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Role of rs670 variant of *APOA1* gene on metabolic response after a high fat vs. a low fat hypocaloric diets in obese human subjects

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ABSTRACT

Background & aims: A common G-to-A transition located 75 base pairs upstream (rs670) from transcription start site of the *APOA1* gene has been related with some metabolic parameters. Our aim was to analyze the effects of *rs670 APOA1 gene* polymorphism on lipid profile and metabolic changes after two different hypocaloric diets.
Methods: 282 obese subjects were randomly allocated during 12 weeks (Diet HF – high fat diet vs. Diet LF – low fat diet). Anthropometric and biochemical status were evaluated.
Results: Body mass index, weight, fat mass, waist circumference, systolic blood pressure, leptin levels and waist circumference decreased in all patients in average after both diets. In A allele carriers after 12 weeks with both diets, insulin levels (Delta diet HF: −5.3 + 1.2 UI/L; P = 0.02 and Delta diet LF: −5.8 + 1.3 UI/L; P = 0.02) and HOMA-IR (Delta diet HF: −2.9 + 0.8 units; P = 0.01 and Delta diet LF: −2.2 + 0.9 units; P = 0.03) improved in a significant way. With the low fat diet, A allele carriers showed a statistical improvement in HDL-cholesterol levels (Delta: 4 + 1 mg/dl; P = 0.03).
Conclusions: Our study showed the association of rs670 *ApoA1* polymorphism with a decrease of insulin resistance induced by both diets and provided additional evidence on HDL-cholesterol increase after a LF hypocaloric diet in A allele carriers.

1. Introduction

Dietary recommendations to control plasma lipid and lipoprotein profiles and reduce the risk of cardiovascular disease are aimed at total calorie, total fat, fat saturation and cholesterol contents of the diet.[1] There is already a growing body of evidence suggesting that the variability in dietary responses has a strong genetic component.

Apolipoprotein ApoA1 is the major protein of HDL-cholesterol and plays an important role in lipoprotein metabolism. For example, ApoA1 is the main activator of the enzyme lecithin cholesterol acyl transferase,[2] and it is the main component of the reverse cholesterol transport pathway.[3] The gene for *ApoA1* is highly polymorphic and common single nucleotide polymorphisms (SNPs) have been described extensively in relation to plasma lipid concentrations.[4] In vitro studies have showed that overexpression of the human *APOA1* gene increased HDL-C levels and reduce the cardiovascular risk secondary to a high fat diet.[5]

A common mutation due to an adenine (A) to guanine (G) transition (G/A) has been reported 75 bp upstream (rs670) from the Apo A-1 gene transcription start site.[6] The presence of the A allele has been associated with higher ApoA1 and HDL-cholesterol levels,[7] but not in all population studies.[8,9] Moreover, rs670 variant has a direct effect on plasma LDL-cholesterol responsiveness to change in the amount of total dietary fat in non-obese subjects.[10] Besides, Philips et al[11] have reported that ApoA1 rs670 may influence metabolic syndrome risk secondary to dietary fat composition, too. Perhaps, the variability of the lipid levels response to changes in the amount of dietary fat or caloric restriction may be explained by this genetic variants. Besides that, in the literature there is few studies evaluating the effect of this SNP of *APOA1* gene on lipid levels modifications after a dietary intervention[11] with a clinical trial design with two different amount of total dietary fat.

Our aim was to analyze the effects of *rs670 APOA1 gene* polymorphism on lipid profile and metabolic changes after two different hypocaloric diets in obese patients.

2. Materials and methods

2.1. Subjects and clinical investigation

We recruited females and males aged 25–65 years (282 Caucasians subjects) with a body mass index ≥30 kg/m^2 from Primary Care Physicians of an urban area of Castilla y Leon (Norwest of Spain). The recruitment of obese patients was a consecutive method of sampling among

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subjects send from Primary Care Physicians to treat obesity. The protocol was approved by the local ethical review boards (Hospital Clinico Universitario Valladolid (HCUVA) ethics committee) and participants provided signed informed consent. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Data of these subjects were collected at the beginning and after 12 weeks of dietary treatment.

Subjects were excluded if had history of cardiovascular disease or stroke during the previous year, history of cancer undergoing active treatment, weight loss of >5% of body weight in the last 3 months, total cholesterol ≥200 mg/dl, triglycerides ≥150 mg/dl, blood pressure ≥ 140/90 mm Hg, as well as the use of any of the treatments of diabetes mellitus, glucocorticoids, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other antidyslipidemic drugs.

2.2. Procedure

After subjects met the above criteria, they were randomly allocated to one of two diets for a period of 12 weeks (diet HF, high fat diet vs Diet LF, low fat diet). The randomization was done with a table of random numbers. Drop-outs was 16 subjects in diet group I and 2 in group II diet. Of the 16 dropouts in the HF diet, 8 were women and 8 were men, the reason did not want to perform a prolonged dietary restriction, with respect to the two patients that left in the LF diet, 1 was female and another male, leaving by the same causes the HF diet. All participants underwent a medical evaluation including physical examination, anthropometric evaluation and a clinical chemistry analysis. Data on anthropometric parameters (weight, height, body mass index (BMI), waist circumference, fat mass by impedance) and blood pressure were recorded at basal time and after dietary intervention. Blood samples were collected in EDTA-treated and plain tubes after a 12 h overnight fast for analysis of insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and serum adipokine levels (leptin, total adiponectin and resistin). These biochemical parameter were measure at basal time and after 12 weeks. The variant of *ApoA1* gene was assessed at basal time by polymerase chain reaction at real-time.

2.3. Dietary intervention

Both diets was designed to provide about 500 kcal/day less than individually estimated total energy expenditure during 12 weeks. As above-mentioned, patients were randomly allocated to one of two diets for a period of 12 weeks. The target macronutrient composition with a Diet HF (high fat diet) was (38% carbohydrates, 24% proteins and 38% fats). Diet LF was low in fats (53% carbohydrates, 20% proteins and 27% fats). The quality of dietary fat was similar in both diets (45% monounsaturated fatty acids, 30% saturated fatty acids and 25% polyunsaturated fatty acids), the main difference was the total amount of fat. All participants had two individual sessions (45 min with diet sheets and example menu plans) with the dietitian at the start of the trial to explain the diet and solve doubts. Food tables were used with a Mediterranean pattern including (legumes, vegetables, poultry, fish and fresh fruit, using olive oil). Sugar-sweetened drinks, alcoholic beverages of high graduation were prohibited. This dietitian assessed the adherence of this diet each 2 weeks days with a phone call. All enrolled subjects were instructed to record their daily dietary intake for three non-consecutive days including a weekend day. These groups of 3 dietary records were collected two weeks prior to the randomization (data to obtain the basal diet) and each month during the randomization (data to obtain the diet intervention). This adherence was assessed each 7 days with a phone call by a dietitian in order to improve compliment. Records were analyzed with a computer-based data evaluation system (Dietosource ®, Ge,Swi). National composition food tables were used as reference.[12]

The recommended physical activity consisted of an aerobic exercise at least two times per week (60 min each). The exercises recommended by the authors were (walking, running, cycling, swimming) and muscular strength exercises such as weight training or harterophilia were contraindicated. The exercise activity was recorded by the patient with a self-reported questionnaire.

2.4. Blood pressure and anthropometric parameters

Weight and height were measured with an electrical scale (Omrom, LA, CA) and a telescopic height measuring instrument (Omrom, LA, CA). Body weight were measured in the morning while the subjects were minimally unclothed and not wearing shoes. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Waist circumferences (WC) was measured with a flexible non-stretchable measuring tape (Type SECA, SECA. Birmingham, UK). A bipolar bioimpedance was used to determine body composition with an accuracy of 5 g[13] (BIA 110) (EFG, Akern, It). Blood pressure was measured by investigators twice after a 10 min rest with a random zero mercury sphygmomanometer, and averaged (Omrom, LA,CA).

2.5. Genotyping

Blood sample was collected at the beginning of the study. DNA was extracted from the buffy coat fraction using a commercial kit (Biorad, LA, CA) according to the manufacturer's protocol. Its quantity and quality were determined with a NanoDrop ND-1000 spectometer (Bio-Rad®, San Diego, CA). Primers were realized with the Sequenom Assay Design v4 (SEQUENOM, Inc.San Diego, California CA). Genotyping for the rs670 polymorphism was performed by polymerase chain reaction real time analysis. Briefly, this polymerase chain reaction (PCR) was carried out with 30 ng of genomic DNA, 0.1–0.15 μl each of oligonucleotide primer for rs670 (primer forward: 5′-ACGTTGGATGAAGTTCCACATTGCCAGGAC-3′ and reverse 5′-ACGTTGGATGCAGGGCCTATTTATGTCTGC-3′ in a 2.5-μl final volume (Termociclador Life Tecnologies, LA, CA). DNA was denatured at 85 °C for 5 min; this was followed by 45 cycles at 65 °C for 15 s, and annealing at 58.1 °C for 45 s. Hardy Weinberg equilibrium was calculated with a statistical test (Chi-square) and the variant of *ApoA1* gene was in Hardy Weinberg equilibrium in diet HF group ($P = 0.34$) and diet LF group ($P = 0.41$).

2.6. Biochemical procedures

Venous blood was collected in EDTA containing tubes after a 12-h overnight fast at two time points (basal and 12 weeks). Glucose, total cholesterol, triglyceride and lipid profile (low density lipoprotein cholesterol (LDL) and high density lipoprotein cholesterol (HDL)) were measured using a biochemical auto-analyzer (Hitachi 7060, Tokyo, Japan). LDL cholesterol was calculated using Friedewald formula.[14] Fasting glucose was determined by the enzymatic colorimetric method (glucose oxidase). Fasting insulin was measured by radio-immunoassay method (RIA) (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5mUI/L (normal range 0.5–30 mUI/L).[15] Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the following equation (**HOMA-IR** = (insulin UI/Lx glucose mmol/l)/22,5).[16]

All the next adipokines were measured by enzyme-Linked Immuno-Sorbent Assay (ELISA). Leptin kit (Diagnostic Systems Laboratories, Inc., Texas, USA) (DSL1023100) has a sensitivity of 0.05 ng/ml and a normal range of 10–100 ng/ml and a CV% 3.5%.[17] Adiponectin kit (R&D systems, Inc., Mineapolis, USA) (DRP300) has a sensitivity of 0.246 ng/ml and a normal range of 8.65–21.43 ng/ml and a CV% 3.8%.[18] Resistin kit (Biovendor Laboratory, Inc., Brno, Czech Republic) (RD191016100) has a sensitivity of 0.2 ng/ml and a normal range of 4–12 ng/ml[19] and a CV% 3.2%. CRP was measured by immunoturbimetry (Roche

Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg/dl) and analytical sensivity 0.5 mg/dl.

2.7. Statistical analysis

All the data were analyzed using SPSS for Windows, version 19.0 software package (SPSS Inc. Chicago, IL). Sample size was calculated to detect differences over 3 ± 0.2 mg/dl in HDL cholesterol levels after dietary intervention with 90% power and 5% significance ($n = 130$, in each diet group). The Kolmogorov–Smirnov test was used to determine variable distribution. The results were expressed as average ± standard deviation. Numerical variables with normal distribution were analyzed with a two-tailed Student's *t*-test. Non-parametric variables were analyzed with the Mann-Whitney's *U*-test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. The statistical analysis to evaluate the gene –diet interaction was a univariate ANCOVA. Time × genotype interactions was evaluated, too. A Chi square test was used to evaluate the Hardy–Weinberg equilibrium. All analysis were performed under a dominant genetic model with rs670 A-allele as the risk allele (AA+AG vs. GG). A P-value < 0.05 was considered significant.

3. Results

Two hundred and eighty two obese subjects were enrolled in the study. The mean age was 49.7 ± 9.2 years (range: 27–64) and the mean BMI 36.5 ± 3.1 kg/m^2 (range: 32.2–39.1). Gender distribution in the all group was 27.8% males and 72.2% females. Sex distribution was similar in both genotype groups, males (28.1% vs 27.2%: ns) and females (71.9% vs 72.8%:ns). In total, 187 subjects (66.3%) had the genotype GG, 88 patients GA (31.2%) and 7 subjects *AA* (2.5%). Age was similar in the three genotype groups (GG; 49.8 ± 9.1 years vs GA; 49.0 ± 10.2 years vs AA; 49.2 ± 8.0 years: ns).

In the 134 subjects (83 GG as wild genotype and 50 GA/1 AA as mutant genotype (GA + AA)) treated with diet HF, basal dietary intakes showed a calorie intake of 1833.1 ± 342.1 kcal/day, a carbohydrate intake of 179.2 ± 21.9 g/day (42.1% of calories), a fat intake of 84.9 ± 9.1 g/day (38.2% of calories) and a protein intake of 88.2 ± 9.0 g/day (19.7% of calories). During the intervention, these subjects reached the recommendations of diet HF (high fat: 1341.1 ± 126.8 kcal/day, 37.4% carbohydrates, 26.1% proteins, 36.5% fats). Physical activity was similar in both genotype groups (58.2 ± 22.3 min/week vs 60.1 ± 20.1 min/week: P = 0.78).

In the 148 subjects (104 GG as wild genotype and 38 GA/6 AA as mutant genotype (GA + AA)) treated with diet LF, basal dietary intake showed a calorie intake of 1789.2 ± 219.9 kcal/day, a carbohydrate intake of 176.3 ± 42.1 g/day (43.0% of calories), a fat intake of 80.1 ± 11.9 g/day (36.4% of calories) and a protein intake of 90.2 ± 8.3 g/day (20.6% of calories). During the intervention, these subjects reached the recommendations of diet LF (low fat: 1321 ± 203.8 kcal/day, 53.2% carbohydrates, 20.8% proteins, 26.0% fats). Physical activity was similar in both genotype groups (60.1 ± 16.3 min/week vs 59.3 ± 26.9 min/week: P = 0.61).

Table 1 details the adiposity parameters and blood pressure of participants at baseline and at week 12 of intervention. In both genotype groups, body weight, body mass index (BMI), fat mass, waist circumference and blood pressure decreased in a significant way. After 12 weeks with a high fat diet (diet HF) (GG vs. GA + AA): BMI (delta:-1.5 ± 0.7 kg/m^2 vs. −1.2 ± 0.6 kg/m^2: P < 0.05), weight (delta:-3.3 ± 1.1 kg vs. −3.4 ± 1.1 kg: P < 0.05), fat mass (delta:-2.0 ± 0.8 kg vs. -2.3 ± 1.0 kg: P < 0.05), waist circumference (delta: −3.2 ± 1.3 cm vs. −2.8 ± 1.2 cm: P < 0.05) and systolic blood pressure (delta:-2.3 ± 1.0 mm Hg vs. −1.6 ± 0.9 mm Hg: P < 0.05) decreased. After dietary intervention with the low fat diet (Diet LF) (GG vs. GA + AA): BMI (delta: −1.3 ± 0.5 kg/m^2 vs. −1.3 ± 0.6 kg/m^2: P < 0.05), weight (delta: −4.0 ± 1.0 kg vs. −3.9 ± 0.7 kg: P < 0.05), fat mass (delta: −2.8 ± 0.9 kg vs. −3.2 ± 1.0 kg: P < 0.05), waist circumference (delta:-5.0 ± 1.9 cm vs. -4.5 ± 1.1 cm: P < 0.05) and systolic blood pressure (delta:-1.3 ± 0.4 mm Hg vs. −1.2 ± 0.6 cm: P < 0.05) decreased, too. All these data shows a significant genotype x time interactions. After 12 weeks with both diets, the improvement of the anthropometric parameters and blood pressure was similar in both genotype groups.

Table 2 details lipid profile, glucose metabolism and CRP levels. The basal levels of HDL-cholesterol were already higher in A allele carriers in both diet groups. In A allele carriers after 12 weeks with diet HF, insulin levels (Delta: -5.3 ± 1.2 UI/L; P = 0.02) and HOMA-IR (Delta: −2.9 ± 0.8 units; P = 0.01) improved in a significant way. The changes in

Table 1
Changes in anthropometric variables and blood pressure after dietary intervention (Diet HF-High fat) and diet LF (Diet low fat) in both genotypes (mean ± SD).

Characteristics	Rs670									
	Diet HF ($n = 134$)				P	Diet LF ($n = 148$)				P
	GG		GA + AA		Time Genotype	GG		GA + AA		Time Genotype
	Basal	12 weeks	Basal	12 weeks	Genotype x time	Basal	12 weeks	Basal	12 weeks	Genotype × time
BMI	36.8 ± 4.6	35.2 ± 3.1*	36.2 ± 5.1	35.0 ± 4.0*	P = 0.009 P = 0.33 P = 0.02	36.5 ± 4.3	35.2 ± 4.1*	36.6 ± 4.1	35.3 ± 4.2*	P = 0.001 P = 0.43 P = 0.02
Weight (kg)	94.6 ± 14.6	91.3 ± 9.1*	95.1 ± 13.1	91.7 ± 12.1*	P = 0.008 P = 0.32 P = 0.02	91.3 ± 11.1	87.3 ± 9.2*	93.4 ± 11.0	89.5 ± 8.1*	P = 0.007 P = 0.39 P = 0.01
Fat mass (kg)	41.6 ± 9.1	39.6 ± 7.1*	39.6 ± 5.2	37.3 ± 5.0*	P = 0.007 P = 0.45 P = 0.01	39.4 ± 6.1	36.6 ± 7.0*	39.8 ± 10.2	36.6 ± 7.1*	P = 0.008 P = 0.38 P = 0.02
WC (cm)	109.7 ± 10.1	106.5 ± 9.1 *	107.9 ± 8.1$	105.1 ± 7.1*	P = 0.02 P = 0.59 P = 0.03	108.4 ± 9.1	103.4 ± 5.0*	109.7 ± 8.1	105.2 ± 8.1*	P = 0.01 P = 0.69 P = 0.03
SBP (mmHg)	126 ± 12	121 ± 121*	130 ± 9	124 ± 8*	P = 0.01 P = 0.59 P = 0.03	128 ± 9	125 ± 5*	129 ± 5	126 ± 4*	P = 0.02 P = 0.62 P = 0.03
DBP (mmHg)	82 ± 5	79 ± 4	83 ± 5	82 ± 5	P = 0.49 P = 0.48 P = 0.59	82 ± 4	81 ± 3	84 ± 5	83 ± 4	P = 0.56 P = 0.71 P = 0.60

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; LF: Low Fat HF: High fat Statistical differences in each genotype group (*BMI, [x]Weight, #fat mass, & WC, **SBP) No statistical differences between genotype groups. Normal distribution was given for every parameter in both genotype groups and at both time points.

Table 2
Biochemical parameters after dietary intervention (Diet HF-High fat) and diet LF (Diet low fat) in both genotypes (mean ± SD).

Characteristics	Rs670									
	Diet HF (n = 134)				P Time Genotype Genotype x time	Diet LF (n = 148)				P Time Genotype Genotype x time
	GG		GA + AA			GG		GA + AA		
	Basal	12 weeks	Basal	12 weeks		Basal	12 weeks	Basal	12 weeks	
Glucose (mg/dl)	100.2 ± 13.1	97.1 ± 11.9$	98.8 ± 7.1	94.5 ± 8.1$	P = 0.49 P = 0.61 P = 0.22	101.3 ± 6.8	97.9 ± 9.1$	101.9 ± 7.2	100.3 ± 9.1$	P = 0.52 P = 0.61 P = 0.23
Total cholesterol (mg/dl)	204 ± 39	190 ± 34	207 ± 18	194 ± 12	P = 0.54 P = 0.69 P = 0.22	199 ± 10	186 ± 13	210 ± 11	200 ± 9	P = 0.53 P = 0.64 P = 0.15
LDL-cholesterol (mg/dl)	131 ± 41	121 ± 33	117 ± 9	106 ± 11	P = 0.56 P = 0.79 P = 0.23	116 ± 9	104 ± 13	133 ± 21	122 ± 9	P = 0.52 P = 0.63 P = 0.19
HDL-cholesterol (mg/dl)	49 ± 8	49 ± 7	54 ± 12$	55 ± 9$	P = 0.44 P = 0.56 P = 0.14	51 ± 11	54 ± 9	55 ± 8$	59 ± 8*$	P = 0.03 P = 0.38 P = 0.04
Triglycerides (mg/dl)	139 ± 52	125 ± 43	111 ± 33	109 ± 30	P = 0.69 P = 0.81 P = 0.31	111 ± 32	105 ± 34	105 ± 13	107 ± 21	P = 0.61 P = 0.82 P = 0.24
CRP (ng/dl)	5.6 ± 3.8	7.0 ± 2.5	5.0 ± 2.0	5.9 ± 1.3	P = 0.41 P = 0.52 P = 0.13	4.9 ± 2.1	4.1 ± 1.8	5.5 ± 1.9	4.3 ± 4.1	P = 0.59 P = 0.71 P = 0.31
Insulin (mUI/l)	18.9 ± 11.1	15.4 ± 7.8	17.7 ± 8.8	12.4 ± 4.8*	P = 0.007 P = 0.13 P = 0.02	15.4 ± 5.8	13.5 ± 4.1	17.2 ± 4.8	11.4 ± 5.1*	P = 0.02 P = 0.34 P = 0.03
HOMA-IR	4.5 ± 3.2	3.6 ± 1.7	5.9 ± 1.6	3.0 ± 1.3*	P = 0.008 P = 0.21 P = 0.01	4.3 ± 1.7	3.9 ± 1.5	4.6 ± 1.1	2.4 ± 1. 2*	P = 0.03 P = 0.13 P = 0.04

CRP: C reactive protein. HOMA-IR (homeostasis model assessment) LF: Low Fat HF: High fat; Statistical differences in each genotype group (*) after intervention. ($) Statistical differences between genotype groups No statistical differences between genotype groups. Normal distribution was given for every parameter in both genotype groups and at both time points.

insulin and HOMA-IR levels in non-A allele carriers were not statistically significant. The remaining parameters didn't change after dietary intervention in both genotype groups. Sex did not influence the results, data not shown.

After dietary intervention with Diet LF, insulin levels (Delta: −5.8 ± 1.3 UI/L; P = 0.02) and HOMA-IR (Delta: −2.2 ± 0.9 units; P = 0.03) improved in A allele carriers, too. With the low fat diet, A allele carriers showed a statistical improvement in HDL-cholesterol levels (Delta: 4 ± 1 mg/dl; P = 0.03). Finally, basal levels of HDL-cholesterol and post-treatment levels of HDL-cholesterol were better in A allele carriers (Table 2). Sex did not influence the results, data not shown.

Table 3 details levels of serum adipokines. No differences were detected among baseline and post-treatment values of adipokines between both genotypes. After weight loss with both diets, leptin levels decreased in a significant way. Resistin and adiponectin levels remained unchanged after dietary intervention.

4. Discussion

In this, 12-weeks randomized weight-loss diet intervention trial; we found an effect of the rs670 polymorphism, located in *ApoA1* gene, in modulating HDL-cholesterol changes after a low-fat diet and insulin resistance after both hypocaloric diets.

A allele frequency of the ApoA1 polymorphism in our study (0.18) was similar to previous literature studies.[20–23] Only one study has shown the double rate (0.32) on such frequency.[24] Other finding of our study was the association between HDL-cholesterol concentrations and A allele. The high levels of HDL-cholesterol in A allele carriers has been reported in most of previous studies.[20,21,25,26] Only one study[8] has shown an inverse relationship of this allele and HDL-cholesterol levels. Perhaps, this contradictory association may be due to differences factors such as; previous pharmacological treatments, basal BMI, gender distribution, different basal dietary intakes or presence of diabetes in obese subjects.[26,27]

Table 3
Adipokines and cytokine levels after dietary intervention (Diet HF-High fat) and diet LF (Diet low fat) in both genotypes (mean ± SD).

Characteristics	Rs670									
	Diet HF (n = 134)				P Time Genotype Genotype × time	Diet LF (n = 148)				P Time Genotype Genotype × time
	GG		GA + AA			GG		GA + AA		
	Basal	12 weeks	Basal	12 weeks		Basal	12 weeks	Basal	12 weeks	
Resistin (ng/dl)	3.9 ± 1.2	4.0 ± 1.1	3.9 ± 1.3	3.8 ± 1.2	P = 0.59 P = 0.71 P = 0.22	3.7 ± 1.4	3.6 ± 1.3	3.6 ± 1.2	3.9 ± 1.2	P = 0.71 P = 0.68 P = 0.22
Adiponectin (ng/dl)	26.8 ± 12.1	25.1 ± 9.5	33.1 ± 13.0	24.8 ± 9.3	P = 0.59 P = 0.89 P = 0.36	40.3 ± 9.0	32.7 ± 8.1	36.6 ± 8.1	39.1 ± 7.8	P = 0.56 P = 0.70 P = 0.21
Leptin (ng/dl)	80.9 ± 33.1$	67.7 ± 12.1*	83.1 ± 19.4	67.2 ± 12.3*	P = 0.03 P = 0.21 P = 0.02	95.9 ± 20.1	66.7 ± 13.5*	96.2 ± 10.4	74.3 ± 11.0*	P = 0.02 P = 0.16 P = 0.03

LF: Low Fat HF: High fat *P < 0.05, in each genotype group. Normal distribution was given for every parameter in both genotype groups and at both time points.

The most relevant data of our study is the improvement of HDL cholesterol levels in A carriers after a hypocaloric low fat diet. A case control study without dietary intervention[10] showed that metabolic syndrome risk appeared to be modified by quality of dietary fat intake and the metabolic deleterious effect was worsened among non A allele carriers consuming high-fat diet disregard the fatty acids distribution. The genotype didn't modify the risk in subjects who consumed a low-fat diet. As we can see in our results, the presence of allele A in subjects who followed a low-fat diet was associated with an increase in HDL cholesterol levels and therefore secondary to the risk of metabolic syndrome.[28] This is a very relevant fact that strengthens the use of low-fat diets to improve the lipid profile such as HDL cholesterol levels. And in further recommendations, advantages of consuming a low-fat hypocaloric diet over a high-fat hypocaloric diet in A allele carriers should be considered in the literature. Other non-interventional study[29] reported that the rs670 SNP of *APOA1* gene interacted with saturated fat intake on LDL-cholesterol levels. In our study, we did not find this type of response with either diet. However, the population of the other study[29] is non-Caucasian and this could be included in the reported association.

A recent interventional trial of 10 weeks[30] reported that there was a relationship between A allele and weight changes after a diet intervention, but no association with lipid changes was identified. In this intervention trial, A allele carriers had a higher decrease of body weight than non A allele carriers. In our design, we did not find this association with the weight modification. Differences between designs could explain this lack of association, for example; the treated population[11] were adolescents and the dietary intervention supposed a caloric restriction that was different from the one proposed in our adult design. Phenotypic differences in the populations studied may also explain these contradictions, for example Philips et al[11] have detected that non A allele carriers obese adolescents showing an increased abdominal obesity and impaired insulin sensitivity after a dietary intervention, but no changes was identified in A allele carriers.

In other interventional design, a non-hypocaloric diet rich in polyunsaturated fatty acids has been shown to induce greater LDL-cholesterol decrease in A allele carriers than non A allele carriers compared with a high saturated fatty acid enriched diet.[31] In our work we did not detect this response. Again, differences in design may explain these differences. The study of Mata et al[31] was performed in non-obese subjects, with an intervention that oscillated between 28 and 35 days, the diet did not present a caloric restriction as used by us and the fat distribution in their different used diets was very different from ours, with a total fat intake of 35%. This percentage of fat makes located the three diets in the range of high fat diets as our Diet HF. Finally, the percentage of polyunsaturated fatty acids may also influence the results as demonstrated in another intervention study.[32]

An interesting finding is the effect of the A allele on the modification of insulin resistance. Philips et al[11] showed in non-A allele carriers with a high dietary fat intakes further impairments to insulin resistance. A recent study[33] has demonstrated that A allele of rs670 variant appear to be protective for metabolic syndrome risk in participants with higher intake of sugar. This unknown association between A allele and glucose metabolism could be explained by molecular pathways. *APOA1* gene is thought to be stimulated by insulin through SP-1 binding elements[34] and ApoA1 rs670 as lying in a sequence homologous to the binding site for this nuclear factor SP-1. Our results show how these metabolic effects are independent of adipokines. Probably the absence of increase of adiponectin after weight modification is related to the amount of weight loss or other unknown factors that have not been monitored in our work.

All the aforementioned data may be interesting considering the effect that this variant of the APOA1 gene and other variants and clusters may have on the usual dietary fat intake of patients.[35,36]

Finally, the levels of leptin decreased with both diets and with both genotypes, in relation to the observed weight loss. These data are those already known in the literature[37] and do not seem to have a relevant relationship with the results obtained in our work. The rest of adipokines studied did not show significant differences

Limitations of our study are; first, it is the lack of ApoA1 levels. The second, it is the short-term of the dietary intervention (12 weeks), this makes that we cannot predict that it can happen in the long term with these responses. Third, another limitation may be the effect of other macronutrients, for example the difference in the percentage of proteins of both diets. Fourth, underestimation of dietary intake is a bias of our design. Finally, the absence in the modification of the percentage of polyunsaturated and monounsaturated fats in both diets that may influence the metabolic response described.

To conclude, our study showed the association of A allele of rs670 *ApoA1* polymorphism with a significant decrease of insulin resistance induced by two different hypocaloric diet and provided additional evidence on HDL-cholesterol increase after a low-fat hypocaloric diet in A allele carriers. The total amount of dietary fat could not be a determinant factor in the weight loss response but showed an important role in improving the lipid profile by increasing the HDL cholesterol levels. The advantages of consuming a low-fat hypocaloric diet over a high-fat hypocaloric diet in A allele carriers should be considered for future recommendations in the literature, although it does not seem to be relevant for losing weight.[38]

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