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Research Article

Role of the Variant Rs3774261 of Adiponectin Gene on Cardiovascular Risk Factors and Adiponectin Levels After a High Fat Hypocaloric Diet with Mediterranean Pattern

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Abstract

Background: The role of *ADIPOQ* gene on serum lipid and adiponectin levels after a dietary intervention remained unclear. Some polymorphisms have been described in this gene that they could interact with metabolic changes after diet intervention.

Objective: Our aim was to analyze the effects of a high fat hypocaloric diet with a Mediterranean dietary pattern on metabolic response and adiponectin levels taking to account of rs3774261 of *ADIPOQ*.

Design: A population of 284 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 high fat hypocaloric diet (12 weeks). All patients were genotyped in the rs3774261.

Results: The genotype distribution was; 78 patients (27.5%) AA, 144 patients AG (50.7%) and 62 patients GG (21.8%). After a significant weight loss secondary to diet intervention (AA vs. AG vs. GG); total cholesterol (delta:-15.1 ± 2.9 mg/dl vs. -7.2 ± 2.1 mg/dl vs. -5.0 ± 1.9 mg/dl; p=0.03), LDL cholesterol (delta:-14.1 ± 2.9 mg/dl vs. -6.4 ± 1.8 mg/dl vs. -3.2 ±

2.0 mg/dl; p=0.01), triglyceride levels (delta:-26.0 ± 4.6 mg/dl vs 6.4 ± 3.3 mg/dl vs. -5.7 ± 2.9 mg/dl; p=0.01), C reactive protein (CRP) (delta:-2.4 ± 0.1 mg/dl vs -1.3 ± 0.2 mg/dl vs. -0.7 ± 0.1 mg/dl; p=0.01) and serum adiponectin levels (delta: 17.6 ± 3.9 ng/dl vs. 1.9 ± 3.3 ng/dl vs. 2.1 ± 1.3 ng/dl; p=0.02) improved only in AA group.

Conclusion: Subjects with AA genotype of *ADIPOQ* variant (rs3774261) showed a significant increase in serum levels of adiponectin and decrease on lipid profile and C-reactive protein (CRP) after weight loss with a hypocaloric high fat Mediterranean diet.

Keywords: Adiponectin; High fat; Mediterranean diet; Single nucleotide polymorphism

1. Introduction

Adipose tissue stores fats during periods of increase in energy supply**,** but this tissue is also an important endocrine organ secreting various adipokines involved in the regulation of metabolism and energy homeostasis [1]. Adiponectin is the most abundant adipokine secreted by this tissue [1]. Usually the adipose tissue secretes inflammatory adipokines, however adiponectin has an anti-inflammatory role and its levels are decreased in obese subjects and are increased after weight reduction [2]. For example, in the literature low adiponectin levels have been associated with a high risk of obesity, diabetes mellitus, hyperlipidemia and insulin resistance [3-5]. Moreover, some studies have shown a potential therapeutic effect with agonists of adiponectin [6]. The production of adiponectin has a strong genetic component associated with different variants of *ADIPOQ*, with heritability estimated at 80% [7]. Single nucleotide polymorphisms (SNPs) are genetic variations that can sometimes have functional implications in the adiponectin C1Q and collagen domain containing (*ADIPOQ*), which located on chromosome 3q27. One of these SNPs, 712 G/A rs3774261 in the *ADIPOQ* has been related with metabolic disturbances [7]. This genetic variant has even been related with cardiovascular events, too [8-9]. The main approach for obesity is dietary treatment, with moderate caloric restrictions and different eating patterns. Despite the importance of the adiponectin pathway and its SNPs on different comorbidities related to obesity and with weight loss, there is only one study in the literature that evaluate the role of rs3774261 after a hypocaloric diet [10] with relevant results on lipid and inflammatory markers. This is a research area with interest, since weight reduction increases serum adiponectin levels [11] and it has potential advantages. It is necessary to take in count, which the beneficial effects of a diet with a Mediterranean pattern can be due to not only weight loss but also to the presence of different nutrients such as type of dietary unsaturated fatty acids and fiber [12]. Unsaturated fatty acids are ligand for the transcription factor PPARgamma [13], which upregulates *ADIPOQ* gene expression and increases adiponectin concentration [14]. Perhaps increasing the amount of unsaturated fat in a hypocaloric diet with a Mediterranean pattern would have greater benefits than a conventional hypocaloric diet and rs3774261 would modulate these changes. Given this lack of studies and that, we realized a study in order to analyze the effects of a high fat hypocaloric diet with a Mediterranean dietary pattern on metabolic response and adiponectin levels taking to account of rs3774261 of *ADIPOQ*.

2. Subjects and Methods

2.1 Subjects

This trial was a single-center trial with 284 obese Caucasian subjects, dietary intervention study conducted in our Hospital. Our Ethics Committee (HCUVA Committee) approved the study and it was in accordance with the guidelines laid down in the Declaration of Helsinki. All participants gave written informed consent. Who were men and women aged between 45-70 y recruited from Primary Care Physicians of an urban area of Spain. Eligibility for the study was based on age from 25 till 70 years and the presence of obesity diagnosis as a body mass index (BMI) $\geq$30 kg/m^2. The exclusion criteria were the following; a history of cardiovascular disease, thyroid disease, renal or hepatic disorders, malignant tumor, and within the 6 months before the study were 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) or nutrient supplements. Data of 284 participants were collected at the beginning and after 12 weeks of dietary treatment, all subjects assigned to the diet completed the study. Systolic and diastolic blood pressure and anthropometric parameters (weight, height, body mass index (BMI), waist circumference, fat mass by impedance) were recorded at both times. Blood samples for the analysis of insulin, total cholesterol, low density lipoprotein (LDL) cholesterol, High density lipoprotein (HDL) cholesterol, triglycerides, serum adipokines levels (leptin, total adiponectin and resistin), C reactive protein (CRP) were drawn in EDTA-treated after a minimum 8-h overnight fast.

2.2 Nutritional treatment

This study was aimed to achieving a calorie reduction of 500 daily calories to the usual intake. The subjects during this interventional study (12 weeks) received individualized counseling on a high fat hypocaloric diet with a Mediterranean dietary pattern diet and exercise. At baseline, the normal dietary habits of subjects were assessed by using 3- day food records. Tables with color food photographs 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) [15]. The percentage of macronutrients was; 38% of carbohydrates, 38% of fats and 24% of proteins. Percentage of fats was 45.0% of monounsaturated fats, 30.0% of saturated fats and 25.0% of polyunsaturated fats. All participants had two individual sessions (90 minutes with diet sheets and example menu plans) with the dietitian at the start of the intervention to explain the diet and solve doubts. The same dietitian assessed the completion of the diet each 7 days. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day, before the dietary intervention and after 12 weeks of the intervention. Records were analyzed with a computer-based data evaluation system (Dietosource ®, Ge,Swi) with national composition food tables were used as reference [15]. The exercise program consisted of an aerobic exercise at least 3 times per week (60 min each, reaching a total of 180 minutes each week) and the patient recorded it with a self-reported questionnaire.

2.3 Genotyping rs3774261 variant

The genotype of SNP rs3774261 of *ADIPOQ* was determined with a polymerase chain reaction at real-time. Genomic DNA was extracted from 150 uL buffy

coat using a blood genomic kit (Bio-Rad®, Hercules, CA) according to the manufacturer´s instructions. Oligonucleotide primers and probes were performed with the Beacon Designer 5.0 (Premier Biosoft International ®, LA, CA). The polymorphic region of adiponectin was amplified using the polymerase chain reaction (PCR) with 50 ng of this genomic DNA, with allele specific sense primers (primer forward: 5'-ACGTTGGATGCTCCTCCTTGAAGCCTTCAT -3' and reverse 5'-ACGTTGGATGCAAGTATTCAAAGTATGGAGC -3' in a 2 µL final volume (Termocicler Life Tecnologies, LA, CA). Ciclyng parameters were as follows: after DNA denaturation at 95ºC for 1 min and annealing at 65ºC for 30 sec. The PCR were run in a 25 µL final volume containing 10.5 µL of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase. The fluorescence signals were detected at excitation an emission wavelengths of 485 nm and 612 nm, respectively.

2.4 Measurements

2.4.1 Lipid metabolism: The methods used for the measurement of plasma lipid levels (total cholesterol and triglycerides) were by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA). HDL cholesterol was measured enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium and finally LDL cholesterol was calculated using Friedewald formula [16].

2.4.2 Glucose metabolism: The method used for measurement of glucose levels were an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L) [17] and the homeostasis model assessment was used to evaluate insulin resistance (HOMA-IR) with this equation (glucose*insulin/22.5) [18].

2.4.3 C reactive protein and adipokines: CRP was determined by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl. Serum adiponectin levels were analyzed by enzyme immunoassay (ELISA) (R&D systems, Inc., Minnesota, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml [19]. Resistin levels were measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml [20]. Leptin levels was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml [21].

2.5 Anthropometric parameters

Height and weight were determined with a telescopic height-measuring instrument (Omrom, LA, CA, USA) and an electrical scale (Omrom, LA, CA, USA), respectively. BMI (body mass index) was calculated as body weight (kg) divided by height (m^2). Waist circumference (WC) was measured with a flexible non-stretchable measuring tape (Type SECA, SECA. Birmingham, UK). Fat mass was assessed by impedance with an accuracy of 5 g [22] (EFG, Akern, It). Blood pressure was determined three times at 5 minutes intervals using a mercury sphygmomanometer (Omrom, LA, CA., USA), and averaged.

2.6 Statistical analysis

Genotype distribution was tested for deviation from the Hardy-Weinberg equilibrium by CHi squared test

with 1 d,f ($p>0.05$). Statistical analyses were carried out using the SPSS for Windows, version 23.0 software package (SPSS Inc. Chicago, IL). Sample size was calculated to detect changes over 5 ng/dl in adiponectin levels after dietary intervention with 90% power and 5% significance (n=280). Normal distribution was evaluated by Q-Q plots. The results were showed as average+/- standard deviation. Numerical variables with normal distribution were analyzed with a two-tailed Student's t-test. Non-parametric variables were analyzed with the Mann-Whitney´s U- test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. 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. Bonferroni test was applied for multiple testing to reduce Type I error in association analysis. Finally, p values in Tables 1, 2 and 3 are as follow; first p, statistical differences after dietary intervention in AA genotype, second p, statistical differences in AG genotype after intervention, third p, statistical differences in GG genotype, fourth p, significance between basal values in different genotypes and fifth p, significance between post-treatment values. A p-value <0.05 was considered significant.

3. Results

3.1 Characteristics of participants

We investigate the role of SNP rs3774261 on the change of biochemical parameters and serum adipokine levels in 284 obese subjects. The mean age of the all population was 49.3 ± 5.1 years (range: 26-63) and the average body mass index (BMI) was 37.3 ± 4.9 kg/m^2 (range: 32.4-39.9). Gender distribution was 208 females (73.2%) and 76 males (26.8%). The genotype distribution of this population was as follow; 78 patients (27.5%) AA, 144 patients AG (50.7%) and 62 patients GG (21.8%). Hardy Weinberg equilibrium in genotype frequencies was confirmed (p=0.31). Gender distribution was similar in all genotype groups (AA; 71.8% females vs. 28.2% males, AG; 72.9% females vs 27.1% females and GG; 75.8% females vs 24.2% males). The average age was similar in all genotype groups (AA; 49.3 ± 4.1 years vs. AG; 49.7 ± 4.2 years vs. GG; 48.1 ± 4.3 years: ns), too. Following the recommendations and sessions of the dietitian, the dietary recommendations were reached as indicated in method section; a total caloric amount of 1433.1 ± 128.2 calories, the percentage of macronutrients was; 39.1% of carbohydrates, 37.4% of fats and 24.5% of proteins. Percentage of fats was 45.4% of monounsaturated fats, 29.6% of saturated fats and 25.0% of polyunsaturated fats, without statistical differences among genotype groups. Basal physical activity was similar in the three groups (AA vs AG vs GG) (128.2 ± 16.3 min/week *vs.* 131.1 ± 21.9 min/week *vs.* 127.1 ± 18.2 min/week; p=0.33). In addition, after the intervention, the physical activity increased, but this activity was similar that basal without differences in total quantity (148.2 ± 21.1 min/week *vs.* 151.9 ± 23.1 min/week *vs.* 150.9 ± 21.2 min/week; p=0.31) or deltas (20.2 ± 1.1 min/week *vs.* 20.8 ± 2.2 min/week *vs.* 23.1 ± 1.9 min/week; p=0.52).

3.2 Anthropometric results

For rs3774261, there were no statistical differences in anthropometric parameters and blood pressure in basal and post-intervention values (AA vs AG vs GG) (Table 1). The following parameters improved after the high fat hypocaloric diet with a Mediterranean dietary pattern in all genotypes (AA vs. AG vs. GG); BMI (delta:-1.5 ± 0.4 kg/m^2 vs. -1.6 ± 0.2 kg/m^2 vs. -1.0 ± 0.4 kg/m^2; p=0.23), weight (delta:-4.2 ± 2.1 kg

vs. -3.9 ± 1.9 kg vs. -3.8 ± 1.5 kg; p=0.31), fat mass (delta:-3.5 ± 1.1 kg vs. -3.3 ± 1.0 kg vs. -3.0 ± 1.1 kg; p=0.30), waist circumference (delta:-4.4 ± 1.2 cm vs. -3.7 ± 1.5 cm -4.0 ± 1.3 cm; p=0.38) and systolic blood pressure (delta:-8.7 ± 2.1 mmHg vs. -9.5 ± 1.8 mmHg vs. -11.5 ± 2.4 mmHg; p=0.36). These statistically significant improvements were similar in all genotype groups.

3.3 Biochemical results

In the second analysis of our design, we studied the effects of dietary intervention on glucose metabolism, lipid profile (Table 2) and serum adipokine levels (Table 3). After weight loss secondary to diet intervention (AA vs. AG vs. GG); insulin levels (delta:-6.4 ± 0.9 UI/L vs 6.4 ± 1.1 UI/L vs 5.7 ± 1.0 UI/L; p=0.38) and HOMA-IR (delta:-2.2 ± 0.2 units vs -2.1 ± 0.3 units vs -2.4 ± 0.4 units; p=0.31) improved in all genotypes without intergroup differences in these statistical improvements. Finally, after 12 weeks with the dietary intervention (AA vs AG vs GG); total cholesterol (delta:-15.1 ± 2.9 mg/dl vs. -7.2 ± 2.1 mg/dl vs. -5.0 ± 1.9 mg/dl; p=0.03), LDL cholesterol (delta:-14.1 ± 2.9 mg/dl vs. -6.4 ± 1.8 mg/dl vs. -3.2 ± 2.0 mg/dl; p=0.01), triglyceride levels (delta:-26.0 ± 4.6 mg/dl vs 6.4 ± 3.3 mg/dl vs. -5.7 ± 2.9 mg/dl; p=0.01) and C reactive protein (CRP) (delta:-2.4 ± 0.1 mg/dl vs -1.3 ± 0.2 mg/dl vs. -0.7 ± 0.1 mg/dl; p=0.01) improved only in AA group.

3.4 Adipokine levels

Table 3 shows modifications on serum adipokines. After weight loss and in AA genotype group (AA vs. AG vs GG) serum adiponectin (delta: 17.6 ± 3.9 ng/dl vs. 1.9 ± 3.3 ng/dl vs. 2.1 ± 1.3 ng/dl; p=0.02) increased. In addition, patients with any genotype showed a significant decrease on leptin levels. Serum resistin levels remained unchanged during the intervention trial in both genotype groups.

Parameters	**AA (n=78)**		**AG+GG (n=144)**		**AG+GG (n=62)**		
	Basal	**3 months**	**Basal**	**3 months**	**Basal**	**3 months**	**P values** **-Time AA** **- Time AG** **- Time GG** **- Basal Genotype** **- 3 months genotype**
BMI	37.6 ± 7.1	35.1 ± 7.7*	37.7 ± 5.7	35.1 ± 5.5*	37.2 ± 6.3	35.2 ± 7.7*	P=0.02 P=0.01 P=0.03 P=0.36 P=0.37
Weight (kg)	90.5 ± 19.6	86.3 ± 18.1$	94.2 ± 17.2	90.3 ± 17.3$	94.7 ± 17.1	90.9 ± 12.3$	P=0.03 P=0.02 P=0.01 P=0.45 P=0.46
Fat mass (kg)	39.5 ± 12.2	36.0 ± 11.1#	39.9 ± 11.1	36.6 ± 11.0#	40.0 ± 12.4	37.0 ± 5.0#	P=0.03 P=0.02 P=0.02 P=0.43 P=0.39
WC (cm)	107.7 ± 15.1	103.3 ± 17.1&	108.6 ± 14.9	104.9 ± 14.1&	108.7 ± 13.1	104.8 ± 10.1&	P=0.03 P=0.01 P=0.02 P=0.48

							P=0.51
SBP (mmHg)	128.0 ± 13.2	119.3 ± 12.9* *	129.1 ± 13.1	120.6 ± 11.2* *	130.1 ± 15.1	122.2 ± 12.2* *	P=0.03 P=0.01 P=0.04 P=0.41 P=0.39
DBP (mmHg)	81.3 ± 6.1	78.1 ± 10.1	83.4 ± 8.0	81.0 ± 9.0	84.1 ± 9.0	82.0 ± 7.0	P=0.51 P=0.62 P=0.63 P=0.55 P=0.45

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 12 weeks in AA genotype, second p, significance of dietary intervention after 12 weeks in AG genotype, third p, significance of dietary intervention after 12 weeks in GG genotype, fourth p, significance among basal parameters and fifth p significance among 12 weeks parameters.

Table 1: Antropometric Parameters And Blood Pressure (mean ± SD).

Parameters	**AA (n=78)**		**AG(n=144)**		**GG(n=62)**		**P values**
	Basal	**3 months**	**Basal**	**3 months**	**Basal**	**3 months**	**-Time AA** **- Time AG** **- Time GG** **- Basal Genotype** **- 3 months genotype**
Glucose (mg/dl)	99.2 ± 15.1	96.5 ± 15.0	101.1 ± 11.0	97.5 ± 12.1	100.9 ± 11.0	97.8 ± 7.1	P=0.37 P=0.56 P=0.42 P=0.43 P=0.48
Total cholesterol (mg/dl)	204.1 ± 6.7	190.3 ± 8.2$	200.9 ± 14.1	193.7 ± 18.2	200.6 ± 14.1	195.6 ± 17.2	P=0.03 P=0.51 P=0.52 P=0.34 P=0.37
LDL-cholesterol (mg/dl)	126.4 ± 6.3	112.3 ± 7.9 #	123.6 ± 12.1	116.5 ± 17.1	119.6 ± 15.1	116.8 ± 19.1	P=0.01 P=0.49 P=0.35 P=0.34 P=0.39
HDL-cholesterol (mg/dl)	55.4 ± 14.1	56.7 ± 13.1	54.7 ± 11.0	52.3 ± 10.1	52.7 ± 13.0	51.3 ± 12.1	P=0.21 P=0.47 P=0.39 P=0.45 P=0.47

Triglycerides (mg/dl)	126.6 ± 14.1	100.6 ± 12.2 +	117.5 ± 15.2	113.9 ± 16.2	116.9 ± 12.2	112.6 ± 11.2	P=0.02 P=0.61 P=0.51 P=0.45 P=0.39
Insulin (mUI/l)	19.2 ± 3.0	12.8 ± 3.1&	18.3 ± 5.2	11.9 ± 3.0&	18.6 ± 4.1&	12.9 ± 4.6&	P=0.02 P=0.03 P=0.01 P=0.41 P=0.38
HOMA-IR	5.5 ± 0.9	3.3 ± 1.1**	5.3 ± 1.1	3.3 ± 1.8**	5.5 ± 0.8	3.1 ± 1.1**	P=0.01 P=0.02 P=0.03 P=0.43 P=0.40
CRP	7.5 ± 1.3	5.1 ± 0.8&&	6.2 ± 1.1	4.9 ± 2.6	6.2 ± 2.1	5.5 ± 3.2	P=0.01 P=0.36 P=0.37 P=0.43 P=0.45

HOMA-IR (homeostasis model assessment). CRP (C reactive protein) Statistical differences P<0.05, in each genotype group (*glucose, total cholesterol $, LDL cholesterol #, triglycerides +,insulin &, HOMA IR **, CRP&&); 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 12 weeks in AA genotype, second p, significance of dietary intervention after 12 weeks in AG genotype, third p, significance of dietary intervention after 12 weeks in GG genotype, fourth p, significance among basal parameters and fifth p significance among 12 weeks parameters.

Table 2: Biochemical parameters (mean ± SD).

Parameters	AA (n=78)		AG(n=144)		GG(n=62)		P values -Time AA - Time AG - Time GG - Basal Genotype - 3 months genotype
	Basal	3 months	Basal	3 months	Basal	3 months	
Resistin (ng/dl)	3.9 ± 2.1	3.8 ± 3.0	3.7 ± 1.7	3.7 ± 1.9	4.0 ± 2.1	3.9 ± 1.9	P=0.52 P=0.61 P=0.54 P=0.45 P=0.40
Adiponectin (ng/dl)	27.7 ± 8.1	34.1 ± 5.0$	36.1 ± 5.1	31.1 ± 5.2	34.8 ± 7.1	36.3 ± 7.0	P=0.02 P=0.51 P=0.52 P=0.56 P=0.49
Leptin (ng/dl)	102.2 ± 19.6	74.7 ± 12.5*	99.2 ± 13.1	67.8 ± 9.1*	94.2 ± 12.1	67.1 ± 7.1*	P=0.02 P=0.01 P=0.03 P=0.33 P=0.45

Statistical differences P<0.05, in each genotype group (* leptin $ adiponectin); First p, significance of dietary intervention after 12 weeks in AA genotype, second p, significance of dietary intervention after 12 weeks in AG genotype, third p, significance of dietary intervention after 12 weeks in GG genotype, fourth p, significance among basal parameters and fifth p significance among 12 weeks parameters.

Table 3: Serum Adipokine levels (mean ± SD).

4. Discussion

We disclosed, in this study on a high fat hypocaloric diet with a Mediterranean pattern, that a decrease in total cholesterol, LDL-cholesterol and Protein C reactive and an increase in serum adiponectin levels was significantly greater in the obese subjects with the AA genotype than those with the AG or GG genotypes of rs3774261. While all subjects in all genotype groups showed a significant decrease or body weight. To our knowledge, there is little evidence for this SNP in relation to dietary modifications. There are some studies studying the relationship between this genetic variant (rs3774261) on *ADIPOQ* gene and diabetes mellitus, obesity and serum adiponectin levels [23-24], with a clear relationship of some adiponectin gene variants and the risk towards the development of type 2 diabetes, obesity and hypoadiponectinemia in non-Caucasian population [24]. This polymorphism has also been associated with other pathologies such as polycystic ovary syndrome, also related to metabolic syndrome and insulin resistance [25]. As we mentioned above, there is a lack of information about the effect of dietary treatment and this genetic variant in interventional designs. A previous study with a hypocaloric Mediterranean diet showed [10] a better improvement in lipid profile, CRP and adiponectin levels in subjects with AA genotype compared in a dominant model with (AG+GG). Our study shows that this genetic variant of *ADIPOQ* is associated with a differential regulation of adiponectin synthesis or secretion after weight loss and this relationship could contribute to our findings related with lipid profile and CRP changes. The amount of lipid profile improvement was similar in both studies [10]; however, the increase in adiponectin levels and the decrease in CRP was two times greater in the current study than in the previous one [10]. The populations were similar, middle-aged Caucasian and predominantly female. The caloric restriction was similar in both studies, about 500 calories to the previous intake. Although both strategies were carried out with a Mediterranean diet pattern, in the current study the percentage of fat in the diet was higher than previous one; 38% vs. 25%, reaching a daily intake of almost 30 grams of monounsaturated fat and 13 of polyunsaturated, compared to 14 grams and 3.6 grams, respectively, in the preceding study [10]. Few other studies have investigated the interaction between *ADIPOQ* variants and dietary intake of fat. In a interventional study with low hypocaloric diets, the Alsahel et al. [26] reported that other genetic variant (-1006G/A) in this gene increased serum adiponectin after a high-MUFA diet, whereas in A-allele carriers it decreased, with no changes after a low-fat diet. As ligands of PPARgama, dietary unsaturated fats, particularly at highest dose were expected to increase adiponectin concentration. Moreover, this relationship could explain our positive effects found in the lipid profile and inflammatory markers such as CPR. CRP is a systemic marker for inflammation and adiponectin contributes more strongly to CRP elevation than for example smoking habit, age and other metabolic parameters [27]. Adiponectin has an anti-inflammatory property in vitro. The NF-kB signaling in endothelium, which would induce CRP; was reportedly inhibited by adiponectin [27].

The SNP rs3774261 within *ADIPOQ* were strongly associated with serum adiponectin levels in Caucasian subjects [23, 28] and this association is independent of the body mass index of the patients [9]. Moreover, this relationship seems to be related to race, since it has only been found within European populations [29]. All these metabolic reactions are important because *a posteriori* they can be related with cardiovascular events. In this sense in the literature, this SNP has been

related to the risk of coronary heart disease [30]. Kanu et al. [26] reported that G allele of the rs3774261 *ADIPOQ* was significantly higher in coronary heart disease patients that controls. Other study with ischemic stroke patients [31] have demonstrated that this polymorphism in *ADIPOQ* are associated with risk for these events. Finally, the effect of this genetic variant on the response of insulin and HOMA-IR levels had also not been described in the literature after a hypocaloric diet. With observational designs, a relationship with the risk of type 2 diabetes mellitus has been clearly described [32]. In addition, patients carrying the A allele have greater insulin sensitivity demonstrated by euglycemic clamp [33]. There are several important strengths of this study as an interventional trial with an important sample size, but also some limitations. First, we only evaluated one SNP of *ADIPOQ*, so other genetic variants could be related with metabolic parameters. Second, the lack of a control group without diet might be a bias. Finally, the self-reported dietary intake is not reliable and it might include bias of under- or over-reporting. Taking into account that recently has described that rs3774261 variant is related to a disinhibition in eating behaviors [34] influencing food uptake, the self-reported intakes could be a biass.

In conclusion, Subjects with AA genotype of *ADIPOQ* variant (rs3774261) showed a significant increase in serum levels of adiponectin and decrease on lipid profile and C-reactive protein (CRP) after weight loss with a hypocaloric high fat Mediterranean diet. Therefore, we find it important not only to base the treatment of weight loss on a caloric restriction, but also on a diet with qualitative and quantitative modifications in fat content and its potential association with genetic variants of ADIPOQ gene.

Author Contributions

Daniel de Luis designed the study and realized statistical analysis. He contributed to the analysis of data for the work and revised it critically for important intellectual content. They 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.

Rosario Bachiller 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 and genotype. He contributed to the analysis of data for the work and revised it critically for important intellectual content. They 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.

Compliance with Ethical Standards

No funding.

Conflict of Interest

Daniel de Luis declares that he has no conflict of interest.

David Primo declares that he has no conflict of interest.

Rosario Bachiller declares that he has no conflict of interest.

Olatz Izaola declares that she has no conflict 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 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 Committee PI7/2017).

Informed Consent

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

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