for the combined GT and TT as a group (T-allele carriers) and GG as second group (non–T-allele carriers). Before and after 12 wk on each hypocaloric diet, an anthropometric evaluation, an assessment of nutritional intake, and a biochemical analysis were realized.
Results: With both dietary interventions, body weight, body mass index (BMI), fat mass, waist circumference, systolic blood pressure, and leptin levels decreased. In non–T-allele carriers after both diets, the decrease in total cholesterol levels −12.3 ± 2.2 mg/dL (T-allele carriers −6.9 ± 2.1 mg/dL; $P = 0.01$ diet HP) and 12.2 ± 3.1 mg/dL (T-allele carriers −4.7 ± 1.2 mg/dL; $P = 0.02$ after diet S), low-density lipoprotein cholesterol −13.2 ± 2.7 mg/dL (T-allele carriers −6.1 ± 2.1 mg/dL; $P = 0.02$ after diet HP) and −9.3 ± 1.8 mg/dL (T-allele carriers −4.8 ± 2.9 mg/dL; $P = 0.01$ after diet S), triacylglycerol levels −12.7 ± 6.1 mg/dL (T-allele carriers −6 ± 2.9 mg/dL; $P = 0.01$ after diet HP) and −16.3 ± 7.2 mg/dL (T-allele carriers −5.3 ± 1.4 mg/dL; $P = 0.03$ after diet S), insulin levels −5 ± 1.1 mUI/L (in T-allele −1.7 ± 0.9 mUI/L; $P = 0.02$ after diet HP) and −3.2 1.1 mUI/L (T-allele carriers −0.7 ± 0.7 mUI/L; $P = 0.02$ after diet S), and homeostatic model assessment of insulin resistance levels −0.4 ± 0.2 units (T-allele group −0.1 ± 0.1; $P = 0.04$ after diet HP) and −0.7 ± 0.1 units (T-allele carriers −0.1 ± 0.5 mg/dL; $P = 0.01$ after diet S) was higher than T-allele carriers. Only no T-allele carriers showed an increase in adiponectin levels after both diets.
Conclusion: After two different hypocaloric diets during 9 mo of intervention, the GG genotype of an *ADIPOQ* gene variant (rs1501299) is related to better improvement in adiponectin levels, insulin resistance, and lipid profile than T-allele carriers.

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Introduction

Adiponectin expressed by adipocyte decreases in obesity and its related pathologies such as diabetes mellitus and metabolic syndrome [1,2]. The biological functions of adiponectin on vascular endothelial cells, smooth muscle cells, and metabolism confer protection against metabolic syndrome and obesity [3]. The adiponectin synthesis has an important genetic component, with heritability estimated >70% [4]. Some single-nucleotide polymorphisms (SNPs) have been associated with adiponectin synthesis and serum adiponectin levels [5,6]. However, the association between SNPs and adiponectin levels are unclear, partly because of the interaction between these genetic variants and nutrients [7].

One of the most relevant SNPs at the *ADIPOQ* locus are a G to T substitution in intron 2 (+ *276 G>T*, rs1501299). The G allele has been negatively [8] and positively [9] associated with obesity in some populations. In addition, this SNP has been associated either with increased or decreased concentrations of plasma adiponectin [9,10]. On the other hand, this genetic variant has been negatively

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[11] and positively [12] associated with obesity in some populations. Another area with uncertain results is the relationship of this genetic variant to glucose metabolism and insulin resistance [13,14].

Initial treatment for obesity typically includes a lifestyle intervention comprising hypocaloric diet and physical activity, which has been shown to reduce both the comorbidities and dependency on pharmacologic treatment [15]. Caloric restriction, in addition to the composition of the diet, may influence adiponectin levels. For example, polyunsaturated and monounsaturated fatty acids (PUFAs and MUFAs, respectively) upregulate *ADIPOQ* gene expression [16,17] and increase adiponectin levels. An interaction between *ADIPOQ* SNPs and dietary intake of PUFAs [18] and MUFAs [19] has been reported. Moreover, other components of diet such as dietary fiber from whole grain products, low-glycemic foods, and the ratio between unsaturated fat and carbohydrates might influence adiponectin levels [20,21]. Some studies with short dietary interventions of 3 mo have shown an influence of this SNP on metabolic response [22] and serum adiponectin changes [23].

In the present study, we evaluated the effect of the genetic variant rs1501299 *ADIPOQ* gene on biochemical changes after weight loss secondary to a high-protein and low-carbohydrate diet versus a standard, severe hypocaloric diet over a 9-mo period as the primary endpoint.

Patients and methods

Participants

We prospectively enrolled 270 obese white individuals. This is a secondary analysis of a prospective randomized controlled trial. Ethical approval for this study was granted by the local ethic committee and written informed consent from participants was obtained, including subsequent genetic studies. The recruitment of participants was a consecutive method of sampling among patients sent from primary care physicians. This study was realized according to the guidelines laid down in the Declaration of Helsinki. Eligibility for entry to the study was assessed with implementation of the exclusion and inclusion criteria described. Inclusion criteria included being 20 to 65 y of age and having a body mass index (BMI) >30 kg/m^2. Exclusion criteria included a hypocaloric diet during the 12 mo before the study, history of cardiovascular or cerebrovascular diseases, insufficient motivation, and the use of any of the following drugs: metformin, dipeptidyl type-4 inhibitors drugs, thiazolidinedione, glucagon-like peptide-1 analogs, sodium-glucose cotransporter 2 inhibitors, insulin, glucocorticoids, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, psychoactive medications, statins, and other lipid drugs.

Design

The study duration was 18 mo, from the inclusion of the first patient to the end of the last patient. Patients were randomly allocated to one of two diets (high-protein and low-carbohydrate [diet HP] versus a standard, severe hypocaloric diet [diet S]) for a period of 9 mo. Randomization was not performed according to the genotype. Venous blood specimens (15 mL) were collected in Ethylenediaminetetraacetic acid (EDTA)-treated tubes after an 8-h fasting period (basal, 3 and 9 mo) from all participants. Basal fasting glucose, lipid metabolism (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], plasma triacylglycerol [TG] concentration), C-reactive protein, insulin, homeostatic assessment model for insulin resistance (HOMA-IR), and adipokine (leptin, resistin, adiponectin) levels were measured at the start of the trial and repeated after 3 and 9 mo during the protocol. Weight, height, and blood pressure measurements were determined at the same times (basal, 3 and 9 mo). A bioimpedance was realized to measure fat mass. Genotype of *ADIPOQ* gene (rs1501299) was studied.

Dietary intervention

In all, 270 obese patients were randomly allocated to one of the next two diets. Diet HP (n = 137; severe hypocaloric diet, high protein and low carbohydrate) consisted of 1050 cal/d, 33% fats (39 g/d), 33% carbohydrates (86.1 g/d), and 34% proteins (88.6 g/d). The distribution of fats was 63.8% monounsaturated fats, 23.5% saturated fats, and 12.6% polyunsaturated fats. Diet S (n = 133; severe hypocaloric diet, standard protein) consisted of 1093 cal/d, 27% fats (32.6 g), 53% carbohydrates (144.3 g/d), and 20% proteins (55.6 g/d). The distribution of fats was 67.4% monounsaturated fats, 20.9% saturated fats, and 11.6% polyunsaturated fats. The exercise recommendations for patients of both groups were the completion of aerobic physical activities at least three times per week (60 min each). Adherence to these diets was recorded every 14 d with a phone call to improve diet's compliance with a dietitian. National composition food tables were used as references [24].

Anthropometric measurements and blood pressure

In all patients, body weight and height were measured in the morning. Weight was measured to an accuracy of 100 g with a calibrated scale (Omrom, Los Angeles, CA, USA) and a stadiometer 0.1 mm (Omrom), respectively. Body mass index (BMI) was calculated as the next formula; body weight (in kg) divided by height (in m^2). Waist circumference (WC; narrowest diameter between xiphoid process and iliac crest) and hip circumference (widest diameter over greater trochanters) to derive waist-to-hip ratio were measured. A bioimpedance was used to determine body composition with an accuracy of 50 g (Akern, EFG, Pissa, Italy) [25]. Finally, blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer and averaged (Omrom).

Assays

Blood samples for analysis were drawn after a minimum of an 8-h overnight fast, and serum was stored at −80 °C until analyzed. Fasting serum concentrations of TC and TG were measured using commercially available kits (Technicon Instruments, Ltd., New York, NY, USA), whereas HDL-C was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate magnesium. LDL-C was calculated using Friedewald formula [26]. Plasma glucose levels were measured using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was determined by RIA (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (range 0.5–30 mUI/L) [27] and the HOMA-IR was calculated using the following formula:

fasting insulin = fasting glucose/22.5 [28].

Serum adiponectin analysis was carried out by enzyme-linked immunosorbent assay (ELISA; R&D systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng/mL, a normal range of 8.65 to 21.43 ng/mL, and a coefficient of variation (CV%) of 3.8% [29]. Resistin was analyzed by ELISA (Biovendor Laboratory, Inc., Brno, Brno, Czech Republic) with a sensitivity of 0.2 ng/mL, a normal range of 4 to 12 ng/mL [30], and a CV% of 3.2%. Leptin was analyzed by ELISA (Diagnostic Systems Laboratories, Inc., Dallas, TX, USA) with a sensitivity of 0.05 ng/mL, a normal range of 10 to 100 ng/mL, and a CV% of 3.5% [31]. C-reactive protein was analyzed by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Baden Wutenbergh, Germany), with a normal range of (0–7 mg/dL) and analytical sensivity of 0.5 mg/dL.

DNA extraction and SNP genotyping

Genomic DNA was extracted from 300 uL buffy coat (blood samples) with a commercial kit extraction (BioRad, Los Angeles, CA, USA). Primers were designed with the Sequenom Assay Design v4 (Sequenom, San Diego, CA, USA). Genotyping for the rs1015299 polymorphism was performed by polymerase chain reaction (PCR) real-time analysis. This PCR was carried out with 20 to 25 ng of genomic DNA, 0.1 to 0.15 μL each of oligonucleotide primer for rs1501299 (primer forward: 5′-ACGTTGGATGAAAGCTTTGCTTTCTCCCTG-3′ and reverse 5′-ACGTTGGATGAAAGCTTTGCTTTCTCCCTG-3′ in a 2-μL final volume; Termociclador Life Technologies, Los Angeles, CA, USA). DNA was denatured at 85°C for 5 min, followed by 45 cycles of denaturation at 95°C for 15 s, and annealing at 58.1°C for 45 s. The PCRs were run in a 2-μL final volume containing 0.1 μL of iPLEx Termination mix (Bio-Rad, San Diego, CA, USA) with hot start Taq DNA polymerase.

Statistical analysis

Sample size was calculated to detect differences over 3 kg in body weight loss after 9 mo of dietary intervention with 90% power and 5% significance (n = 130, in each diet group). The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's *t* test. A χ^2 test was used to evaluate the Hardy–Weinberg equilibrium. Non-parametric parameters were analyzed with the Wilcoxon test. A mixed model has been used within and between participants to test the interaction between the polymorphism groups and outcome variables due to the repeated-measures study design. Qualitative parameters were analyzed with the χ^2 test, with Yates correction as necessary, and Fisher exact test. The statistical analysis to evaluate the gene–diet interaction was an univariate analysis of covariance with Bonferroni post hoc test. The statistical analysis was performed

for the combined TT and GT as a group and GG genotype as second group (wildtype genotype), with a dominant model. $P < 0.05$ was considered significant.

Results

Two hundred and seventy obese individuals were enrolled in the study (141 GG [52.2%], 108 GT [40%], and 21 TT [7.8%]). All patients completed the 9-mo follow-up period without dropouts. The mean age of the group was 49.8 ± 7.1 y (range 26–62 y) and the mean BMI 34.9 ± 4 kg/m^2 (range 30.1–40.2 kg/m^2). Sex distribution was 200 women (74.1%) and 70 men (25.9%). Age was similar in both genotype groups (wildtype GG versus mutant type GT + TT: 50.2 ± 7.1 versus 47.9 ± 5.1 y: ns). Sex distribution was similar in genotype groups (males 26.2% versus 25.3% and females 73.8% versus 74.7%).

The basal determination of dietary intake with a 3-d written food questionnaire showed the following in the 137 obese patients (65 GG genotype and 72 T-allele carriers) randomized to diet HP: basal calorie intake of 1995.1 ± 422.8 kcal/d, fat intake of 88.2 ± 14.3 g/d (39.8% of calories), carbohydrate intake of 211.31 ± 29.4 g/d (41% of calories), and a protein intake of 78.2 ± 41.2 g/d (27.2% of calories). During the intervention (9 mo), these patients reached the objectives of dietary intervention. Physical activity in min/wk was similar in both genotype groups (68.1 ± 17.2 versus 69.1 ± 19.3 min/wk; $P = 0.47$).

The basal assessment of dietary intake with the same aforementioned methodology in the 133 obese patients (65 GG genotype and 72 T-allele carriers) randomized to diet S reported the following: basal calorie intake of 1998.2 ± 312.2 kcal/d, fat intake of 89.2 ± 16.3 g/d (38.5% of calories), carbohydrate intake of 207.2 ± 29.9 g/d (42.7% of calories), and protein intake of 90 ± 10.2 g/d (19.8% of calories). During the intervention, these patients reached the recommendations of diet. Finally, physical activity was similar in both genotype groups (71.1 ± 8.1 versus 68.9 ± 31.3 min/wk; $P = 0.46$).

Anthropometric parameters and blood pressure levels of enrolled patients at baseline, at 3 mo, and at 9 mo of intervention are reported in Table 1. In both genotype groups (GG versus GT + TT) and with both diets (diet HP versus diet S), body weight, BMI, fat mass, WC, and systolic blood pressure (SBP) decreased. Table 1 shows that after a dietary intervention (9 mo) with diet HP, individuals with both genotypes (GG versus GT + TT) showed a similar decrease in body weight, BMI, fat mass, WC and SBP. After the standard hypocaloric diet (diet S), obese patients with both genotypes (GG versus GT + TT) had a similar decrease in body weight, BMI, fat mass, WC and SBP.

After dietary intervention (Table 2) with both diets, non–T-allele carriers showed a significant decrease in TC, LDL-C, TGs, insulin, and HOMA-IR values. Changes in HDL-C did not show statistical differences. After diet HP and in non–T-allele carriers, the decrease in TC levels (−12.3 ± 2.2 mg/dL) was greater than in T-allele carriers (−6. 9 ± 2.1 mg/dL; $P = 0.01$). Moreover, the decrease in LDL-C levels was greater in non–T-allele carriers (−13.2 ± 2.7 mg/dL) than in T-allele carriers (−6.1 ± 2.1 mg/dL; $P = 0.02$). The decrease in TG levels also was greater in non–T-allele carriers (−12.7 ± 6.1 mg/dL) than in T-allele carriers (−6 ± 2.9 mg/dL; $P = 0.01$). Similarly, the decrease of insulin levels in non–T-allele carriers was greater (−5 ± 1.1 mUI/L) than in T-allele carriers (−1.7 ± 0.9 mUI/L; $P = 0.02$). Finally, the decrease of HOMA-IR levels also was greater in non–T-allele carriers (−0.4 ± 0.2 units) than in T-allele carriers (−0.1 ± 0.1; $P = 0.04$).

In non–T-allele carriers after diet S, the decrease in total cholesterol levels was greater (−12.2 ± 3.1 mg/dL) than in T-allele carriers (−4.7 ± 1.2 mg/dL; $P = 0.02$). The decrease in LDL-C levels was greater in non–T-allele carriers (−9.3 ± 1.8 mg/dL) than in T-allele carriers (−4.8 ± 2.9 mg/dL; $P = 0.01$). The decrease in TG levels also was greater in non–T-allele carriers (−16.3 ± 7.2 mg/ dL) than in T-allele carriers (−5.3 ± 1.4 mg/dL; $P = 0.03$). Therefore, the decrease of insulin levels in non–T-allele carriers was greater (−3.2 ± 1.1 mUI/L) than in T-allele carriers (−0.7 ± 0.7 mUI/L; $P = 0.02$). Finally, the decrease of HOMA-IR levels was greater in non–T-allele carriers (−0.7 ± 0.1 units) than in T-allele carriers (−0.1 ± 0.5 mg/dL; $P = 0.01$).

Table 3 reports levels of serum adipokines. Leptin levels decreased in both genotypes after diets HP and S. Only non–T-allele carriers showed an increase in adiponectin levels after dietary intervention (10.1 ± 0.3 ng/ dL; T-allele group 2.6 ± 0.3 ng/dL; $P = 0.03$ after diet HP and 10.9 ± 0.2 ng/dL; T-allele carriers 0.6 ± 0.3 ng/dL; $P = 0.01$ after diet S).

Discussion

The most important data in the present study was the association between this genetic variant and changes in lipid profile, insulin levels, HOMA-IR, and adiponectin concentrations after significant weight loss secondary to two different hypocaloric diets. Obese individuals with the T allele of this genetic variant did not show an improvement in adiponectin levels, lipid profile, insulin levels, or HOMA-IR despite presenting weight loss.

The association of rs1015299 on the *ADIPOQ* gene with insulin levels and adipokines concentrations have been studied in some cross-sectional studies [10–14], but the results after intervention studies are scarcer [22,23]. Furthermore, the results of the association between this SNP and metabolic parameters are unclear; T allele has been associated with higher insulin levels and HOMA-IR [8], and in other studies, opposite results have been reported [12].

Table 1
Changes in anthropometric parameters (mean ± SD)

	DIET HP (n = 137)						DIET S (n = 133)					
	GG(n = 65)			*GG + GT* (n = 72)			*GG* (n = 57)			*GG+ GT (n = 75)*		
Characteristics	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths
BMI	34.8 ± 5.1	32.7 ± 4.0*	31.8 ± 4.0*	34.9 ± 5.0	32.8 ± 4.1*	31.9 ± 5.0*	34.9 ± 5.1	32.9 ± 4.3*	31.8 ± 5.0*	35.0 ± 4.0	32.9 ± 4.1*	31.9 ± 4.0*
Weight (kg)	91.6 ± 9.3	86.2 ± 8.2*	83.5 ± 10.1*	90.6 ± 11.4	85.9 ± 9.1*	83.8 ± 8.4*	89.9 ± 11.6	86.5 ± 8.0*	83.6 ± 8.2*	90.4 ± 12.3	86.1 ± 10.2*	83.9 ± 9.1*
Fat mass (kg)	35.1 ± 5.0	32.4 ± 4.0*	30.1 ± 5.1*	35.7 ± 9.1	33.2 ± 8.1*	30.8 ± 8.1*	35.8 ± 5.1	33.2 ± 7.0*	31.0 ± 9.1*	36.5 ± 7.0	34.2 ± 8.1*	31.4 ± 8.1*
WC (cm)	110.9 ± 9.1	106.1 ± 7.2*	102.7 ± 6.2*	112.2 ± 7.1	107.2 ± 8.1*	103.8 ± 7.1*	110.5 ± 8.1	106.3 ± 7.1*	104.7 ± 8.2*	113.3 ± 9.1	108.8 ± 7.0*	106.9 ± 8.0*
SBP (mmHg)	126.9 ± 10.1	123.3 ± 6.7*	123.1 ± 8.2*	127.1 ± 10.2	124.3 ± 9.0*	123.1 ± 10.0*	126.3 ± 9.0	123.3 ± 8.0*	121.1 ± 8.1*	129.2 ± 8.1	126.1 ± 9.1*	124.56 ± 8.0*
DBP (mmHg)	81.4 ± 8.1	78.9 ± 7.0	77.1 ± 9.0	80.3 ± 9.1	79.1 ± 8.1	78.2 ± 7.0	80.3 ± 9.1	78.6 ± 4.1	78.3 ± 5.0	80.1 ± 5.0	79.3 ± 8.0	79.2 ± 7.0

HP: high protein/low carbohydrate. S: standard. DBP: Diastolic blood pressure. Mths: Months BMI: body mass index. SBP: Systolic blood pressure. DBP: Diastolic blood pressure WC: Waist circumference.

*p < 0.05, in each genotype group with basal values. No differences between genotypes groups.

Table 2
Biochemical parameters (mean ± SD)

Characteristics	DIET HP (n = 137)						DIET S (n = 133)					
	GG(n = 65)			*GG+ GT* (n = 72)			*GG* (n = 57)			*GG+ GT* (*n = 75*)		
	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths
Glucose (mg/dl)	103.5 ± 8.0	98.7 ± 8.1*	98.4 ± 7.1*	106.9 ± 10.2	104.9 ± 8.0	104.6 ± 4.1	101.7 ± 8.0	97.8 ± 9.0*	97.4 ± 8.1*	100.7 ± 9.1	98.5 ± 6.2	98.3 ± 7.1
Total ch. (mg/dl)	211.1 ± 9.1	200.1 ± 10.1*	198.7 ± 22.7*	206.2 ± 22.0	199.2 ± 11.0	199.4 ± 9.1	205.3 ± 21.9	195.1 ± 18.4*	193.3 ± 10.4*	218.5 ± 10.2	214.5 ± 9.7	213.8 ± 20.9
LDL-ch. (mg/dl)	135.3 ± 10.1	124.5 ± 12.1*	122.1 ± 11.3*	126.4 ± 21.2	120.3 ± 19.1	121.1 ± 13.1	127.1 ± 10.5	117.3 ± 10.2*	115.8 ± 11.0*	124.1 ± 12.1	119.3 ± 20.2	118.3 ± 19.1
HDL-ch. (mg/dl)	52.1 ± 9.1	52.7 ± 8.1	53.8 ± 7.0	50.1 ± 8.0	50.9 ± 9.2	49.8 ± 8.1	51.8 ± 10.2	52.2 ± 9.1	52.1 ± 9.0	55.0 ± 12.3	54.9 ± 8.9	55.3 ± 11.0
TG (mg/dl)	120.8 ± 18.1	116.3 ± 12.4*	108.1 ± 12.2*	127.6 ± 12.1	123.1 ± 11.2	121.9 ± 30.3	125.1 ± 22.6	115.4 ± 23.1*	108.1 ± 11.1*	124.1 ± 21.3	119.3 ± 32.3	118.9 ± 31.9
Insulin (mUI/L)	11.1 ± 5.1	8.8 ± 3.1*	7.1 ± 4.1*	10.9 ± 7.0	10.1 ± 9.0	9.2 ± 7.4	10.9 ± 5.1	8.9 ± 3.1*	7.7 ± 3.0*	11.1 ± 5.0	10.3 ± 4.1	10.5 ± 7.1
HOMA-IR	2.1 ± 0.9	1.9 ± 0.5	1.7 ± 0.8*	2.1 ± 1.2	2.0 ± 2.1	2.0 ± 1.3	2.2 ± 1.1	2.0 ± 0.9*	1.5 ± 1.0*	2.3 ± 1.0	2.2 ± 1.1	2.1 ± 1.4
CRP (mg/dl)	5.0 ± 3.0	4.9 ± 2.9	4.7 ± 3.1	4.9 ± 3.0	5.0 ± 3.1	4.9 ± 3.3	5.0 ± 4.1	4.9 ± 3.0	5.0 ± 4.0	5.0 ± 4.1	5.0 ± 3.8	5.1 ± 3.1

HP: high protein/low carbohydrate. S: standard. Ch: Cholesterol. TG: Triglycerides CRP: c reactive protein. HOMA-IR: Homeostasis model assessment. LDL: low density lipoprotein,. HDL: High density lipoprotein. Mths: months.
*$p < 0.05$, in each group with basal values. No statistical differences among genotypes in each diet or in different diet groups.

In the present study, there was no relationship between this allele and the basal levels of these parameters, but we detected an association between the modification of the lipid, insulin, and adiponectin levels.

Adiponectin is a well-known adipokine with anti-inflammatory properties that has been shown to increase insulin sensitivity by increasing fatty acid oxidation and inhibition of hepatic glucose metabolism [32]. This fact may be what explains the findings of insulin resistance improvement found in the same genotype group in our study (non–T-allele carriers) with both diets. The present results suggested that rs1501299 variant of the *ADIPOQ* gene is associated with differential regulation of adiponectin synthesis and secretion in adipose tissue secondary to weight loss with a better metabolic improvement in non–T-allele carriers. This is an important fact, taking into account the relationship between adiponectin and insulin resistance. It is possible that not only the restriction of calories with the secondary loss of weight but also the components of the diet influence this relationship. For example, in a non-interventional study with healthy women with this variant [17], non–T-allele carriers showed higher adiponectin levels than T carriers under conditions of low-fiber intake. Also, in a one-branch interventional design [33], after a 12-wk weight loss intervention in obese individuals, the significant decreases in insulin resistance and increase in adiponectin levels were observed in non–T-allele carriers, as in the present study results. Moreover, some interventional studies [34] showed a lack of interaction of rs1501299 and diet with biochemical parameters.

On the other hand, in addition to the caloric restriction and the composition of the diet, the duration of the intervention is also an important factor. Most of the previous studies were short duration. For example, in one study with three different diets for 4 wk each (saturated fatty acid–rich diet versus carbohydrate-rich diet versus MUFA-rich diet) [35], rs1501299 did not show a statistical relationship with metabolic changes secondary to diet modifications. In one study with a dietary intervention of 3 mo with a hypocaloric diet with Mediterranean pattern, T-allele carriers of this SNP showed a lack of response of insulin resistance, fasting glucose, and LDL-C without effect on adiponectin levels [22]. In another 3-mo randomized clinical trial of a moderate carbohydrate hypocaloric diet versus a low-fat hypocaloric diet, only non–T-allele carriers showed a significant decrease in insulin resistance and adiponectin levels [23]. Finally, the RISCK (Reading, Imperial, Surrey, Cambridge and Kings) [36] study, a randomized controlled trial of 24 wk with three different diets (reference versus high MUFA and low-fat diet), did not show a statistical relationship with lipid or glycemic metabolism and this SNP.

In addition to the duration of the intervention, the intensity of the weight loss also is another variable to consider. For example, GG genotype in morbidly obese individuals is associated with increases of adiponectin levels and better decrease of HOMA-IR and insulin levels after a massive weight loss with a biliopancreatic diversion surgery and 3 y of follow up [37]. The molecular mechanisms responsible for the interaction between dietary intake and weight loss with this genetic variant (rs1501299) remained unclear. First, we can hypothesize an activation of peroxisome proliferator-activated receptor-γ by some fatty acids from the diet; this fact could change serum adiponectin levels because of the potential linkage disequilibrium between peroxisome proliferator response element and the *ADIPOQ* gene. Second, perhaps the rs1501299 variant could directly influence metabolic parameters, as demonstrated with LDL-C levels [38,39] and blood pressure [40,41].

Limitations of the present study included the fact that epigenetic modifications also can play a role in the results found, and in

Table 3
Circulating adypocitokines (mean ± SD)

	DIET HP (n = 137)						DIET S (n = 133)					
	GG (n = 65)			*GG+ GT* (n = 72)			*GG* (n = 57)			*GG+ GT (n = 75)*		
Characteristics	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths
Adiponectin (ng/ml)	10.0 ± 3.1	15.6 ± 2.2*	20.1 ± 5.2*	10.2 ± 4.1	12.1 ± 6.1	12.8 ± 5.3	11.0 ± 4.9	16.9 ± 2.1*	21.9 ± 6.2*	9.8 ± 5.4	10.2 ± 4.6	10.4 ± 5.1
Resistin (ng/ml)	6.1 ± 3.0	6.0 ± 2.1	6.3 ± 4.1	6.1 ± 3.2	6.0 ± 3.3	6.3 ± 3.1	8.1 ± 2.3	7.9 ± 3.5	7.8 ± 2.3	7.9 ± 3.0	7.6 ± 4.1	7.2 ± 4.1
Leptin (ng/ml)	31.0 ± 12.1	15.9 ± 8.3*	12.3 ± 4.1*	33.1 ± 8.1	16.8 ± 6.1*	15.3 ± 7.3*	33.9 ± 13.0	16.3 ± 9.0*	12.0 ± 6.1*	40.1 ± 6.9	18.1 ± 7.2*	14.3 ± 10.1*

*$p < 0.05$, in each group with basal values. No statistical differences among genotypes in each diet or in different diet groups.

the present study, an epigenetic analysis was not carried out. The determination was of total adiponectin rather than the most bioactive high-molecular-weight form, but a strong correlation has been described between the two measures. The study included a relatively small sample size. The study population was a white, obese adult sample without comorbidities; these factors could modulate the response to dietary intervention. Recently, a study [42] with obese individuals and metabolic syndrome showed a greater response to the lifestyle intervention in T-allele carriers than in non−T-allele carriers. Some factors could be implied to understand this apparent discrepancy, for example, differences in the lifestyle intervention such as caloric deficit or improvement in diet quality and length of intervention. And finally, the *ADIPOQ* gene effect of this study is purely statistical but not mechanistic. There could be other unknown factors responsible for the results.

Conclusion

After two different hypocaloric diets during 9 mo of intervention, the GG genotype of *ADIPOQ* gene variant (rs1501299) was associated with a better improvement in adiponectin levels, insulin resistance, and lipid profile than T-allele carriers.

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