European Review for Medical and Pharmacological Sciences 2019; 23: 2960-2970

Single nucleotide polymorphisms at the ADIPOQ gene locus *rs1501299* interact with different type of dietary fatty acids in two hypocaloric diets

D.A. DE LUIS, O. IZAOLA, D. PRIMO, R. ALLER

Center of Investigation of Endocrinology and Nutrition, School of Medicine, Department of Endocrinology and Nutrition, Hospital Clinico Universitario, University of Valladolid, Valladolid, Spain

Abstract. – OBJECTIVE: Some adiponectin gene (ADIPOQ) and single nucleotide polymorphisms (SNPs) have been related to adiponectin levels and metabolic parameters. Few studies of interaction gene-nutrient have been realized in this topic area. The aim of our study was to analyze the effect of the rs1501299 ADIPOQ gene polymorphism, and the dietary intake on total adiponectin levels and the insulin resistance changes after an enriched-monounsaturated fat (Diet M) *vs.* an enriched-polyunsaturated fat hypocaloric diet (Diet P).

PATIENTS AND METHODS: A Caucasian population of 363 obese patients was enrolled in a randomized clinical trial with two hypocaloric diets. Before and after 12 weeks on each hypocaloric diet, an anthropometric evaluation, an assessment of nutritional intake and a biochemical analysis were realized. The statistical analysis was performed for the combined GT and TT as a group (mutant) and GG as second group (wild).

RESULTS: With both caloric restriction strategies, body weight, body mass index (BMI), fat mass, waist circumference, systolic blood pressure and leptin levels decreased. After Diet P, only subjects with GG genotype showed a significant improvement in the insulin levels (GG *vs.* GT±TT) (-3.2±1.0 mU/L *vs.* -0.6±0.4 mU/L: *p*=0.01) and in the homeostasis model assessment (HOMA-IR) (-1.1±0.2 units *vs.* -0.3±0.4 units: *p*=0.02). The same improvement in both parameters was reported after Diet M: insulin levels (-3.7±0.9 mU/L *vs.* -0.4±0.5 mU/L: *p*=0.01) and HOMA-IR (-1.0±0.2 units *vs.* -0.4±0.3 units: *p*=0.03). After weight loss with diet M, both genotypes (GG *vs.* GT±TT) showed similar decrease in total cholesterol and LDL-cholesterol. Only subjects with GG genotype showed a significant increase of the adiponectin levels after both diets: (Diet P: 9.3±3.0 ng/dl *vs.* Diet M: 8.2±2.9 ng/dl: *p*=0.38).

CONCLUSIONS: The GG genotype of ADIPOQ gene variant (rs1501299) is associated to a significant improvement in the adiponectin levels and a decrease of insulin and HOMA-IR after two different hypocaloric diets with different profile of unsaturated dietary fats.

Key Words

Rs1501299, ADIPOQ, Adiponectin, Weight loss, Insulin resistance, Unsaturated fatty acids.

Introduction

Adipose tissue is an important endocrine organ secreting various biologically active adipokines involved in the regulation of energy homeostasis, immune status, appetite and metabolism[1]. Adiponectin levels secreted by adipocyte decreases in obesity and its related pathologies such as diabetes mellitus and hypertension[2-4] Activities of adiponectin in vascular endothelial cells, smooth muscle cells, macrophages and lipid, and glucose metabolism confer protection against metabolic syndrome and obesity[3-4].

The adiponectin synthesis has a strong genetic component, with heritability estimated above 70%[5]. Some single nucleotide polymorphisms (SNPs) have been associated with serum adiponectin levels[6,7]. However, there is a controversy between the association of these genetic variants and the adiponectin levels. These discrepancies may relate to the interaction between these genetic variants and nutrients. In particular, heterogeneity of response to fish oil fatty acids within different patients' groups has been attributed to genetic variation[8]. One of the most important SNPs at the adiponectin gene (ADIPOQ) locus is a G to T substitution in intron 2 (±276G>T, rs1501299). The G allele has been associated either with increased or decreased concentrations of plasma adiponectin[9-10]. On the other hand, this genetic variant has been negatively[11] and positively[12] associated with obesity in some populations. Another area of controversy is the relationship between this variant with insulin levels and insulin resistance and the secondary increase of cardiovascular risk[13-15].

Corresponding Author: Daniel Antonio de Luis, MD; e-mail: dadluis@yahoo.es

Moreover, unsaturated fatty acids (polyunsaturated and monounsaturated) are important ligands for the transcription factor PPAR-gamma[16] which up-regulates ADIPOQ gene expression[17] and increases adiponectin levels. Thus, adiponectin levels could be influenced by the interaction between ADIPOQ SNPs, (and) dietary intake of polyunsaturated fatty acids (PUFAs)[18] and monounsaturated fatty acids (MUFAs)[19]. However, other components of the diet, such as dietary fiber from whole-grain products, foods with a low glycemic index, caloric restriction and the ratio between unsaturated fat and low in carbohydrates might influence adiponectin levels[18-21].

The aim of our study was to analyze the effect of rs1501299 ADIPOQ gene polymorphism and dietary intake on total adiponectin levels and insulin resistance changes after an enriched-monounsaturated fat *vs.* an enriched-polyunsaturated fat hypocaloric diet.

Patients and Methods

Patients

Ethical approval for this study was granted from the local Ethics Committee and written informed consent from participants was obtained, including subsequent genetic studies. Subjects were recruited from the general population who attended a clinic in a fasting state. The eligibility for the entry into the study was assessed through the implementation of inclusion and exclusion criteria described as follows. The inclusion criteria were an adult age ranged from 20 to 65 years, body mass index ≥30 kg/m^2, and absence of diet during the previous 12 weeks before the study. The exclusion criteria included active tumor, total cholesterol >200 mg/dl, triglycerides >200 mg/dl, history of cardiovascular disease or stroke during the previous 6 months, blood pressure >140/90 mmHg, as well as the use of thiazolidinedione, insulin, metformin, sulfonylurea, dipeptidyl type IV inhibitors drugs, glucocorticoids, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other antidyslipidemic drugs.

Finally, a sample of 363 Caucasians subjects with obesity (body mass index ≥30 kg/m^2) was enrolled in a randomized clinical trial with two arms; Diet P (enriched PUFAs) fat hypocaloric diet and Diet M (enriched MUFAs) fat hypocaloric diet, for 12 weeks.

Design

The next biochemical parameters were determined at basal time and after 12 weeks of dietary intervention; fasting glucose, c-reactive protein (CRP), insulin, insulin resistance as homeostasis model assessment (HOMA-IR), total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol), plasma triglycerides concentration and adipokines levels (leptin, adiponectin and resistin). At the same times (basal and 12 weeks), weight, height, fat mass by bioimpedance and blood pressure were measured too. Genotype of ADIPOQ gene (rs1501299) was studied.

Dietary Intervention

The study duration was 12 months, from the inclusion to the first patient until the end of the last patient. As mentioned above, patients were randomly allocated to one of the two diets for a period of 12 weeks. The target percentage of energy derived from the carbohydrates, the fats and the proteins in the two diets were: Diet P (45.7% of carbohydrates, 34.4% of lipids and 19.9% of proteins) and Diet M (46.6% of carbohydrates, 34.1% of lipids and 19.2% of proteins). The distribution of fats in Diet P was: 21.8% of saturated fats, 55.5% of monounsaturated fats and 22.7% of polyunsaturated fats (7 g per day of w-6 fatty acids, 2 g per day of w-3 fatty acids and a ratio w6/w3 of 3.5). The distribution of fats in Diet M was: 21.7% of saturated fats, 67.5% of monounsaturated fats and 10.8% of polyunsaturated fats. The recommended physical activity consisted of an aerobic exercise at least two times per week (60 min each). All the enrolled subjects received instruction to record their daily dietary intake for three non-consecutive days including a weekend day. This adherence was assessed every seven days with a phone call by a dietitian in order to improve compliment of dietary intervention. Dietary records were analysed using a computer-based data evaluation system, including national composition food tables as a reference[22]. The exercise activity was recorded by the patients with a self-reported questionnaire.

Assays

Blood samples for analysis were drawn after a minimum of an 8 h overnight fast, and serum was stored at -80ºC until analyzed. Fasting serum concentrations of total cholesterol and triglyceride were measured using commercially available kits (Technicon Instruments, Ltd., New York, N.Y.,

USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using the Friedewald formula[23]. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5mUI/L (normal range 0.5-30 mUI/L)[24] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values (fasting insulin=fasting glucose/22.5)[25]. CRP was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Baden Gutenberg, Germany), with a normal range of (0-7 mg/dl) and analytical sensitivity 0.5 mg/dl. Serum adiponectin analysis was carried out at ELISA (R&D Systems, Inc., Minneapolis, MN, USA) (DRP300) with a sensitivity of 0.246 ng/ml, a normal range of 8.65-21.43 ng/ml and a CV% 3.8%[26]. Resistin was determined by ELISA (Biovendor Laboratory, Inc., Brno, Brno, Czech Republic) (RD191016100) with a sensitivity of 0.2 ng/ml, a normal range of 4-12 ng/ml[27] and a CV% 3.2%. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Dallas, TX, USA) (DSL1023100) with a sensitivity of 0.05 ng/ml, a normal range of 10-100 ng/ml and a CV% 3.5%[28].

Anthropometric Measurements and Blood Pressure

Body weight and height were measured in the morning while the subjects were unclothed. Weight was measured to an accuracy of 100 g with a calibrated weight (Omron, Los Angeles, CA, USA) and a stadiometer 0.1 mm (Omron, Los Angeles, CA, USA), respectively. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Waist (narrowest diameter between the xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to-hip ratio (WHR) were measured, too. An ea was used to determine body composition with an accuracy of 50 g[29]. Blood pressure was measured twice after a 10 minutes rest with a random-zero mercury sphygmomanometer and averaged (Omron, Los Angeles, CA, USA).

DNA Extraction and SNP Genotyping

Buffy coats removed from blood samples were stored in EDTA coated tubes at -20°C. Genomic DNA was extracted from 300 µl buffy coat with and commercial kit extraction (Bio-Rad®, Los Angeles, CA, USA). Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc., San Diego, CA, USA). Genotyping for the rs1015299 polymorphism was performed by chain reaction real-time analysis. This polymerase chain reaction (PCR) was carried out with 20-25 ng of genomic DNA, 0.1-0.15 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; this was 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

The genotype distribution was tested for deviation from Hardy-Weinberg equilibrium by a Chi-square test with 1 df ($p>0.05$). The variant of all ADIPOQ gene was in Hardy Weinberg equilibrium ($p=0.39$). Statistical analysis was carried out with SPSS version 15.0 (Chicago, IL, USA). The sample size was calculated to detect differences over 3 kg in body weight loss with 90% power and 5% significance (n=175). Each variable was examined for normality with the Kolmogorov-Smirnov test. For descriptive purposes, results were expressed as average ± standard deviation. In within-groups, we conducted paired *t*-tests for biochemical parameters at baseline and after caloric restriction. In between-groups, independent *t*-test was used to compare the differences in both times. Non-parametric variables were analyzed with the Mann-Whitney U test. The categorical variables were analyzed with the Chi-square test, with Yates correction as necessary, and Fisher's test. For the statistical analysis to evaluate the gene-diet interaction, it was used a univariate ANCOVA with Bonferroni post-hoc test. Moreover, the Chi-square test was used to evaluate the categorical parameters. The statistical analysis was performed for the combined TT and GT as a group and GG genotype as second group (wild type genotype), with a dominant model. A p-value <0.05 was considered significant.

Results

Three hundred and sixty-three obese subjects were enrolled in the study (199 GG (54.8%), 130 GT (35.8%) and 34 TT (9.5%)). All patients completed the 12-weeks follow-up period without dropouts. The mean age of all groups after was 49.7±7.9 years (range: 26-61) and the mean BMI 37.6±4.1 kg/m^2 (range: 30.2-41.3). Sex distribution was 261 women (71.9%) and 102 men (28.1%). The age was similar in both genotype groups (wild type (GG) *vs.* mutant type (GT±TT)) (50.1±7.9 years *vs.* 46.9±6.1 years: ns). Sex distribution was similar in both genotype groups, males (27.1% *vs.* 29.1%) and females (72.9% *vs.* 70.9%).

In the 177 obese patients (95 GG as wild genotype and 67 GT/15 TT as mutant genotype (GT±TT)) treated with diet P, basal evaluation of dietary intakes with a 3 days written food record showed a calorie intake of 1969.8±512.8 kcal/day, a carbohydrate intake of 216.1±50.9 g/day (44.0% of calories), a fat intake of 80.1±28.3 g/day (35.7% of calories) and a protein intake of 86.1±30.1 g/day (20.3% of calories). During the 3 months of dietary intervention, these obese subjects reached the recommendations of diet P: 1435.9 calories per day (45.0% of carbohydrates, 34.4% of lipids and 20.6% of proteins). The distribution of fats was: a 20.4% of saturated fats, a 54.0% of monounsaturated fats and a 23.6% of polyunsaturated fats (7.0 g per day of w-6 fatty acids, 1.9 g per day of w-3 fatty acids and a ratio w6/w3 of 3.7). Physical activity was similar in both genotype groups (58.1±17.3 min/week *vs.* 60.1±13.2 min/week: p=0.56).

In the 186 subjects (104 GG as wild genotype and 63 GT/19 TT as mutant genotype (GT±TT)) treated with diet M, basal dietary intake with a 3 days written food record showed a calorie intake of 2013.6±309.1 kcal/day, a carbohydrate intake of 213.1±29.3 g/day (43.9% of calories), a fat intake of 82.1±16.3 g/day (34.7% of calories) and a protein intake of 88.9±17.9 g/day (22.4% of calories). During the dietary intervention, these patients reached the right recommendations of the diet M: 1449.1 calories per day (45.1% of carbohydrates, 34.0% of lipids and 20.9% of proteins). The distribution of dietary fats was: 20.2% of saturated fats, 68.0% of monounsaturated fats and 11.8% of polyunsaturated fats. Physical activity was similar in both genotype groups (57.1±12.3 min/week *vs.* 59.3±13.9 min/week: p=0.54).

As reported in Table I, there were no significant genotype related differences (baseline and after a dietary intervention) in anthropometric parameters and blood pressure. After both dietary caloric restriction strategies with two different profiles of dietary fatty acids, body weight, body mass index (BMI), fat mass, waist circumference and systolic blood pressure decreased. Obese patients with both genotypes (GG *vs.* GT±TT) after Diet P showed similar improvement in body weight (-4.3±1.2 kg *vs.* -5.2±1.3 kg: p=0.39), BMI (-0.5±0.1 kg/m^2 *vs.* -0.7±0.4 kg/m^2:p=0.18), fat mass (-3.4±1.3 kg *vs.* -3.1±1.3 kg: p=0.48), waist circumference (-4.6±1.4 cm *vs.* -4.8±1.2 cm: p=0.21) and systolic blood pressure (-6.2±3.1 mmHg *vs.* -5.9±2.2 mmHg: p=0.41). After caloric restriction with Diet M, both groups (GG *vs.* GT±TT) also showed a similar decrease in body weight (-4.3±1.1 kg *vs.* -4.1±1.2 kg: p=0.23), BMI (-0.4±0.1 kg/m^2 *vs.* -0.5±0.4 kg/m^2: p=0.38), fat mass (-3.6±1.1 kg *vs.* -3.5±1.3 kg: p=0.61), waist circumference (-5.3±1.3 cm *vs.* -4.7±1.1 cm: p=0.42) and systolic blood pressure (-7.0±3.9 mmHg *vs.* -7.1±1.2 mmHg: p=0.71), too. The improvements in all below-mentioned parameters were similar with both diets.

Table II reports biochemical parameters (inflammatory status, lipid profile and glucose metabolism). No statistical differences were detected among baseline and post-treatment values of these variables between both genotypes (GG *vs.* GT±TT). A significant improvement was detected in the insulin levels and the insulin resistance, measured as HOMA-IR after both caloric restrictions in no T allele carriers. After dietary intervention with a polyunsaturated enriched hypocaloric diet (Diet P), only subjects with GG genotype showed a significant improvement in the insulin levels (GG *vs.* GT±TT) (-3.2±1.0 mU/L *vs.* -0.6±0.4 mU/L: p=0.01) and HOMA-IR (-1.1±0.2 units *vs.* -0.3±0.4 units: p=0.02). The same improvement in both parameters was reported after a monounsaturated enriched hypocaloric diet (Diet M) and only subjects with GG genotype showed a significant improvement in the insulin levels (GG *vs.* GT±TT) (-3.7±0.9 mU/L *vs.* -0.4±0.5 mU/L: p=0.01) and HOMA-IR (-1.0±0.2 units *vs.* -0.4±0.3 units: p=0.03). The improvement in both markers after two diets was similar: insulin concentrations (Diet P: -3.2±1.0 mUI/L *vs.* Diet M: -0.6±0.4 mUI/L: p=0.71) and HOMA-IR (Diet P: -1.1±0.2 units *vs.* Diet M: -1.0±0.2 units: p=0.59). After weight loss with Diet M, both genotypes showed a similar decrease in total cholesterol (GG *vs.* GT±TT) (-9.2±5.1 mg/dl *vs.* -10.0±4.1 mg/dl: p=0.36) and LDL-cholesterol (-12.0±3.6 mg/dl *vs.* -11.9±6.0 mg/dl: p=0.29).

Table I. Changes in anthropometric variables (mean ± SD).

Characteristics	Rs1501299									
	Diet P (n=177)				P Time Genotype Genotype x time	Diet M (n=186)				P Time Genotype Genotype x time
	GG		GT±TT			GG		GT±TT		
	Basal	3 months	Basal	3 months		Basal	3 months	Basal	3 months	
BMI	37.8±6.0	37.31±5.0*	38.0±5. 1	37.3±4.2*	*p*=0.05 *p*=0.28 *p*=0.03	37.5±4.3	37.1±4.0*	37.3±4.0	36.8±4.8*	*p*=0.01 *p*=0.44 *p*=0.02
Weight (kg)	95.0±9.6	90.7±7.1 x	96.4±9.0	91.2±8.1^{x}	*p*=0.01 *p*=0.40 *p*=0.02	96.0±12.0	91.7±9.1x	95.9±8.1	91.8±7.1^{x}	*p*=0.01 *p*=0.51 *p*=0.02
Fat mass (kg)	40.8±8.1	37.4±7.0$^{\#}$	39.4±8.1	36.4±9.0$^{\#}$	*p*=0.02 *p*=0.47 *p*=0.03	39.9±6.0	36.3±5.2$^{\#}$	38.8±7.9	35.3±6.0$^{\#}$	*p*=0.01 *p*=0.54 *p*=0.03
WC (cm)	112.4±8.1	107.8 ±4.2$^{\&}$	110.4±6.1	105.6±8.1$^{\&}$	*p*=0.01 *p*=0.61 *p*=0.02	116.9±8.0	111.6 ±6.9$^{\&}$	115.8±8.1	111.1±4.1$^{\&}$	*p*=0.01 *p*=0.60 *p*=0.02
SBP (mmHg)	129.1±3.0	122.9±4.0**	127.9±4.0	122.0±7.0**	*p*=0.01 *p*=0.55 *p*=0.03	128.8±12.1	121.8±7.1**	127.8±7.2	120.7±7.1**	*p*=0.01 *p*=0.43 *p*=0.03
DBP (mmHg)	83.8±6.0	84.3±3.0	82.9±7.1	83.2±7.0	*p*=0.49 *p*=0.58 *p*=0.51	83.9±5.1	80.4±5.0	83.7±5.1	80.1±5.2	*p*=0.59 *p*=0.78 *p*=0.48

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; Statistical differences in each genotype group (*BMI, ^{x}Weight, $^{\#}$fat mass, & WC, **SBP) No statistical differences between genotype groups.

Table II. Biochemical parameters (mean±SD).

Characteristics	Rs1501299									
	Diet P (n=177)				P Time Genotype Genotype x time	Diet M (n=186)				P Time Genotype Genotype x time
	GG		GT±TT			GG		GT±TT		
	Basal	3 months	Basal	3 months		Basal	3 months	Basal	3 months	
Glucose (mg/dl)	99.9±10.2	99.2±10.1	100.9±8.0	98.7±7.2	p=0.51 p=0.61 p=0.22	99.6±13.1	95.2±8.0	100.1±11.2	98.9±8.2	p=0.51 p=0.70 p=0.21
Total cholesterol (mg/dl)	209.3±14.1	203.5±15.2	200.4±23.1	193.3±21.2	p=0.06 p=0.37 p=0.14	203.7±8.1	194.5±9.1$	202.9±10.9	192.8±12.1$	p=0.01 p=0.16 p=0.02
LDL-cholesterol (mg/dl)	129.3±20.3	127.9±21.1	125.9±22.1	120.7±16.2	p=0.03 p=0.46 p=0.02	125.7±12.0	113.1±9.8%	124.4±24.1	112.5±12.1%	p=0.02 p=0.16 p=0.03
HDL-cholesterol (mg/dl)	53.9±7.0	53.1±5.2	50.5±6.1	53.7±7.6	p=0.04 p=0.53 p=0.03	49.7±6.0	51.5±5.1	50.7±9.1	52.3±7.0	p=0.12 p=0.49 p=0.22
Triglycerides (mg/dl)	126.6±21.1	123.5±18.2	131.2±20.2	121.5±19.2	p=0.61 p=0.89 p=0.30	139.2±13.1	127.8±9.3	137.1±10.9	122.2±11.1	p=0.61 p=0.89 p=0.24
CRP (ng/dl)	6.6±3.0	6.3±2.9	7.0±3.1	5.7±2.8	p=0.40 p=0.50 p=0.18	4.9±3.2	5.3±2.1	5.1±3.0	4.7±3.0	p=0.52 p=0.76 p=0.34
Insulin (mUI/l)	12.3±7.1	9.1±7.0#	12.2±6.0	11.6±7.3	p=0.01 p=0.36 p=0.02	14.8±5.2	10.1±3.9#	12.2±6.1	11.8±8.9	p=0.01 p=0.31 p=0.03
HOMA-IR	3.7±2.1	2.6±1.8&	3.2±2.5	2.9±2.0	p=0.02 p=0.31 p=0.03	3.9±1.1	2.9±1.1&	3.3±2.0	2.9±2.1	p=0.03 p=0.19 p=0.04

CRP: C reactive protein. HOMA-IR (homeostasis model assessment); Statistical differences in each genotype group (#Insulin, &HOMA IR, $Total cholesterol, %LDL cholesterol). No Statistical differences between genotype groups.

2965

Table III shows levels of adipokines. No differences between both genotypes were detected among baseline and post-treatment values of serum adipokine levels. Only subjects with GG genotype showed a significant increase of the adiponectin levels after both diets: (Diet P: 9.3±3.0 ng/dl *vs*. Diet M: 8.2±2.9 ng/dl: p=0.38). After both hypocaloric diets, leptin levels decreased in both genotypes (Table III). This improvement was similar with both diets without any relation to genotype.

Discussion

The most important data of our study was the effect of this genetic variant for the changes in the insulin levels, the HOMA-IR and the adiponectin concentrations, followed by a significant weight loss secondary to two hypocaloric diets with a different unsaturated fat dietary profile. Obese subjects with T allele of this genetic variant did not show an improvement of the adiponectin, the insulin levels and the HOMA-IR.

The relationship between the rs1015299 on ADIPOQ gene and the insulin resistance or adipokines concentrations have been evaluated in a lot of cross-sectional studies[9-15], but there is a lack of information about the influence of dietary intervention on this association in the interventional designs. Furthermore, the results of the relationship between this SNP and the metabolic parameters are contradictory. For example, while in a small population T allele was associated with higher insulin levels and HOMA-IR[11], in other studies opposite results have been reported[13,30,31].

Adipose tissue secretes various pro- and anti-inflammatory adipokines both to regulate inflammation and insulin resistance; the obesity-induced insulin resistance and diabetes mellitus type 2 may partly result from an imbalance in the expression of anti- and pro-inflammatory adipokines. Adiponectin is an anti-inflammatory hormone that has been shown to increase the insulin sensitivity by increasing fatty acid oxidation and inhibition of hepatic glucose metabolism[32]. This fact may be the one that explains the findings of the insulin resistance improvement found in the same genotype group in our study. Secondly, our results suggest that the rs1501299 variant of ADIPOQ gene is related to differential regulation of the adiponectin synthesis and secretion in adipose tissue, secondary to weight loss with a better metabolic improvement in the non-T-allele carriers. As we have previously commented, nutritional intervention studies which evaluate the effects of this gene variant after weight loss are scarce. The first data in the literature has been reported in a non-interventional study on healthy women with this variant[33]: GG homozygotes showed higher adiponectin levels compared to T carriers under the conditions of lower fiber intake. Moreover, the first interventional study[34] based on the replacement of dietary saturated fatty acids (SFAs) with monounsaturated fatty acids (MFAs) or carbohydrates, showed a lack of interaction of (rs1501299) and diet. On the other hand, in a one-branch interventional design[35], after 12-weeks weight loss intervention in obese subjects, the significant decrease in insulin resistance and the increase in the adiponectin levels were observed in GG homozygotes, which were not shown in carriers of T allele, as our above-mentioned results. On the other hand, the resistin levels did not vary in our study, and the leptin levels decreased as expected, after having decreased the weight and after the intervention in both groups.

On the other hand, the intervention studies comparing several diets are even scarcer. For example, one study with three different diets for 4 weeks each (saturate fatty acid-rich diet *vs*. carbohydrate-rich diet *vs*. monounsaturated fatty acid-rich diet) was realized in non-obese subjects[36]. In this design, rs1501299 did not show a statistical relationship with metabolic changes secondary to diet modifications; in addition, only subjects with CC genotype of the -11377C>G SNP at ADIPOQ gene had significantly less insulin resistance after the last two diets, compared with saturated fatty acid-rich diet. The RISCK (Reading, Imperial, Surrey, Cambridge and Kings)[37] study is a randomized controlled trial of 24 weeks with three different diets (reference *vs*. high monounsaturated fatty acid and low-fat diet). Again, rs1501299 did not show a statistical relationship with lipid or glycemic metabolism and only subjects rs182052 variant of ADIPOQ gene showed an association between the increase of adiponectin levels and the high monounsaturated fatty acid diet. In other randomized clinical trials[38] with two hypocaloric diets (I: moderate carbohydrates *vs* II: low fat). Only in subjects with GG genotype, both diets improved fasting insulin levels, HOMA-IR and adiponectin levels.

Table III. Adipokines and cytokine levels (mean±SD).

Characteristics	Rs1501299									
	Diet P (n=177)				P Time Genotype Genotype x time	Diet M (n=186)				P Time Genotype Genotype x time
	GG		GT±TT			GG		GT±TT		
	Basal	3 months	Basal	3 months		Basal	3 months	Basal	3 months	
Resistin (ng/dl)	3.9±1.0	4.0±2.1	3.7±2.2	4.1±2.9	p=0.59 p=0.72 p=0.24	3.9±1.2	4.1±1.1	3.8±1.1	4.1±0.9	p=0.69 p=0.70 p=0.23
Adiponectin (ng/dl)	9.6±4.0	18.9±5.3*	10.5±3.1	13.1±6.0	p=0.59 p=0.812 p=0.31	11.9±6.4	21.1±5.9*	11.0±8.9	13.7±9.0	p=0.59 p=0.73 p=0.21
Leptin (ng/dl)	90.1±21.6	74.1±12.5*	85.9±13.1	69.9±12.1*	p=0.02 p=0.21 p=0.02	88.3±11.1	67.8±9.3*	90.7±13.1	68.2±19.1*	p=0.01 p=0.18 p=0.03

*p<0.05, in each genotype group.

2967

The influence of other strategies on weight loss with ASIPOQ gene effects on metabolic parameters has been evaluated. For example, GG genotype in morbid obese subjects is associated to the increases of the adiponectin levels and a better decrease of HOMA-IR and insulin levels, after a massive weight loss with a biliopancreatic diversion surgery and 3 years of follow up[39].

The exact molecular mechanisms responsible for the interaction between the dietary intake of different profiles of unsaturated fatty acids and this genetic variant (rs1501299) remained unclear. Firstly, we can hypothesize an activation of PPAR-gamma by unsaturated fatty acids, this fact increased expression of ADIPOQ gene and serum adiponectin levels and an (a) unknown linkage disequilibrium between peroxisome proliferator response element and ADIPOQ gene. Secondly, perhaps this SNP could directly influence metabolic response, some investigations have shown an association of this SNP and LDL cholesterol levels[40,41] and blood pressure[42,43]. Thirdly, T allele of ADIPOQ rs1501299 could alter the sequence of one of the transcriptional stimulatory protein binding sites and reduce adiponectin promoter activity. Finally, the results of increased levels of adiponectin and decreased leptin levels are related to weight loss and fat mass, a common feature in the literature[44].

The strength of our study was its design as a randomized controlled trial with high adherence with practical relevance to the general population. Limitations included: firstly, the determination of total adiponectin rather than the most bioactive high molecular weight form, but a strong correlation has been described between the two measures. Secondly, epigenetic modifications can also play a role in the results found and, in our study, an epigenetic analysis has not been carried out. Thirdly, a relatively small sample size. Finally, our population represents a Caucasian obese adult sample without comorbidities; these factors could modulate the response to dietary intervention.

Conclusions

We showed that the GG genotype of ADIPOQ gene variant (rs1501299) is associated to a significant improvement in the adiponectin levels and decreases of insulin and HOMA-IR after two hypocaloric diets with different unsaturated dietary fats. There is no doubt that this is an area of knowledge that requires future research to clarify the role of SNPs in obesity, taking into account the large amount of data that appear in the literature[45-46].

Ethical Approval

All the procedures performed in the 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.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1) Sheng T, Yang K. Adiponectin and its association with insulin resistance and type 2 diabetes. J Genet Genomics 2008; 35: 321-326.
2) Gable DR, Hurel SJ, Humphries SE. Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. Atherosclerosis 2006; 188: 231-244.
3) Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab 2007; 9: 282-289.
4) Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, Mantzoros CS, Hu FB. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. Ann Intern Med 2008; 149: 307-316.
5) Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE, Maas D, Takahashi M, Kihara S, Tanaka S, Matsuzawa Y, Blangero J, Cohen D, Kissebah A. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab 2001; 86: 4321-4325.
6) Jee SH, Sull JW, Lee JE, Shin C, Park J, Kimm H, Cho EY, Shin ES, Yun JE, Park JW, Kim SY, Lee SJ, Jee EJ, Baik I, Kao L, Yoon SK, Jang Y, Beaty TH. Adiponectin concentrations: a genome-wide association study. Am J Hum Genet 2010; 87: 545-552.
7) Croteau-Chonka DC, Wu Y, Li Y, Fogarty MP, Lange LA, Kuzawa CW, McDade TW, Borja JB, Luo J, AbdelBaky O, Combs TP, Adair LS, Lange EM, Mohlke KL. Population-specific coding variant underlies genome-wide association with adiponectin level. Hum Mol Genet 2012; 21: 463-471.
8) Kasim-Karakas SE, Tsodikov A, Singh U, Jialal I. Responses of inflammatory markers to a low-fat, high-carbohydrate diet: effects of energy intake. Am J Clin Nutr 2006; 83: 774-779.
9) Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, Verrienti A, Fanelli M, Fallarino M, Sorropago G, Baroni MG. The adiponectin gene SNP±276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age <or = 50 years). J Mol Med 2005; 83: 711-719.

10) Kyriakou T, Collins LJ, Spencer-Jones NJ, Malcolm C, Wang X, Snieder H, Swaminathan R, Burling KA, Hart DJ, Spector TD, O'Dell SD. Adiponectin gene ADIPOQ SNP associations with serum adiponectin in two female populations and effects of SNPs on promoter activity. J Hum Genet 2008; 53: 718-727.
11) Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, Boutin P, Vaxillaire M, Leprêtre F, Dupont S, Hara K, Clément K, Bihain B, Kadowaki T, Froguel P. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002; 11: 2607-2614.
12) Salmenniemi U, Zacharova J, Ruotsalainen E, Vauhkonen I, Pihlajamäki J, Kainulainen S, Punnonen K, Laakso M. Association of adiponectin level and variants in the adiponectin gene with glucose metabolism, energy expenditure, and cytokines in offspring of type 2 diabetic patients. J Clin Endocrinol Metab 2005; 90: 4216-4223.
13) Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 2002; 51: 536-540.
14) Szopa M, Malczewska-Malec M, Kiec-Wilk B, Skupien J, Wolkow P, Malecki MT, Sieradzki J. Variants of the adiponectin gene and type 2 diabetes in a Polish population. Acta Diabetol 2009; 46: 317-322.
15) Nikolajević-Starčević J, Pleskovič A, Santl Letonja M, Jenko Pražnikar Z, Petrovič D. Polymorphisms +45T>G and +276G>T of the adiponectin gene does not affect plasma adiponectin level and carotid intima-media thickness in patients with diabetes mellitus type 2. Int Angiol 2014; 33: 434-440.
16) Lithander FE, Keogh GF, Wang Y, Cooper GJ, Mulvey TB, Chan YK, McArdle BH, Poppitt SD. No evidence of an effect of alterations in dietary fatty acids on fasting adiponectin over 3 weeks. Obesity (Silver Spring) 2008; 16: 592-599.
17) Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism 2008; 57: 824-830.
18) Qi L, Rimm E, Liu S, Rifai N, Hu FB. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. Diabetes Care 2005; 28: 1022-1028.
19) Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003; 289: 1799-1804.
20) Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am J Clin Nutr 2005; 81: 780-786.
21) Xita N, Georgiou I, Chatzikyriakidou A, Vounatsou M, Papassotiriou GP, Papassotiriou I, Tsatsoulis A. Effect of adiponectin gene polymorphisms on circulating adiponectin and insulin resistance indexes in women with polycystic ovary syndrome. Clin Chem 2005; 51: 416-423.
22) Mataix J, Mañas M. Tablas de composición de alimentos españoles. Ed: University of Granada, 2003
23) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
24) Duart Duart MJ, Arroyo CO, Moreno Frígols JL. Validation of a kinetic model for the reactions in RIA. Clin Chem Lab Med 2002; 40: 1161-1167.
25) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.
26) Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50: 1511-1525.
27) Pfützner A, Langenfeld M, Kunt T, Löbig M, Forst T. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin Lab 2003; 49: 571-576.
28) Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem 2004; 50: 219-221.
29) Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985; 41: 810-817.
30) Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Häring H. Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. Diabetes 2002; 51: 37-41.
31) Jang Y, Lee JH, Kim OY, Koh SJ, Chae JS, Woo JH, Cho H, Lee JE, Ordovas JM. The SNP276G>T polymorphism in the adiponectin (ACDC) gene is more strongly associated with insulin resistance and cardiovascular disease risk than SNP45T>G in nonobese/nondiabetic Korean men independent of abdominal adiposity and circulating plasma adiponectin. Metabolism 2006; 55: 59-66.
32) de Luis DA, Sagrado MG, Pacheco D, Terroba MC, Martin T, Cuellar L, Ventosa M. Effects of C358A missense polymorphism of the endocannabinoid degrading enzyme fatty acid hydrolase on weight loss and cardiovascular risk factors 1 year after biliopancreatic diversion surgery. Surg Obes Relat Dis 2010; 6: 516-520.

33) Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism 2008; 57: 824-830.
34) Alsaleh A, Crepostnaia D, Maniou Z, Lewis FJ, Hall WL, Sanders TA, O'Dell SD, MARINA study team. Adiponectin gene variant interacts with fish oil supplementation to influence serum adiponectin in older individuals. J Nutr 2013; 143: 1021-1027.
35) Shin MJ, Jang Y, Koh SJ, Chae JS, Kim OY, Lee JE, Ordovas JM, Lee JH. The association of SNP276G>T at adiponectin gene with circulating adiponectin and insulin resistance in response to mild weight loss. Int J Obes (Lond) 2006; 30: 1702-1708.
36) Pérez-Martínez P, López-Miranda J, Cruz-Teno C, Delgado-Lista J, Jiménez-Gómez Y, Fernández JM, Gómez MJ, Marín C, Pérez-Jiménez F, Ordovás JM. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. J Nutr 2008; 138: 1609-1614.
37) AlSaleh A, O'Dell SD, Frost GS, Griffin BA, Lovegrove JA, Jebb SA, Sanders TA; RISCK Study Group. Single nucleotide polymorphisms at the ADIPOQ gene locus interact with age and dietary intake of fat to determine serum adiponectin in subjects at risk of the metabolic syndrome. Am J Clin Nutr 2011; 94: 262-269.
38) de Luis DA, Izaola O, Primo D, Aller R, Ortola A, Gomez E, Lopez JJ. The association of SNP276G>T at adiponectin gene with insulin resistance and circulating adiponectin in response to two different hypocaloric diets. Diabetes Res Clin Pract 2018; 137; 93-98.
39) de Luis DA, Pacheco D, Primo D, Izaola O, Aller R. The association of snp276g>t at adiponectin gene with insulin resistance and circulating adiponectin in morbid obese patients after a biliopancreatic diversion surgery. Obes Surg 2017; 27: 3247-3252.
40) Melistas L, Mantzoros CS, Kontogianni M, Antonopoulou S, Ordovas JM, Yiannakouris N. Association of the ±45T>G and ±276G>T polymorphisms in the adiponectin gene with insulin resistance in nondiabetic Greek women. Eur J Endocrinol 2009; 161: 845-852.
41) Jang Y, Lee JH, Chae JS, Kim OY, Koh SJ, Kim JY, Cho H, Lee JE, Ordovas JM. Association of the 276G→T polymorphism of the adiponectin gene with cardiovascular disease risk factors in nondiabetic Koreans. Am J Clin Nutr 2005; 82: 760-767.
42) Leu HB, Chung CM, Lin SJ, Jong YS, Pan WH, Chen JW. Adiponectin gene polymorphism is selectively associated with the concomitant presence of metabolic syndrome and essential hypertension. PLoS One 2011; 6(5): e19999.
43) Gupta V, Khadgawat R, Ng HK, Walia GK, Kalla L, Rao VR, Sachdeva MP. Association of TCF7L2 and ADIPOQ with body mass index, waist-hip ratio, and systolic blood pressure in an endogamous ethnic group of India. Genet Test Mol Biomarkers 2012; 16: 948-951.
44) de Luis DA, Perez Castrillón JL, Dueñas A. Leptin and obesity. Minerva Med 2009; 100: 229-236.
45) de Luis DA, Izaola O, Primo D, Aller R. Effect of the rs1862513 variant of resistin gene on insulin resistance and resistin levels after two hypocaloric diets with different fat distribution in subjects with obesity. Eur Rev Med Pharmacol Sci 2018; 22: 3865-3872.
46) Zhu ZL, Yang QM, Li C, Chen J, Xiang M, Chen MM, Yan M, Zhu ZG. Association between the resistin gene-420 C>G polymorphism and obesity: an updated meta-analysis. Eur Rev Med Pharmacol Sci 2016; 20: 4922-4929.