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# Role of the variant rs3774261 of adiponectin gene on adiponectin levels and ratio adiponectin/leptin after biliopancreatic diversion in morbid obese subjects

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**Abstract. – OBJECTIVE: Single nucleotide variants (SNVs) of *ADIPOQ* gene on different comorbidities are related to obesity and weight loss. Despite, there are no studies evaluating the effect of rs3774261 on metabolic variables after bariatric surgery. We evaluated the effect of SNV rs3774261 of *ADIPOQ* gene on biochemical changes after biliopancreatic diversion surgery in morbidly obese subjects for 3 years follow-up.**

**PATIENTS AND METHODS: One hundred and forty-nine patients (111 females/38 males) with morbid obesity (body mass index >40 kg/m2) without diabetes mellitus type 2 were enrolled. Biochemical and anthropometric evaluation were registered before and after 1, 2, and 3 years. Genotype of rs3774261 has been studied.**

**RESULTS: Total cholesterol, LDL-cholesterol and triglyceride levels decreased in all genotype groups during the study. Although the improvement in glucose, insulin and HOMA-IR was significant in two genotypes (AA and AG); these changes were earlier in the AA genotype than in Ag and GG genotypes. Adiponectin levels increased in a significant way in subjects with AA genotype in the 3 follow-up periods (first year delta: 16.4±0.5 ng/ml; *p*=0.03, second year delta: 21.3±0.5 ng/ mL; *p*=0.02 and third year delta: 23.6±0.7 ng/mL; *p*=0.01) and at 3 years in subjects with AG genotype (delta: 18.3±0.4 ng/ mL; *p*=0.03). The ratio adiponectin/leptin increased in a significant way in subjects with AA genotype in the 3 follow-up times (first year delta: 0.40±0.1 units; *p*=0.02, second year delta: 0.58±0.1 units; *p*=0.01 and third year delta: 0.65±0.1 ng/mL; *p*=0.01) and at 3 years in subjects with AG genotype (delta: 0.61±0.1 ng/ mL; *p*=0.02). Subjects with GG genotype did not show a significant improvement in these parameters during the follow-up.**

**CONCLUSIONS: G allele carriers of rs3774261 showed a delay in the improvement of glucose metabolism parameters, adiponectin and adiponectin/leptin ratio.**

*Key Words:*
*ADIPOQ* gene, Adiponectin, Biliopancreatic diversion, Rs3774261, SNVs.

## Introduction

Human adiponectin is a well-described adipokine. This molecule shows anti-inflammatory[1] and insulin-sensitizing effects[2]. Adiponectin has an inverse relationship to body mass index (BMI) and obesity with beneficial effects for insulin sensitivity[3]. Some nutritional compounds may influence adiponectin levels[4], too. In addition, genetic factors, such as ARL15 (ADP-ribosylation factor like 15 gene locus), also influence the levels of this adipokine, besides several other loci. The strongest genetic determinants influencing serum adiponectin levels have been identified within the *ADIPOQ* locus[5]. The production of adiponectin has an important genetic component associated with different polymorphisms of *ADIPOQ* gene, with a heritability estimated at 80%[6]. Single nucleotide variant (SNVs) are genetic variations with functional implications in the adiponectin C1Q (*ADIPOQ*), which located on chromosome 3q27. One of these SNVs, 712 G/A rs3774261 in the *ADIPOQ* has been related with metabolic disturbances[7] and cardiovascular events, too[8-9].

Obesity is a worldwide health problem with many associated comorbidities, such as diabetes mellitus type 2, high blood pressure and hyperlipidemia, reaching pandemic proportions. Weight loss interventions based on low energy intake have proven limited effectiveness; moreover, bariatric surgery guarantees an important weight loss and a significant reduction in comorbidities[10].



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There are a lot of factors implied in the response after bariatric surgery and many peripheral parameters have been relates in the regulation of metabolism and energy homeostasis, especially the adipokines such as adiponectin[11-12]. Despite the importance of this metabolic pathway and its SNVs on different comorbidities related to obesity and with weight loss, there is only one study in the literature that evaluate the role of rs3774261 on this area after a hypocaloric diet[13]. As far as we know, there are no studies evaluating the effect of this SNV on metabolic variables after bariatric surgery in obese patients. This is a research area with interest, since weight reduction increases serum adiponectin levels[14] and it has potential metabolic advantages. Serum levels of adiponectin has been shown to be involved in glucose metabolism[15] and with potential central effects[16].

Given this lack of studies in this topic area, we evaluated the effect of the genetic variant rs3774261 of *ADIPOQ* gene on biochemical changes after biliopancreatic diversion surgery in morbidly obese subjects for 3 years of follow-up.

## Materials and Methods

### Subjects and Bariatric Surgery Procedure

A group of 149 obese subjects has been consecutively enrolled. These patients have been previously admitted to the Department of Endocrinology and Nutrition for weight loss treatment (Table I). Individuals with the following criteria were excluded: severe systemic disease, diabetes mellitus type 2, systemic inflammatory diseases, cancer, coagulopathy, severe liver or chronic renal diseases, pregnancy or lactation, and patients with serious eating disorders. The Local Ethical Committee (HCUVA Clinico Universitario Valladolid-Committee-4/2016) approved the experimental design. All volunteers signed the written informed consent to participate in the study. All procedures performed in this study were in accordance with the Declaration of Helsinki.

All participants underwent biliopancreatic diversion (BPD) surgery. The BPD surgery consisted in the set-up of 175-cm alimentary limb and 70-cm common limb with the addition of a partial gastrectomy. In addition, other step was a transection of the small bowel half-way form the Treitz angle to the ileocecal valve followed by a Roux on Y gastroenterostomy on the distal bowel loop. The final step was an end-to-side enteroileostomy of the proximal bowel loop on the ileum 50-75 cm before the ileocecal valve. After four weeks of BPD surgery, all patients followed the same diet based on the intake of 1200-1400 calories, non-protein calories were distributed among fats (35% total fats; 10% saturated, 20% monounsaturated and 5% polyunsaturated) and carbohydrates (65%). Protein consumption was 1.4 g per kg of ideal weight (BMI) 23 kg/m$^2$.

### Design of Study

Plasma samples were obtained by venipuncture after an overnight fast (minimum 8 hrs). All individuals underwent clinical assessment by a multidisciplinary team. This team reported the next parameters: blood pressure and anthropometric parameters [body weight, waist circumference and percent excess weight loss (EWL%)]. The following biochemical parameters were measured: serum lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), insulin, fasting glucose, (homeostasis model assessment of insulin resistance) HOMA-IR and adipokines (leptin, adiponectin, ratio leptin/adiponectin). We recorded co- morbidities (percentage of patients with low HDL-cholesterol, hypertension or hyperlipidemia), too. All these parameters were obtained at the last visit prior to surgery (baseline, time 0) and at each postoperative visit at 1, 2 and

**Table I.** Preoperative and postoperative epidemiological and adiposity characteristics of the patients.

| | Basal time | 1 year | 2 years | 3 years |
|---|---|---|---|---|
| | n=149 | n=146 | n=139 | n=137 |
| Morbid obese | 136 | 69 | 50 | 20 |
| Super Morbid obese | 13 | 5 | 2 | 0 |
| Gender (female/male) | 111/38 | 109/37 | 105/34 | 104/33 |
| Age (years) | 49.7 ± 3.1 | 50.3 ± 4.9 | 51.1 ± 5.1 | 52.2 ± 5.3 |
| BMI (kg/m$^2$) | 46.5 ± 5.1 | 37.7 ± 4.2 | 34.0 ± 3.1 | 31.3 ± 4.1 |

Morbid: BMI > 40 kg/m$^2$ and <50 kg/m$^2$. Super morbid obese > 50 kg/m$^2$.

3 years. Genotype of rs3774261 of *ADIPOQ* gene was evaluated in all subjects.

### Clinical Procedures

At baseline, 1, 2 and 3 years, body weight, height, and waist circumference (WC) were measured in the morning before breakfast. Body mass index (BMI) was calculated as weight in kg divided by the height in meters squared. BMI $\geq$30 kg/m$^2$ was considered obesity. Fat mass was estimated by body electrical bioimpedance (Akern, EFG, Pisa, Italy) with an accuracy of 50 g[17]. WC was determined in the narrowest diameter between xiphoid process and iliac crest. Percent excess weight loss (EWL%) was determined using the formula; (preoperative weight – current weight x 100 / preoperative weight – ideal weight). Ideal weight was calculated with an ideal BMI 22 kg/m$^2$. Blood pressure was determined three times and averaged after a 5-minute rest with a random zero mercury sphygmomanometer (Omrom, Los Angeles, CA, USA).

Comorbidities were defined as hypertriglyceridemia (triglycerides > 150 mg/dl), hypertension (systolic or diastolic blood pressures higher than 130 and 85 mmHg, respectively) and low HDL cholesterol (<40 mg/dl and 50 mg/dl for men and women, respectively).

### Biochemical Procedures

Total cholesterol and triglyceride concentrations were determined by enzymatic spectrophotometric assay (Technicon Instruments, LTd., New York, NY, USA), while HDL cholesterol was determined by colorimetric method in a Bech Synchron® CX analyser (Beckman Instruments, LTd., Bucks, UK). LDL cholesterol was calculated using Friedewald formula [LDL cholesterol = total cholesterol-HDL cholesterol-(triglycerides/5)][18].

Glucose levels were analysed by an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, LTd., Bucks, UK). Insulin was measured by radioimmunoassay (RIA) (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L)[19] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values[20]. Serum adiponectin levels were analyzed by enzyme immunoassay (ELISA) (R&D systems, Inc., Mineapolis, MN, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml[21]. Leptin levels was measured by ELISA (Diagnostic Systems Laboratories, Inc., Houston, TX, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml[22]. The adiponectin/leptin ratio was calculated with adiponectin expressed in ng/ml and leptin in ng/ml, too.

The genotype of SNV rs3774261 of *ADIPOQ* was determined with a polymerase chain reaction at real-time from peripheral blood leucocytes. Genomic DNA was extracted from 150 uL buffy coat using a blood genomic kit (Bio-Rad®, Los Angeles, CA, USA) according to the manufacturer's instructions. Oligonucleotide primers and probes were performed with the Beacon Designer 5.0 (Premier Biosoft International®, Los Angeles, CA, USA). 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). Cycling parameters were as follows: after DNA denaturation at 95ºC for 1 min and annealing at 65º C for 30 sec. The PCR were run in a 25 µL final volume containing 10.5 µL of IQTM Supermix (Bio-Rad®, Los Angeles, CA, USA) with hot start Taq DNA polymerase. The fluorescence signals were detected at excitation an emission wavelength of 485 nm and 612 nm, respectively.

### Statistical Analysis

IBM SPSS Statistics version 23.0 (Chicago, IL, USA) was used for statistical analysis. Power analysis suggested at least 140 subjects. We used the following premises: improvement in weight of 30% EWL%, polymorphism frequency (40%), a type I error of 0.05 and type II error of 0.10 (power=0.9). The statistical analysis was performed with three genotypes (AA *vs.* AG *vs.* GG). The results were expressed as average $\pm$ standard deviation. The normal distribution of variables was studied with the Kolmogorov-Smirnov test.

Non-parametric variables were analyzed with the Mann-Whitney test and Wilcoxon test. Parametric test was analyzed with ANOVA test and Bonferroni post-hoc test. The presence of comorbidities was analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test as necessary. A *p*-value under 0.05 was considered statistically significant.

## Results

A total of 149 morbid obese subjects were evaluated. Table I shows the epidemiological and

adiposity characteristics. As expected, there was a gradual loss of patients during the three-year follow-up period. A total of 111 patients were females (74.5%) and 38 were males (25.5%). The genotypic frequency was 24.3% (37 patients) with AA genotype, 50.4% (75 patients) with AG genotype and 24.3% (37 patients) with GG genotype. The allelic frequency was 53.6% A and 46.4% G. Hardy Weinberg equilibrium in genotype frequencies was confirmed ($p$=0.431).

The gender distribution was similar in all genotypes (AA; 29.7% (n=11) males and 70.3% (n=26) females), (AG; 22.7% (n=17) males and 77.3% (n=58) females) and (GG; 27.0% (n=10) males and 73.0% (n=27) females). Average age was similar in all genotype groups (AA: 50.0 ± 5.2 years *vs.* AG 49.6 ± 6.1 years vs. AG 49.8 ± 6.1 years: ns), too.

Table II shows blood pressure, classical adiposity parameters, fat mass by bioimpedance and percentage of excess of weight loss. No statistical differences were detected among genotype groups. When the evolution of adiposity parameters over time was revised, we detected similar pattern between genotypes. Body weight, waist circumference and fat mass showed a statistically significant reduction after surgery at 1, 2 and 3 years. Blood pressures showed a statistically significant decrease, too. Improvements in these parameters were similar in all genotypes. Percentage of excess of weight loss (EWL%) showed a significant improvement during the following in the three genotype groups.

Table III shows the improvements in all biochemical parameters. Basal glucose parameters (fasting glucose levels, HOMA-IR and insulin), lipid profile (total cholesterol, HDL-cholesterol, LDL cholesterol and triglyceride levels) and serum adipokines (leptin, adiponectin and ratio leptin/adiponectin) were similar in all genotype groups. Total cholesterol, LDL-cholesterol and triglyceride levels decreased in all genotype groups. Although the improvement in glucose, insulin and HOMA-IR was significant in two genotypes (AA and AG), this change was earlier in the AA genotype than the rest of genotype groups. We alone detected the improvement at 3 years in AG. Subjects with GG genotype did not show a significant improvement in these parameters during the follow-up.

Basal glucose levels improved the first year in AA subjects (delta: -11.2 ± 2.1 mg/dL; $p$=0.02), the second year (delta: -17.1 ± 2.1 mg/dL; $p$=0.01) and third year (delta: -19.9 ± 2.3 mg/dL; $p$=0.02), too. Glucose improvement was significant at 3 years in subjects with AG genotype (delta: -15.9 ± 3.1 mg/dL; $p$=0.02). The decrease of fasting insulin levels showed a similar pattern at first year (AA) (delta: -6.7 ± 1.5 mUI/L; $p$=0.01), the second year (delta: -7.1 ± 1.5 mUI/L; $p$=0.02), and the third year (delta: -8.8 ± 1.3 mUI/L; $p$=0.01), too. Finally, insulin improvement was significant at 3 years in subjects with AG genotype (delta: -5.8 ± 1.1 mUI/L; $p$=0.03). On the other hand, the improvement of HOMA-IR (homeostasis model assessment of insulin resistance) levels was earlier in AA genotype than the remaining genotypes as reported: at year one (AA subjects (delta: -2.3 ± 0.2 units; $p$=0.02), at the second year (delta: -2.7 ± 0.2 units; $p$=0.03) and the last year (delta: -2.8 ± 0.1 units; $p$=0.03). Finally, HOMA-IR improved at 3 years of follow-up intabl AG genotype (delta: -2.5 ± 0.4 units; $p$=0.04). Fasting glucose, HOMA-IR and insulin levels did not change in subjects with GG genotype.

Table III shows adipokine levels. Leptin levels decreased in all genotype's groups. Adiponectin levels increased in subjects with AA genotype during the 3 times (first year delta: 16.4 ± 0.5 ng/ mL; $p$=0.03, second year delta: 21.3 ± 0.5 ng/ mL; $p$=0.02 and third year delta: 23.6 ± 0.7 ng/ mL; $p$=0.01). This improvement was detected at 3 years of follow-up in subjects with AG genotype (delta: 18.3 ± 0.4 ng/ mL; $p$=0.03). Finally, adiponectin/leptin ratio increased in the same way. This ratio increased in subjects with AA genotype during the 3 times (first year delta: 0.40 ± 0.1 units; $p$=0.02, second year delta: 0.58±0.1 units; $p$=0.01 and third year delta: 0.65 ± 0.1 ng/ mL; $p$=0.01) and at 3 years in subjects with AG genotype (delta:0.61 ± 0.1 ng/ mL; $p$=0.02). Subjects with GG genotype did not show a statistical modification in either adiponectin levels or ratio adiponectin/leptin.

Table IV reports the improvement in obese comorbidities (percentage of hypertriglyceridemia, hypertension and low-HDL levels). These improvements were similar in all genotype groups.

## Discussion

To our knowledge, this is the first study in the literature that evaluates the effect of the SNV rs3774261 of *ADIPOQ gene* on the metabolic response secondary to a biliopancreatic diversion surgery (BPD). Our data shows an early effect of AA genotype on glucose, insulin, HOMA-IR, adiponectin and ratio adiponectin/leptin after 3 years of follow-up.

**Table II.** Changes in anthropometric variables rs3774261 (mean ± SD).

| Characteristics | AA (n=37) | | | | AG (N=75) | | | | GG (n=37) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 time | At 1 year | At 2 year | At 3 year | 0 time | At 1 year | At 2 year | At 3 year | 0 time | At 1 year | At 2 year | At 3 year |
| **BMI** | 46.7 ± 5.1 | 37.8 ± 5.2* | 33.9 ± 5.2* | 31.2 ± 5.0* | 46.5 ± 4.3 | 37.7 ± 4.1 | 34.1 ± 3.9* | 31.3 ± 3.0* | 46.3± 4.1 | 37.6 ± 3.8* | 34.0 ± 3.3* | 31.2 ± 3.9* |
| **Weight (kg)** | 127.1 ± 9.6 | 94.9 ± 8.9* | 85.1 ± 8.4* | 80.0 ± 5.2* | 126.1 ± 5.1* | 95.8 ± 9.2* | 86.1 ± 5.2* | 79.9 ± 4.1* | 128.1 ± 8.9 | 96.9 ± 5.0* | 87.0 ± 5.4* | 79.3 ± 5.1* |
| **Fat mass (kg)** | 47.9 ± 4.4 | 35.7± 5.2* | 34.1 ± 4.2* | 31.2 ± 3.1* | 48.9 ± 3.9* | 36.7 ± 7.0* | 34.2 ± 6.1* | 31.9 ± 2.9* | 47.5 ± 3.9* | 36.8 ± 3.2* | 33.8 ± 3.3* | 30.8 ± 3.1* |
| **WC (cm)** | 120.2 ± 9.1 | 112.1 ± 5.0* | 102.1 ± 5.2* | 98.2 ± 5.0* | 119.9 ± 4.3* | 112.2 ± 6.1 | 102.1±4.9* | 99.9±5.7* | 118.9 ± 4.5* | 110.9 ± 3.4* | 99.9 ± 3.9* | 97.9 ± 4.2* |
| **EWL%** | - | 58.8 | 62.2 | 68.1 | - | 59.0 | 62.1 | 67.9 | - | 59.3 | 62.4 | 68.0 |
| **SBP (mmHg)** | 148.1 ± 7.0 | 134.1 ± 6.1* | 128.1 ± 7.0* | 127.2 ± 6.2* | 149.1 ± 5.9 | 135.0 ± 4.1 | 129.1 ± 4.1* | 127.9 ± 4.6 | 149.4 ±3 .2* | 135.1 ± 3.3* | 128.0 ± 3.9* | 127.3 ± 4.5* |
| **DBP (mmHg)** | 90.1 ± 4.1 | 87.1 ± 4.3* | 84.1 ±4.2* | 83.5 ± 5.0* | 90.9 ±3.0 | 86.6 ± 3.1* | 84.0 ± 3.1* | 83.2 ± 3.4* | 89.9 ± 2.6* | 87.7 ±3.2* | 83.6 ± 3.1* | 83.6 ± 3.9* |

DBP: Diastolic blood pressure. SBP: Systolic blood pressure. WC: Waist circumference. EWL%: Percent excess weight loss (*) $p<0.05$, in each genotype group with basal values in the same genotype group. There are no statistical differences in demographic, anthropometric and metabolic characteristics between the three-genotype groups.

**Table III.** Changes in biochemical parameters (mean±SD) rs3774261.

| Characteristics | AA (n=37) | | | | AG (N=75) | | | | GG (n=37) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 time | At 1 year | At 2 year | At 3 year | 0 time | At 1 year | At 2 year | At 3 year | 0 time | At 1 year | At 2 year | At 3 year |
| **Glucose (mg/dl)** | 106.1 ± 5.8 | 94.9 ± 6.1* | 89.0 ± 7.1* | 87.2 ± 4.9* | 105.0 ± 3.9 | 99.5 ± 4.8 | 91.9 ± 4.1 | 88.1 ± 4.2 * | 101.7 ± 5.1 | 96.7 ± 5.1 | 90.7 ± 5.2 | 89.7 ± 5.0 |
| **Total ch. (mg/dl)** | 198.3 ± 11.2 | 138.1 ± 14.9* | 128.0 ± 13.1* | 125.2 ± 7.1* | 194.1 ± 8.9* | 138.9 ± 10.4 | 127.2 ± 9.3 * | 124.9 ± 9.1* | 197.9 ± 8.7 | 137.1 ± 7.1* | 129.1 ± 7.0* | 124.9 ± 6.1* |
| **LDL-ch. (mg/dl)** | 121.0 ± 10.1 | 73.5 ± 9.2* | 64.7 ± 8.1* | 62.9 ± 4.0* | 122.9 ± 9.9* | 74.9 ± 10.0 | 65.0 ± 5.1 * | 60.1 ± 5.1* | 120.8 ± 5.2 | 79.9 ± 4.1* | 65.9 ± 5.8* | 61.9 ± 5.8* |
| **HDL-ch. (mg/dl)** | 49.4 ± 1.1 | 51.1 ± 5.1* | 52.3 ± 5.2* | 52.4 ± 4.1* | 47.7 ± 3.2 | 51.9 ± 3.2* | 52.4 ± 2.0* | 52.9 ±3.0* | 48.8 ± 2.1 | 52.0 ±5.0* | 52.9 ± 6.1* | 54.0 ± 3.0* |
| **TG (mg/dl)** | 172.9 ± 18.1 | 131.1 ± 14.2* | 110.1 ± 13.6* | 90.1 ± 8.1* | 178.2 ± 15.1 | 130.1 ± 11.8 | 108.2 ± 12.0* | 91.1 ± 9.8* | 176.7 ± 12.1* | 130.1 ± 12.1* | 109.1 ± 7.9* | 90.2 ± 7.2* |
| **Insulin (mUI/L)** | 19.9 ± 2.4 | 13.2 ± 3.0* | 12.8 ± 3.8* | 11.1 ± 4.0* | 18.0 ± 3.8 | 16.9 ± 2.2# | 15.1 ± 4.0 | 12.2 ± 3.1* | 17.9 ± 3.9 | 15.9 ± 2.8 | 14.1 ± 3.9 | 14.2 ± 2.8 |
| **HOMA-IR** | 4.9 ± 1.0 | 2.6 ± 1.1* | 2.2 ± 1.2* | 2.1 ± 0.4* | 4.8 ± 1.4 | 3.7 ± 1.8 | 3.0 ± 0.8 | 2.5 ± 0.6* | 4.7±1.6 | 3.1 ± 1.5 | 3.0 ± 1.2 | 2.6 ±1.2 |
| **Adiponectin (ng/mL)** | 17.7 ± 8.1 | 34.1 ± 5.1$ | 39.1 ± 5.2$ | 41.3 ± 4.9$ | 18.8 ± 8.1 | 26.3 ± 9.0# | 30.7 ± 8.3# | 37.1 ± 8.0$ | 18.1 ± 6.1 | 31.1 ± 9.2# | 32.8 ± 9.1# | 33.3 ± 10.1# |
| **Leptin (ng/mL)** | 83.2 ± 12.6 | 54.7 ±12.5$ | 49.2 ± 10.1$ | 47.8 ± 8.1$ | 84.2 ± 10.1 | 57.1 ± ± 7.0$ | 50.0 ± 9.6$ | 44.7 ± 8.5$ | 89.2 ± 10.1 | 55.8 ± 9.3$ | 48.8 ± 6.1$ | 47.1 ± 7.2$ |
| **Ratio Adiponectin/leptin** | 0.21 ± 0.09 | 0.61 ± 0.10$ | 0.79 ± 0.11$ | 0.86 ± 0.02$ | 0.22 ± 0.13# | 0.46 ± 0.11# | 0.51 ± 0.9 | 0.83 ± 0.12$ | 0.23 ± 0.13 | 0.45 ± 0.21# | 0.47 ± 0.18# | 0.49 ± 0.14# |

LDL. Low density lipoprotein. HDL High density lipoprotein. Chol: Cholesterol. TG: Triglycerides. HOMA-IR (homeostasis model assessment). Glucose and lipid metabolism (*) $p<0.05$, in each genotype group with basal values. Adipokine levels $ $p<0.05$ in each genotype group with basal values. # $p<0.05$ differences between data of AG genotype vs AA genotype and GG genotype *vs.* AA genotype

In the literature, some studies have been evaluated the relationship between this genetic variant (rs3774261) and metabolic disturbances, such as diabetes mellitus and obesity[23,24]. These studies[23,24] reported a clear association of this genetic variant and the risk towards the development of type 2 diabetes, overweight and hypoadiponectinemia. Despite the interestingness of this area of knowledge, there is only one study involving a dietary intervention[13]. This short-term study (3 months) showed a better improvement in lipid profile, C reactive protein and adiponectin levels in subjects with AA after a hypocaloric Mediterranean[13]. Our present study shows that this genetic variant of *ADIPOQ* (rs3774261) is associated with a differential regulation of adiponectin levels and glucose metabolism after weight loss in subjects with AA genotype. The amount of lipid profile improvement was similar in the dietary intervention study[13] and in the present surgical intervention study. Moreover, the changes in adiponectin, glucose, insulin and HOMA-IR were greater in the current bariatric intervention study than in the previous one[13]. This difference is probably due to the greater weight loss obtained with the bariatric surgery compared to the hypocaloric diet. The populations were similar in both designs: middle-aged Caucasian and predominantly female.

Other research studies have reported a relationship between other SNVs in the *ADIPOQ* gene and the metabolic response after a (Biliopancreatic diversion) BPD[11,12]. In the first one[11], the rs1501299 variant was associated with different glucose metabolic response and adiponectin increase after bariatric surgery. In the second study[12], the rs266729 variant modulated glucose metabolism, lipid profile and adiponectin levels after a massive weight loss secondary to bariatric surgery. Nevertheless, in these previous investigations, the adiponectin/leptin ratio was not analyzed. These studies evaluated leptin concentrations and they reported a decrease proportional to postoperative weight loss[11,12,25]. Nonetheless, the adiponectin/leptin ratio is a marker of adipose tissue dysfunction[26] and it has been correlated with insulin resistance. For example, a ratio below 0.5 may indicates an increase in the metabolic risk[27]. Our study shows a ratio lower than this value in patients with the GG genotype and in the first years after surgery in patients with the AG genotype.

To explain our findings, we can raise several hypotheses to analyze the improvement in the metabolism of glucose, adiponectin and adiponectin/leptin ratio only in patients with AA genotype and in those with AG genotype but three years after surgery. Firstly, perhaps G allele modifies the sequence for one transcriptional stimulatory protein binding sites and secondary this fact reduces adiponectin promoter activity. We must consider that adiponectin was shown to be involved in enhanced glucose uptake *via* (glucose transporter 4) GLUT-4[28]. According to a second hypothesis, there could exist a possible molecule that relates this genetic SNV with insulin sensitivity. Finally, adiponectin has a well-known anti-inflammatory property *in vitro* with the pathway of NF-kB

**Table IV.** Preoperative and postoperative comorbidities of the patients.

| | Basal time | 1 year | 2 years | 3 years |
|---|---|---|---|---|
| Low levels HDL Cholesterol | n=149 | n=146 | n=139 | n=137 |
| AA | 48.6% | 24.3%* | 18.9%* | 16.2%* |
| AG | 44.0% | 26.6%* | | 20.3%* |
| 20.0%* | | | | |
| GG | 45.9% | 22.2%* | 16.4%* | 14.3%* |
| High Levels TG | | | | |
| AA | 40.5% | 29.9%* | 27.0%* | 21.6%* |
| AG | 48.0% | 23.4%* | 20.6%* | 16.0%* |
| GG | 37.8% | 27.0%* | 24.3%* | 19.9%* |
| Blood Hypertension | | | | |
| AA | 24.3% | 13.5%* | 10.8%* | 10.8% |
| AG | 24.0% | 20.0% | 18.6% | 16.1%* |
| GG | 21.6% | 16.2%* | 10.8%* | 8.1%* |

(*) $p<0.05$, in each group with basal values. HyperTG Hypertriglyceridemia (triglycerides > 150 mg/dL), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg respectively), low HDL cholesterol (<40 mg/dL and 50 mg/dL for men and women, respectively.

signaling in endothelium[29]. This powerful anti-inflammatory action can improve insulin sensitivity in subjects without G allele.

### Limitations

Several limitations need to be highlighted in the present study. Firstly, we only analysed one genetic variant of *ADIPOQ* gene, so other SNVs in this gene could be interacted with our observations. Secondly, other non-genetic factors could modify the findings in our design (i.e., smoke habit, exercise, hormone status, and so on) and epigenetic factors, too. Thirdly, the lack of a dietary assessment throughout the study in order to measure macronutrients intakes could be a bias[30]. Finally, our group of patients is a morbid obese adult sample (BMI $\geq$ 40 kg/m$^2$) without diabetes mellitus; so, the data is not generalizable to other groups such as: non-morbid obese, overweight patients or obese patients with diabetes mellitus type 2.

## Conclusions

The improvement of glucose, insulin levels, HOMA-IR, adiponectin levels and ratio leptin/adiponectin was significant in subjects with AA genotype during the follow-up period. This improvement was significant at 3 years of follow-up in subject with AG genotype. Subjects with GG genotype did not show a significant improvement in these parameters. This different response must be considered after bariatric interventions to implement glycemic control measures in risk patients with GG genotype. Besides, this genetic variant has been related with cardiovascular risk[31].

### Statement of Ethics

The Local Ethical Committee (HCUVA Clinico Universitario Valladolid-Committee-4/2016) approved the experimental design. All volunteers signed the written informed consent to participate in the study. All procedures performed in this study were in accordance with the Declaration of Helsinki.

### Conflicts of Interest

The authors have no conflicts of interest to declare.

No funding has been received to realize this study.

### Authors' Contributions

Daniel de Luis wrote the article and made statistical analysis. David Primo and Olatz Izaola made anthropometric evaluation. David Primo made biochemical evaluation. David Pacheco realized surgeries and made statistical analysis.

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