Interaction of the variant in the adiponectin gene rs3774261 with serum lipid profile and adiponectin levels after 9 months with a high monounsaturated fat hypocaloric diet with Mediterranean pattern

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Abstract. – OBJECTIVE: The role of ADIPOQ gene variants on weight loss and serum lipid changes after a dietary intervention is an important topic area with little scientific evidence. We designed a study in order to analyze the effects of rs3774261 of ADIPOQ gene on metabolic response and adiposity parameters after a hypocaloric Mediterranean diet pattern for 9 months and with a high amount of monounsaturated fatty acids.

PATIENTS AND METHODS: 133 patients with obesity were enrolled. Adiposity parameters, blood pressure, and serum parameters (lipid profile, insulin, HOMA-IR, glucose, C reactive protein, adiponectin, resistin, and leptin levels) were measured, at basal time and after dietary intervention (3 and 9 months). All patients were genotyped rs3774261 and evaluated in a dominant model (AA vs. GA+AA).

RESULTS: Genotype frequencies were 46 (34.6%) AA, 66 (49.6%) AG, and 21 (15.8%) GG. After dietary intervention and in both genotypes, BMI, weight, fat mass, systolic blood pressure, waist circumference, glucose, insulin, HOMA-IR, and leptin decreased. In patients with the AA genotype, there was a significant improvement at (3 and 9 months) in low-density lipoprotein (LDL) cholesterol levels (-10.1±0.9 mg/dl vs. -5.6±1.7 mg/dl, p=0.01) (-19.1±0.9 mg/dl vs. -6.9±0.7 mg/dl, p=0.03), total cholesterol (-9.4±0.8 mg/dl vs. -5.8±0.9 mg/dl, p=0.02) (-17.4±1.8 mg/dl vs. -9.8±1.9 mg/dl, p=0.02), triglycerides (-12.3±0.8 mg/dl vs. -8.0±0.9 mg/dl, p=0.01) (-26.1±0.8 mg/dl vs. -11.0±0.3 mg/dl, p=0.01), C reactive protein (CRP) (-0.8±0.2 mg/dl vs. -0.4±0.3 mg/dl, p=0.01) (-1.1±0.2 mg/dl vs. -0.7±0.1 mg/dl, p=0.01) and adiponectin (28.2±11.1 ng/ml vs. 4.1±2.8 ng/ml, p=0.02) (30.1±8.1 ng/ml vs. 7.1±4.8 ng/ml, p=0.02). Finally, higher values of adiponectin and adiponectin/leptin ratio were detected at 3- and 9-months post-treatment in patients with AA genotype.

CONCLUSIONS: G allele carriers of ADIPOQ gene variant (rs3774261) showed no improvement in metabolism levels of adiponectin, adiponectin/leptin ratio, total-cholesterol, LDL-cholesterol, triglycerides, and CRP after weight loss with a hypocaloric fat monounsaturated diet.

Key Words: Adiponectin gene, Adiponectin, Lipid profile, Mediterranean diet, Monounsaturated fatty acids, rs3774261.

Introduction

Adiponectin is the most important adipokine released by adipose tissue. Adiponectin, unlike most adipokines, has an anti-inflammatory role, and its levels are elevated after weight loss and its levels are reduced in overweight subjects. Some investigations have demonstrated that adiponectin is related to lipid and glucose metabolism, in two different ways. First of all, low serum adiponectin levels may increase risks of metabolic syndrome, obesity, diabetes mellitus type 2, hyperlipidemia and insulin resistance. On the other hand, the second way, adiponectin is a potential target for therapeutic intervention in diabetes and obesity with analogues or different strategies to increase its serum circulating levels.

The serum circulating levels of adiponectin are dependent on certain variants of the gene ADIPOQ, this gene is located on chromosome 3q27. Even in some studies, ADIPOQ single-nucleotide polymorphisms (SNPs) have been associated with obesity and metabolic syndrome. One of these SNP, rs3774261 in the ADIPOQ gene, has been evaluated in the literature with different designs. It has been related even to cardiovascular events. Despite the importance of SNP on different comorbidities related to obesity and the potential improvement of these comorbidities with weight loss, studies in the literature...
are scarce. These interventional designs\textsuperscript{10-12} reported significant results on serum lipid profile and inflammatory markers after a dietary intervention of 3 months with different hypocaloric diets with Mediterranean patterns. The effect of macronutrient distribution and dietary fat profile in these hypocaloric diets could also explain the interesting results, with different responses in lipid levels and adiponectin. Perhaps the metabolic effects found in these studies\textsuperscript{8-12} and their relationship with this genetic variation are due to both factors; weight loss and the Mediterranean diet pattern used. The beneficial effects of a hypocaloric diet with a Mediterranean style can be due to the presence of different foods and nutrients, such as unsaturated fatty acids, and the duration of the nutritional intervention\textsuperscript{13}. In the present study, we will increase the contribution of monounsaturated fats in the diet, reduce energy intake, and increase the duration of dietary intervention, compared to previous studies in the literature\textsuperscript{10-12}. Perhaps increasing the ratio of monounsaturated fat in a hypocaloric diet and increasing the time of the dietary intervention would have greater benefits than a conventional hypocaloric diet, and rs3774261 would modulate these changes. Although it’s possible that monounsaturated fatty acids may have an impact and interact with the genetic variant of the \textit{ADIPOQ} gene, this theory has not been tested yet.

We designed a study in order to analyze the effects of rs3774261 of \textit{ADIPOQ} gene on metabolic response and adiposity parameters after a hypocaloric Mediterranean diet pattern for nine months and with a high amount of monounsaturated fatty acids.

\section*{Patients and Methods}

\subsection*{Subjects and Clinical Investigation}

A total of 133 Caucasian subjects with obesity from Valladolid (Northwest of Spain), volunteered to participate in the study and underwent a medical examination. The inclusion criteria were age between 20 and 60 years old, obesity diagnosis as a body mass index (BMI) $\geq$30 kg/m$^2$ with body weight stability over the last 3 months. Exclusion criteria included a history of cardiovascular disease or thyroid disease, renal or hepatic disorders, history of alcoholism, and malignant tumor. Subjects under medication for hyperlipidemia, hypertension, hyperuricemia, or other illness were not included in the study. All participants received written and verbal information about the nature and purpose of the study, and all provided written informed consent. The Ethics Committee (HCUVA Committee) approved the study and was in accordance with the guidelines laid down in the Declaration of Helsinki.

This study was designed as a 9-month controlled body weight-loss intervention, and data of the subjects were collected at the beginning and after 3 and 9 months of dietary treatment. After an overnight fast, waist circumference, hip circumference, body weight, height, and blood pressure were measured, and BMI was calculated. Blood samples were collected in EDTA-tube treated for analysis of insulin, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, serum adipokine levels (leptin, total adiponectin and resistin), C reactive protein (CRP) and measured homeostasis model assessment for insulin resistance (HOMA-IR). Adiponectin/leptin ratio was calculated as a quotient. The variant rs3774261 of \textit{ADIPOQ} gene was determined by polymerase chain reaction in real-time.

\subsection*{Diet Intervention}

133 subjects met the above criteria and were enrolled in the dietary intervention. Body weight reduction was induced by a low-energy mixed Mediterranean [33\% of carbohydrates (86.1 g/day), 33\% of fat (39.0 g/day), and 34\% of proteins (88.6 g/day)] diet pattern providing 700 kcal fewer calories than required. The percentages of different fat were 63.8\% of monounsaturated fats, 23.6\% of saturated fat, and 12.6\% of polyunsaturated fats. To optimize compliance, dietary instructions were reinforced with a weekly phone call by a dietician. Records of daily dietary intake for 3 days were evaluated with a computer-based data evaluation system (Dietsource\textsuperscript{®}, Geneve, Switzerland), and national composition food tables were used as reference\textsuperscript{16}. Food tables were used with a Mediterranean pattern, including (legumes, vegetables, poultry, whole grains, fish, fresh fruit, using olive oil, and limiting unhealthy fats). Indications of physical activity for patients were aerobic physical activity at least 3 times each week (45 min each). The exercises recommended by the protocol were (running, walking, cycling, and swimming). The patient recorded the exercise activity with a self-reported questionnaire.
Genotyping

Genomic DNA was isolated from the buffy coat of centrifuged whole blood using a blood genomic kit (Bio-Rad®; Hercules, CA, USA) according to the manufacturer’s instructions. Genotyping was carried out using TaqMan probes. The polymerase chain reaction (PCR) was carried out with 50 ng of this genomic DNA, 0.5 µL of each oligonucleotide primer (primer forward: 5’- ACGTTGGAATGCCTCTCTTGAAGCCTTCT -3’ and reverse 5’- ACGTTGGATGCAAGTATTCAAGTATGGAGC -3’) in a 2 µL final volume (Thermocycler Life Technologies, Los Angeles, CA, USA). The PCR was run in a 25 µL final volume containing 10.5 µL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed genotype frequencies. The variant was in Hardy-Weinberg equilibrium (p=0.31). The genotyping success rate was 100%, and no discordant genotypes were observed in duplicate samples.

Biochemical Analysis

Serum biochemistry analysis for glucose, insulin, C reactive protein (CRP), total cholesterol, HDL-cholesterol, and triglyceride levels was measured using the COBAS INTEGRA 400 analyser (Roche Diagnostic, Basel, Switzerland). LDL cholesterol was calculated using Friedewald formula (LDL cholesterol=total cholesterol-HDL cholesterol – triglycerides/5)17. Based on glucose and insulin levels, HOMA-IR was obtained using the next equation (glucose x insulin/22.5)18. Finally, all adipokine levels were determined by enzyme-linked immunosorbent assay (ELISA); resistin (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml, leptin (Biovendor Laboratory, Inc., Webster, Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml19, and adiponectin (R&D systems, Inc., Minneapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml20. Adiponectin/leptin ratio was calculated as a quotient.

Adiposity Parameters and Blood Pressure

Blood pressure was measured three times after a 10-minute rest time with a mercury sphygmomanometer (Omrom, Los Angeles, CA, USA) and the results were averaged. Body weight was measured through a scale with a precision of 50 g (Omrom, Los Angeles, CA, USA) and the body mass index was calculated as weight (kg)/height (m^2), classifying as obese patients with a body mass index greater than 30 kg/m^2. The waist circumference was also measured with a tape measure (Type SECA, SECA. Birmingham, UK) (narrowest diameter between the xiphoid process and the iliac crest). The hip circumference was also measured with a tape measure (Type SECA, SECA, Birmingham, UK) (the iliac crest). Total fat mass was obtained by impedance with an accuracy of 5 g21 (EFG BIA 101 Anniversary, Akern, Pontassieve, Italy). This formula was used (0.756 Height^2/Resistance) + (0.110 x Body mass) + (0.107 x Reactance) – 5.463.

Statistical Analysis

The sample size was calculated to detect differences over 4.5 kg in body weight loss with 90% power and 5% significance (n=130). The Kolmogorov-Smirnov test was used to analyze variable distribution. The results were shown 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 Mann-Whitney U test. Categorical variables were analyzed with the Chi-square test, with Yates correction if 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), 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 in AA genotype, second p, significance between AA genotypes vs. AG + GG baseline values, third p, significance of dietary intervention in AG + GG genotype, fourth p, significance between AA genotypes vs. AG + GG post-treatment values (3 months) and fifth p significance between AA genotypes vs. AG + GG post-treatment values (9 months). A Chi-square test was used to evaluate the Hardy-Weinberg equilibrium. All analysis was performed under a dominant genetic model rs3774261 G- allele as the risk allele (AG+GG vs. AA). All the data were analyzed using SPSS for Windows, version 23.0 software package (IBM Corp., Armonk, NY, USA). A p-value <0.05 was considered significant.
Results

We evaluated the effects of this SNP on the change of adiposity, classical biochemical parameters, and adipokines parameters in 133 subjects with obesity. Genotype frequencies were 46 (34.6%) AA, 66 (49.6%) AG, and 21 (15.8%) GG. Genotype distribution did not deviate from Hardy-Weinberger expectations. The mean age of the population was 47.6±8.1 years (range: 25-60), and the average body mass index (BMI) was 34.9±1.3 kg/m² (range: 31.4-37.2). The gender distribution was 101 females (75.9%) and 32 males (24.1%). The gender distribution was similar in the two genotype groups (AA vs. AG+GG), men (27.5% vs. 27.8%) and women (72.5% vs. 72.2%). Age was similar in both groups (47.7±7.0 years vs. 47.2±5.0 years: ns).

During the dietary intervention, the patients with AA genotype reached the recommendations of the prescribed diet; 1,016.4±65.9 calories per day with the following distribution of macronutrients; 37% carbohydrates (98.1±22.9 g/day), 33% fat (37.1±2.3 g/day) and 33% protein (85.6±7.7 g/day). The percentages of different fats in this diet were 63.1% monounsaturated fats, 23.9% saturated fats, and 12.6% polyunsaturated fats. In the group with genotype (AG+GG), the patients reached the dietary recommendations; 1,028.6±64.9 calories per day with the following distribution of

<table>
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<th>Parameters</th>
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<th>AG+GG (n=87)</th>
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<td>9 months</td>
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<tr>
<td><strong>BMI</strong></td>
<td>34.9±1.1</td>
<td>32.9±1.2*</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>89.0±1.2</td>
<td>84.1±1.1</td>
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<td><strong>Fat mass (kg)</strong></td>
<td>34.9±1.0</td>
<td>33.0±0.8*</td>
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<tr>
<td><strong>WC (cm)</strong></td>
<td>110.1±2.3</td>
<td>105.1±3.1</td>
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<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>129.1±2.2</td>
<td>122.1±3.1</td>
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<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>81.0±2.0</td>
<td>78.9±3.2</td>
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</table>

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, & WC, **SBP). First p, significance of dietary intervention in AA genotype; second p, significance between AA genotypes vs. AG + GG baseline values; third p, significance of dietary intervention in AG + GG genotype; fourth p, significance between AA genotypes vs. AG + GG post-treatment values 3 months, fifth p, significance between AA genotypes vs. AG + GG post-treatment values 9 months.

Table I. Antropometric parameters and blood pressure (mean ± SD).

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Table II. Biochemical parameters (mean±SD).

<table>
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<th>AG+GG (n=87)</th>
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<td></td>
<td>Basal</td>
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<td>9 months</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>105.8±2.0</td>
<td>100.1±2.1+</td>
<td>98.0±1.1+</td>
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<td>Total cholesterol (mg/dl)</td>
<td>216.4±2.1</td>
<td>207.1±1.0$</td>
<td>197.2±3.1$</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>138.9±4.1</td>
<td>128.8±3.2 #</td>
<td>119.9±2.9 #</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>54.4±2.6</td>
<td>55.9±1.9</td>
<td>56.1±1.3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>146.2±7.0</td>
<td>124.9±5.2*</td>
<td>120.1±3.2*</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>12.2±2.0</td>
<td>9.5±1.1&amp;</td>
<td>8.6±1.1&amp;</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0±0.4</td>
<td>2.1±0.3**</td>
<td>1.8±0.2**</td>
</tr>
<tr>
<td>CRP</td>
<td>5.2±0.3</td>
<td>4.3±0.2++</td>
<td>4.1±0.4++</td>
</tr>
</tbody>
</table>

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++). First p, significance of dietary intervention in AA genotype; second p, significance between AA genotypes vs. AG + GG baseline values; third p, significance of dietary intervention in AG + GG genotype; fourth p, significance between AA genotypes vs. AG + GG post-treatment values 3 months; fifth p, significance between AA genotypes vs. AG + GG post-treatment values 9 months.

macronutrients; 38% carbohydrates (101.1±22.3 g/day), 33% fat (35.1±12.3 g/day), and 28% protein (81.2±8.1 g/day). The percentages of different fats in this diet were 63.1% monounsaturated fat, 23.9% saturated fat, and 12.6% polyunsaturated fat. Basal dietary exercise was similar in both genotype groups (AA vs. AG+GG) (121.3±23.4 min/week vs. 118.9±21.2 min/week, p=0.67). No differences were detected at 3 months (125.4±11.8 min/week vs. 121.1±11.9 min/week, p=0.37) and at 9 months (126.3±13.8 min/week vs. 122.4±19.1 min/week, p=0.35).
We did not find significant interaction effects between the rs3774261 polymorphism and diet-induced changes on adiposity parameters and blood pressure (Table I). In both genotypes, BMI, weight, fat mass, waist circumference, and systolic blood pressure decreased at 3 months and 9 months. The decrease at 3 months was similar in both genotype groups (AA vs. AG+GG) (BMI: -2.0±0.3 kg/m² vs. -1.9±0.2 kg/m², p=0.28), weight (-4.9±1.1 kg vs. -5.1±1.0 kg, p=0.38), fat mass (-1.9±0.2 kg vs. -1.3±0.2 kg, p=0.58) and systolic blood pressure (-7.0±2.0 mmHg vs. -6.8±1.2 mmHg, p=0.34). The decrease at 9 months was similar in both genotype groups (AA vs. AG+GG) (BMI: -2.4±0.3 kg/m² vs. -2.2±0.1 kg/m², p=0.29), weight (-6.5±1.1 kg vs. -6.2±1.0 kg, p=0.35), fat mass (-2.9±0.2 kg vs. -3.1±0.9 kg, p=0.64) and systolic blood pressure (-7.9±2.3 mmHg vs. -7.7±1.8 mmHg, p=0.36). There were no significant differences in diastolic blood pressure levels throughout the study. The pre-treatment and post-treatment nutritional values were similar in both groups.

Moreover, we observed significant interaction effects between the rs3774261 polymorphism and diet-induced changes on classical biochemical parameters (Table II). In patients with the AA genotype, there was a significant decrease at 3 months in LDL cholesterol levels (-10.1±0.9 mg/dl vs. -5.6±1.7 mg/dl, p=0.01), total cholesterol (-9.4±0.8 mg/dl vs. -5.8±0.9 mg/dl, p=0.02), triglycerides (-12.3±0.8 mg/dl vs. -8.0±0.9 mg/dl, p=0.01) and CRP (-0.8±0.2 mg/dl vs. -0.4±0.3 mg/dl, p=0.01). At 9 months, the decrease in these parameters was also significant in patients with the AA LDL cholesterol genotype (-19.1±0.9 mg/dl vs. -6.9±0.7 mg/dl, p=0.03), total cholesterol (-17.4±1.8 mg/dl vs. -9.8±1.9 mg/dl, p=0.02), triglycerides (-26.1±0.8 mg/dl vs. -11.0±0.3 mg/dl, p=0.01) and CRP (-1.1±0.2 mg/dl vs. -0.7±0.1 mg/dl, p=0.01). Moreover, in both genotypes, glucose, insulin and HOMA-IR levels decreased significantly at 3 and 9 months. The decrease at 3 months of (insulin levels -2.7±0.8 IU/L vs. -2.9±0.3 IU/L, p=0.24), HOMA-IR (-0.9±0.2 units vs. -0.8±0.1 units, p=0.56), and basal glucose (-5.7±0.7 mg/dl vs. -5.5±0.6 mg/dl, p=0.42) was significant in the AA genotype group.

### Table III. Serum Adipokine levels (mean±SD).

<table>
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<th>Parameters</th>
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<th>AG+GG (n=87)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>3 months</td>
<td>9 months</td>
</tr>
<tr>
<td>Resistin (ng/dl)</td>
<td>4.7±1.1</td>
<td>4.8±1.3</td>
<td>4.7±1.5</td>
</tr>
<tr>
<td>Adiponectin (ng/dl)</td>
<td>29.1±5.1</td>
<td>57.1±6.1*</td>
<td>59.1±4.2$</td>
</tr>
<tr>
<td>Leptin (ng/dl)</td>
<td>78.8±4.6</td>
<td>51.2±5.5*</td>
<td>48.2±4.3*</td>
</tr>
<tr>
<td>Ratio adiponectin/leptin (ng/dl)</td>
<td>0.36±0.2</td>
<td>1.11±0.2#</td>
<td>1.22±0.2#</td>
</tr>
</tbody>
</table>

Statistical differences p<0.05, in each genotype group (*leptin Sadiponectin). First p, significance of dietary intervention in AA genotype; second p, significance between AA genotypes vs. AG + GG baseline values; third p, significance of dietary intervention in AG + GG genotype; fourth p, significance between AA genotypes vs. AG + GG post-treatment values 3 months; fifth p, significance between AA genotypes vs. AG + GG post-treatment values 9 months.
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...were similar in both diets. The decrease at 9 months of (insulin levels -3.6±0.4 IU/L vs. -3.5±0.5 IU/L, p=0.27), HOMA-IR (-1.2±0.1 units vs. -1.2±0.2 units; p=0.46), and basal glucose (-7.8±0.9 mg/dl vs. -7.5±0.7 mg/dl, p=0.43) were similar in both diets. Pre-treatment and post-treatment values were similar in both groups.

Table III shows the levels of adipocytokines. In both genotypes, leptin levels decreased at 3 months and 9 months. Resistin levels did not change, and adiponectin increased only in patients with AA genotype. In both genotypes, leptin levels decreased at 3 months (2.2±12.1 ng/ml vs. -22.1±10.8 ng/ml, p=0.41) and 9 months (-30.6±10.1 ng/ml vs. -31.1±9.8 ng/ml, p=0.24). The elevation of adiponectin levels at 3 months (28.2±11.1 ng/ml vs. 41±2.8 ng/ml, p=0.02) and 9 months (30.1±8.1 ng/ml vs. 71±4.8 ng/ml, p=0.02) were higher in patients with the AA genotype, as was the adiponectin/leptin ratio. Finally, higher values of adiponectin and adiponectin/leptin ratio were detected at 3- and 9-months post-treatment in patients with AA genotype.

Discussion

We reported, in this study on a high-monounsaturated-fat hypocaloric diet with a Mediterranean pattern, an improvement in LDL-cholesterol, triglycerides, C reactive protein, adiponectin levels and ratio adiponectin/leptin that were significant in subjects with obesity and genotype AA of rs3774261. Additionally, all subjects in both genotype groups revealed a significant improvement in adiposity parameters and systolic blood pressure.

There are one study24 evaluating the association between this genetic variant (rs3774261) on ADIPOQ gene and diabetes mellitus, obesity and serum adiponectin levels. For example, Wassel et al22 have reported that rs3774261 within ADIPOQ gene were strongly related with serum adiponectin levels in Caucasian subjects. This genetic variant lies in intron regions of the gene, there is evidence that introns of the protein-coding gene transcripts can modulate gene expression by repressing translation RNA transcripts25. This association of rs3774261 with serum adiponectin levels is independent of the BMI of the patients2, and seems to be related to ethnicity, since it has only been found with European populations and no relationship was reported in African Americans26.

Another area of scientific interest is the relationship of this SNP with high cardiovascular risk27. This author27 hypothesized the anti-inflammatory effects that play adiponectin and its roles in protecting against atherosclerosis. Other types of epidemiological studies28 have reported the association of the G allele with an increment of the risk of diabetes mellitus type 2. This association could be explained through insulin resistance due to decreased levels of adiponectin, although in some work29, with different ethnic groups, the results have been contradictory. Finally, some investigations30 have described that rs3774261 variant is related to disinhibition in eating behaviors influencing food uptake in minor allele carriers and energy expenditure in the arcuate hypothalamus, becoming an area of potential interest in the evaluation of this polymorphism and dietary intervention. Despite all the previously mentioned findings23-30, the nutritional intervention studies10-13 that have evaluated the effect of this polymorphism on the response to weight loss are of short duration (3 months) and are scarce.

A short-term study31 (3 months) with a hypo-caloric Mediterranean diet showed better changes in lipid profile, CRP, and adiponectin levels in subjects with AA genotype. This study shows very similar data to our current design, with a caloric restriction that reached around 1,500 calories/day with a lower predominance in fats than our present work (carbohydrates 54%, lipids 25%, and proteins 21%) and with a percentage of monounsaturated fats also lower than around 50%. In another interventional design31, the response of lipid levels, CRP, and adiponectin was better in AA genotype than in GA or GG genotypes, with an improvement in adiponectin/leptin ratio as well. This second study31 was a short-term study of 3 months with a caloric restriction that reached around 1,500 calories/day with a similar percentage in fats to our investigation (carbohydrates 38%, lipids 38%, and proteins 25%), with a percentage of monounsaturated fats lower (45%) than the present study. The third short-term study of 3 months32 with a similar caloric restriction that reached around 1,500 calories/day with a similar percentage in fats to our investigation (carbohydrates 38%, lipids 38%, and proteins 25%), with a percentage of monounsaturated fats lower (45%) than the present study. The third short-term study of 3 months32 with a similar caloric restriction than previous10-11 and a different macronutrient distribution (carbohydrates 45.7%, lipids 34.4%, and proteins 19.9%) reported the same changes in lipid profile and adiponectin levels. In this design, a high ratio of polyunsaturated fatty acids (21%) and a low of monounsaturated one (21%) were present in the diet. In the present study, the duration of the intervention was longer (9 months), the caloric restriction achieved was also higher (1,000 calories/day), and the percentage of mono-
unsaturated fats (63%) was the highest of all the studies mentioned\textsuperscript{10-11}. The results were similar in the improvement of the levels of LDL cholesterol, triglycerides, CRP, and adiponectin, although the improvement of adiponectin was more than doubled compared to the previous studies\textsuperscript{10-13}. The real mechanism to explain this association is not well understood, but caloric restriction, the duration of the diet, and the higher percentage of monounsaturated grass may be playing an important role in this greater improvement in serum adiponectin levels.

The beneficial metabolic effects obtained in our study on the levels of LDL cholesterol, triglycerides and CRP may be related to a better response of adiponectin levels in non-carriers of A allele. In this way, adiponectin regulated the Lipoprotein lipase activity, and this fact could increase the hydroxylation of triglycerides with a high rate of catabolism of these particles. Additionally, the association of circulating adiponectin with Hepatic lipase activity could also explain the relation with LDL cholesterol levels\textsuperscript{31}. In the literature, adiponectin levels have been related with CRP levels secondary to an inhibition of the NF-\kappaB pathway in the endothelium, and it is in accordance with the anti-inflammatory effects that play adiponectin in protecting against atherosclerosis\textsuperscript{30}. It seems that this genetic variance is related to a pro-inflammatory status, not only related to ischemic heart disease\textsuperscript{8} but also to ischemic stroke\textsuperscript{32} and nonalcoholic fatty liver disease\textsuperscript{33}. In clinical practice, we can assess this reduction in cardiovascular risk with the adiponectin/leptin ratio. The adiponectin/leptin ratio is a marker of adipose tissue dysfunction and inflammation\textsuperscript{14}. It is well-known that an adiponectin/leptin quotient higher than the unit is considered normal, whereas a ratio below or near 0.5 units may show an increase in cardiovascular risk\textsuperscript{35}. In our study, only subjects with AA genotype increased ratio adiponectin/leptin above 1 point.

There are several important strengths of this study with an interventional design but also some limitations. First, we only analyzed one SNP of \textit{ADIPOQ} gene so that other genetic variants could be related to our findings. Second, many uncontrolled factors could influence our results (epigenetic, hormonal status, and so on). Third, the lack of a control group without diet might be a potential bias. Fourth, the self-reported dietary intake is not reliable, and it might include bias of under- or over-reporting. Finally, we only studied a sample of Caucasian patients with moderate obesity, we cannot generalize the results to other ethnicities, or patients with morbid obesity or other complications related to obesity.

**Conclusions**

In conclusion, G allele carriers of \textit{ADIPOQ} gene variant (rs3774261) showed no improvement in serum levels of adiponectin, adiponectin/leptin ratio, total-cholesterol, LDL-cholesterol, triglycerides, and CRP after weight loss with a hypocaloric fat monounsaturated diet. This study with three times longer intervention than those previously published in the literature which uses a hypocaloric diet with a Mediterranean pattern, makes our data of interest to be applied in clinical practice in medical consultations that attend patients with obesity.

**Authors’ Contributions**

DA de Luis designed the study and realized statistical analysis.

O Izaola R Aller realized anthropometric evaluation and control of dietary intake.

D Primo realized biochemical evaluation and genotype.

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**Conflict of Interest**

The authors declare no conflict of interest.

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**Ethics 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. This study was approved by the Ethics Committee of Hospital Clinico Universitario de Valladolid Area Este (HCUVA Committee) (code 5/2021).

**Informed Consent**

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

**Data Availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.
rs3774261 of ADIPOQ gene and high-fat Mediterranean diet

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