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APOA-5 genetic variant and a hypocaloric diet enriched in ω-6 fatty acids with Mediterranean pattern

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Abstract. – OBJECTIVE: The *APOA5*-1131C allele is related to a worse lipid profile and metabolic response to diet interventions. The present study was designed to investigate the effect of SNP *rs662799* on the lipid profile of patients with obesity after a hypocaloric diet with a Mediterranean pattern enriched in ω-6 polyunsaturated fatty acids (PUFA).

PATIENTS AND METHODS: A population of 362 Caucasian patients with obesity was evaluated. Anthropometric evaluation and serum parameters (lipid profile, insulin, homeostasis model assessment (HOMA-IR), glucose, C reactive protein, and adipokines) were measured at basal time and after 12 weeks. All subjects were genotyped *rs662799*.

RESULTS: The *APOA5* variant distribution among the 362 patients with obesity was the following: 87.2% (n=316) (TT) were homozygous for the T allele, 12.2% (n=44) (TC) were heterozygous, and 0.6% (n=2) (CC) were homozygous for the C allele. There were only significant differences in triglyceride levels between genotype groups. After 12 weeks of intervention, the following parameters improved in both genotype groups: adiposity parameters, systolic blood pressure, total cholesterol, LDL cholesterol, leptin, adiponectin, and ratio leptin/adiponectin. Insulin levels (delta: -3.5±0.2 UI/L *vs.* -1.2±0.6 UI/L; *p*=0.03), HOMA-IR (delta: -1.6±0.1 units *vs.* -0.3±0.2 units; *p*=0.01) and triglyceride levels (delta: -18.8±4.1 mg/dl *vs.* -3.7 ±3.0 mg/dl; *p*=0.02) decreased in non-C allele carriers.

CONCLUSIONS: Our data demonstrate that the minor C allele of the *APOA5* gene (*rs662799*) produces a worse response in triglyceride levels, insulin levels, and HOMA-IR after a ω-6 PUFA enriched hypocaloric diet with Mediterranean pattern.

Key Words:
ApoA5, *rs662799*, Mediterranean diet, Triglycerides, Ω-6 polyunsaturated fatty acids.

Introduction

Dyslipidemia is a common problem detected in patients with obesity[1], and this lipid profile typically shows a decrease in HDL-cholesterol and an increase in triglyceride levels[2]. In this context, the role of Apolipoprotein A5 (ApoA5) in the regulation of lipid metabolism is very important. ApoA5 is secreted from the liver, and this protein with 336 amino acids is contained in high-density lipoprotein (HDL) particles[3,4]. Secondarily, ApoA5 maintains the normality of serum triglycerides with two actions; this protein decreases the synthesis of very low-density lipoprotein rich in triglycerides (VLDL) and ApoA5 maximizes hydrolysis of VLDL triglycerides produced by lipoprotein lipase[3,4].

Scholars have shown that *APOA5* gene is related to metabolic syndrome[5] and cardiovascular risk[6]. In addition, single nucleotide polymorphisms (SNPs) in *APOA5* gene have been associated with a high risk of metabolic syndrome and its entities, and particularly with the elevation of serum triglyceride levels[7-11]. Specifically, the *rs662799* variant of the *APOA5* gene (-1131T>C) is considered a functional-tag SNP with a clear association with metabolic syndrome[12]. This functional genetic variant is related to a higher serum triglyceride level, as the C allele impairs ribosomal translation efficiency. This produces a low level of ApoA5 and lipoprotein lipase activity[7,8]. The potential interaction of this SNP with the response to hypocaloric diets has been scarcely evaluated in the literature. In addition to being scarce, these intervention studies are heterogeneous in their design, type of diet, and duration. For example, in an interventional trial[12] (standard hypocaloric diet) with patients with hypertriglyceridemia, TT ho-

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Table I. Average daily intakes and physical activity at basal time and after 12 weeks of intervention (mean ± SD).

	n=362			
	TT (n=316)		TC±CC (n=46)	
Parameters	Basal	3 months	Basal	3 months
Calorie intake (kcal/day)	1,993.9±211.8	1,412.1±198.1*	1,988.1±128.2	1,399.1±118.1*
Carbohydrate intake (g/day) (PTC%)	207.8±50.2 (50.1%)	137.5±18.1$ (45.3%)	211.1±39.1 (50.8%)	133.1±19.1$ (43.7%)
Fat intake (g/day) (PTC%)	58.1±9.7 (29.9%)	49.1±7.1# (35.9%)	60.4±7.3 (30.1%)	30.2±4.3# (31.9%)
Protein intake (g/day) (PTC%)	88.1±13.1 (20.0%)	50.6±7.3& (18.8%)	89.1±11.1 (19.1%)	51.2±6.9& (17.4%)
Saturated Fat intake (g/day) (PFC%)	36.1±5.0 (61.5%)	6.9±4.7** (19.5%)	37.1±4.1 (60.8%)	5.9±2.2** (19.5%)
Monounsaturated Fat intake (g/day) (PFC%)	17.2±3.9 (25.7%)	31.3±2.9$$ (60.2%)	16.8±5.1 (24.2%)	29.9±4.0$$ (60.9%)
Polyunsaturated Fat intake (g/day) (PFC%)	4.5±3.2 (13.8%)	9.3±2.1## (20.3%)	4.8±2.2 (15.0%)	9.9±2.2## (20.4%)
ω-3 Polyunsaturated Fat intake (g/day)	1.5±2.1	2.1±1.9	1.8±0.2	2.2±0.8
ω-6 Polyunsaturated Fat intake (g/day)	3.1±2.0	7.3±2.1##	3.0±0.9	7.4±1.2##
Physical activity (min/week)	129.8±18.1	128.8±17.5	127.9±14.2	131.1±10.2

PTC: Percentage of total calorie; PFC: Percentage of Fat calorie. Statistical differences $p<0.05$, in each genotype group (*Daily Calorie intake, $Daily Carbohydrate intake, #Daily fat intake, &Daily protein intake, **Saturated fat intake, $$monounsaturated fat intake, ##Polyunsaturated fat intake).

mozygotes showed a higher decrease in triglyceride levels. In another trial[13] in obese females with a low fat/high carbohydrate diet, T allele carriers reported a better improvement in lipid profile. In an interventional design[14] with a high/low-fat diet, the T allele modulated the postprandial triglyceride elevation in healthy subjects. Finally, other investigations with a hypocaloric diet with Mediterranean pattern[15,16] reported a lack of decrease in triglyceride levels after weight loss secondary to the dietary intervention in carriers of the C allele. These two last interventional trials with the Mediterranean diet had different durations, 3 months[15] and 9 months[16], respectively. In both trials, the Mediterranean diet had a standard profile with vegetables, legumes, fruits, whole grains, olive oil, and small quantities of animal proteins (beef, pork and sheep). Protein was obtained from seafood, fish, chickens, and turkeys. Finally, the C allele of *rs662799* could be associated with a more atherogenic lipid profile in subjects with higher ω-6 polyunsaturated fatty acids (PUFA) intakes[17].

Taking into account the previously mentioned data, the present study was designed to investigate the effect of SNP *rs662799* on lipid profile of patients with obesity after a hypocaloric diet with the Mediterranean pattern enriched in ω-6 PUFA.

Patients and Methods

Patients

The current research included 380 individuals from the Health National Service who underwent routine obesity check-ups (body mass index >30 kg/m^2). Out of these, 362 subjects consented to take part in the study and provided written informed consent. The protocol was approved by the Ethics Committee "Comité De Ética De La Investigación Con Medicamentos Área De Salud Valladolid" (May 2017 code of registration PI-5-2017) following the principles of the Declaration of Helsinki. All participants gave their informed consent.

The study included participants who had a body mass index greater than 30 kg/m^2 and were over 30 years old. Those with previous coronary events, chronic kidney or liver disorders, alcohol intake exceeding 20 grams per day for women and 30 grams per day for men, as well as those who had taken medications known

to affect lipid profile (such as statins, fibrates, hormonal therapy, glucocorticoids and anti-inflammatory drugs) in the nine months before the study, were excluded.

All assessments were conducted in the nutrition department by medical professionals, both at the beginning of the protocol and after three months of dietary intervention. Anthropometric measures, such as weight, height, body mass index, and waist circumference were recorded. Body fat percentage was determined using an electric impedance analysis. To perform biochemical tests and genotyping, a 10 ml sample of venous blood was collected after a 10-hour overnight fast and aliquoted into EDTA-coated tubes. Subsequent analyses included lipid profile measurements (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides), C-reactive protein levels, adipokine concentrations (leptin, total adiponectin, resistin, and adiponectin/leptin ratio), as well as fasting glucose and insulin levels.

Adiposity Parameters and Blood Pressure

Height (in centimeters) and waist girth (in centimeters) were assessed using a non-stretch measuring tape (Omrom, LA, CA, USA). Body weight was measured in the morning with subjects minimally clothed and not wearing shoes, utilizing digital scales (Omrom, LA, CA, USA) accurate to the nearest 50 grams. Body mass index (BMI) was computed as body weight (in kilograms) divided by height squared (in meters). Fat mass was determined through electric impedance measurement with an accuracy of 5 grams[18] using EFG BIA 101 Anniversary equipment (Akern, Pisa, Italy). The fat mass was obtained using the

Table II. Adiposity parameters and blood pressure at basal time and after dietary intervention (mean ± SD).

	TT (n=316)		TC±CC (n=46)		*p*-values – Time TT – Basal Genotype – Time TC±CC – 3 months genotype
Parameters	Basal	3 months	Basal	3 months	
BMI	37.2±1.1	35.9±1.2*	37.1±1.1	35.8±0.9*	*p*=0.01 *p*=0.39 *p*=0.02 *p*=0.35
Weight (kg)	95.9±1.0	91.5±1.1$^{\$}$	96.0±2.0	91.5±1.1$^{\$}$	*p*=0.02 *p*=0.44 *p*=0.01 *p*=0.50
Fat mass (kg)	39.8±1.2	36.0±1.1$^{\#}$	40.1±1.4	36.2±1.3$^{\#}$	*p*=0.02 *p*=0.40 *p*=0.02 *p*=0.43
WC (cm)	112.2±4.2	108.1±2.1$^{\&}$	112.7±3.8	107.9±3.2$^{\&}$	*p*=0.03 *p*=0.39 *p*=0.02 *p*=0.51
SBP (mmHg)	129.1±2.1	124.2±2.2**	129.0±2.2	125.1±3.1**	*p*=0.02 *p*=0.41 *p*=0.02 *p*=0.42
DBP (mmHg)	83.2±3.1	83.0±3.1	82.9±5.0	81.8±4.1	*p*=0.44 *p*=0.62 *p*=0.63 *p*=0.67

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; Statistical differences $p<0.05$, in each genotype group (*BMI, $^{\$}$Weight, $^{\#}$fat mass, & WC, **SBP). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs.* TC ± CC baseline values, third *p*, significance of dietary intervention after 12 weeks in TC ± CC genotype, fourth *p*, significance between TT genotypes *vs.* TC ± CC post-treatment values.

Table III. Biochemical parameters at basal time and after dietary intervention (mean±SD).

Parameters	TT (n=316)		TC±CC (n=46)		*p*-values – Time TT – Basal Genotype – Time TC±CC – 3 months genotype
	Basal	3 months	Basal	3 months	
Glucose (mg/dl)	99.7±4.2	96.2±7.0	98.7±4.0	96.3±3.1	*p*=0.16 *p*=0.50 *p*=0.28 *p*=0.43
Total cholesterol (mg/dl)	207.1±3.7	194.1±3.2$^{\$}$	209.9±3.3	195.4±3.1$^{\$}$	*p*=0.02 *p*=0.50 *p*=0.03 *p*=0.39
LDL-cholesterol (mg/dl)	128.9±4.1	114.1±4.2$^{\#}$	130.9±4.0	109.8±3.1$^{\#}$	*p*=0.02 *p*=0.41 *p*=0.01 *p*=0.39
HDL-cholesterol (mg/dl)	50.1±1.9	52.1±1.3	53.2±2.0	52.7±1.9	*p*=0.11 *p*=0.38 *p*=0.51 *p*=0.47
Triglycerides (mg/dl)	118.1±5.1	100.3±4.2*	131.9±4.2	123.9±3.1$^{\pm}$	*p*=0.01 *p*=0.02 *p*=0.31 *p*=0.02
Insulin (mUI/l)	16.5±1.3	13.0±1.2$^{\&}$	13.1±3.0	11.9±2.2	*p*=0.02 *p*=0.44 *p*=0.19 *p*=0.51
HOMA-IR	4.6±1.1	3.0±1.0**	3.1±0.9	2.8±1.2	*p*=0.01 *p*=0.37 *p*=0.12 *p*=0.48
CRP	4.8±1.1	4.6±1.4	4.7±2.0	4.4±2.4	*p*=0.27 *p*=0.34 *p*=0.39 *p*=0.47

HOMA-IR (homeostasis model assessment). CRP (C reactive protein) Statistical differences $p<0.05$, in each genotype group (total cholesterol$^{\$}$, LDL cholesterol$^{\#}$, insulin$^{\&}$, HOMA IR**) (Triglyceride between genotypes$^{\pm}$). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs.* TC ± CC baseline values, third *p*, significance of dietary intervention after 12 weeks in TC ± CC genotype, fourth *p*, significance between TT genotypes *vs.* TC ± CC post-treatment values.

equation: (0.756 * Height^2/Resistance) ± (0.110 * Body mass) ± (0.107 * Reactance) - 5.463.

Average systolic and diastolic blood pressures were derived by averaging three measurements taken after the subjects had been at rest for ten minutes using equipment (Omrom, LA, CA, USA).

Biochemical Parameters

The lipid profile, including total cholesterol, HDL-cholesterol, and triglyceride levels, was measured using the COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, Canada). LDL-cholesterol was calculated using the Friedewald formula (LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides / 5)[19]. Glucose levels were determined by an automated hexokinase oxidase method on the same analyzer. Insulin levels were assessed using electrochemiluminescence assay on the COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, Canada) as well. The homeostasis model assessment for

insulin resistance was obtained using this equation: glucose x insulin/22.5)[20]. C-reactive protein measurements were conducted *via* immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany).

Leptin was assessed using a commercially available kit (Diagnostic Systems Laboratories, Inc., TX, USA) (DSL1023100). Adiponectin levels were measured using another commercial kit by R&D Systems, Inc. (Minneapolis, MN, USA) (DRP300), and resistin levels were measured using (Biovendor Laboratory, Inc., Brno, Czech Republic) (RD191016100). The adiponectin/leptin ratio was calculated directly based on the measurements of both adipokines.

Genotyping of APOA5 Gene Polymorphism

Genomic DNA was extracted from white blood cells using a commercially available kit (primer forward: 5′-GAGCCCCAGGAACTG-GAGCGAAAGT-3′ and reverse 5′-AGATTTG-CCCCATGAGGAAAAGCTG-3′ in a 3.0-µl final volume (Thermocycler Lifetecnologies, LA, CA, USA). The primers were designed with SEQUENOM's Assay Design v4 (SEQUENOM, Inc. San Diego, CA, USA). Genotyping for the SNP was carried out through Real-Time Polymerase Chain Reaction Analysis. This PCR involved 30 ng of genomic DNA and 0.15-0.20 µl each of forward and reverse oligonucleotide primers for *rs662799* in a final volume of 3.0 µl (primer forward: 5′-GAGCCCCAGGAACTG-GAGCGAAAGT-3′ and reverse 5′-AGATTTGC-CCCATGAGGAAAAGCTG-3′ in a 3.0-µl final volume (Thermocycler Lifetecnologies, LA, CA, USA). The denaturation process occurred at 90°C for 5 minutes, followed by 45 cycles at 65°C for 15 seconds and annealing at 59°C for 40 seconds. Lastly, the PCRs ran in a final volume of 2.5 µl containing iPLEx Termination mix (Bio-Rad®, San Diego, CA, USA) along with hot start Taq DNA polymerase.

Nutritional Intervention

Participants were instructed to follow a Mediterranean diet pattern while reducing their daily calorie intake by 500-700 calories for 12 weeks. The macronutrient distribution was as follows: carbohydrates at 45.7%, lipids at 34.4%, and proteins at 19.9%. Additionally, the dietary fats were distributed as follows: saturated fats at 21.8%,

Table IV. Serum adipokines at basal time and after dietary intervention (mean±SD).

Parameters	TT (n=316)		TC±CC (n=46)		*p*-values – Time TT – Basal Genotype – Time TC±CC – 3 months genotype
	Basal	3 months	Basal	3 months	
Resistin (ng/dl)	3.8±1.5	3.9±1.9	4.0±2.0	3.9±1.8	*p*=0.51 *p*=0.60 *p*=0.32 *p*=0.49
Adiponectin (ug/dl)	25.7±4.1	45.9±4.0$^{\$}$	25.0±3.1	46.9±4.1$^{\$}$	*p*=0.02 *p*=0.19 *p*=0.01 *p*=0.38
Leptin (ng/dl)	80.1±4.6	68.2±3.1*	79.1±4.1	62.1±3.1*	*p*=0.01 *p*=0.24 *p*=0.02 *p*=0.38
Ratio adiponectin/leptin	0.33±0.2	0.72±0.3$^{\#}$	0.31±0.3	0.75±0.2$^{\#}$	*p*=0.02 *p*=0.29 *p*=0.01 *p*=0.45

Statistical differences $p<0.05$, in each genotype group ($^{\$}$adiponectin, *leptin, $^{\#}$ratio adiponectin/leptn). (±adiponectin, ±± adiponectin/leptin ratio between genotypes). First *p*, significance of dietary intervention after 3 months in TT genotype, second *p*, significance between TT genotypes *vs.* TC ± CC baseline values, third *p*, significance of dietary intervention after 12 weeks in TC ± CC genotype, fourth *p*, significance between TT genotypes *vs.* TC ± CC post-treatment values.

monounsaturated fats at 55.5%, and polyunsaturated fats at 22.7% (with specific amounts of ω-6 and w-3 fatty acids). Throughout the study, participants maintained detailed dietary records, which were then analyzed using software (DietSource®) along with national food composition tables as reference[21].

The designed diet included three main meals, one morning snack, and an afternoon snack following a Mediterranean dietary pattern rich in ω-6 PUFAs, such as legumes, vegetables, poultry, whole grains, fish, fresh fruit, and olive oil, including over 40 grams per day of nuts. Participants also had two individual sessions with a dietitian where they received instructions on meal planning along with physical exercise recommendations of at least three times per week for aerobic exercises lasting between 45 to 60 minutes each time. Compliance was monitored throughout through weekly phone interviews conducted by the dietitian.

Statistical Analysis

The sample size was determined to detect differences of over 10 mg/dl in triglyceride levels with 90% power and a significance level of 5%. Statistical analysis was conducted using the SPSS for Windows software package, version 25.0 (IMB Corp., Armonk, NY, USA). A dominant genetic model involving the *rs662799* C-allele (TT *vs.* TC±CC) was employed. All variable values were expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. Parametric variables underwent analysis through a two-tailed Student's *t*-test, while categorical variables were analyzed using the Chi-square test with Yates's correction or Fisher's test when necessary. The Bonferroni test was used for multiple tests in order to minimize Type I error in association analysis. For evaluating the interaction between the gene and dietary intervention, ANCOVA (covariance analysis) adjusted by age, sex, and BMI modeling representing dependent variable starting values was performed statistically. The *p*-values presented in Tables I, II, and III are: first *p* – significance of dietary intervention after 12 weeks in TT genotype; second *p* – significance between TT genotypes *vs.* TC±CC baseline values; third *p* – significance of dietary intervention after 12 weeks in TC ± CC genotype; fourth *p* – significance between TT genotypes *vs.* TC±CC post-treatment values. Hardy Weinberg equilibrium evaluation involved comparing expected and observed data using a statistical Chi-square test. The statistical significance of the *p*-value was <0.05.

Results

The *APOA5* variant distribution among the 362 patients with obesity was as follows: 87.2% (n=316) (TT) were homozygous for the T allele, 12.2% (n=44) (TC) were heterozygous and 0.6% (n=2) (CC) were homozygous for the C allele. The allele frequency was T (0.93) and C (0.07). Taking into account that two patients were detected with CC genotype (homozygous), we joined carriers of the less common allele C, as a dominant model (TC±CC). This variant of *APOA5* gene was in Hardy Weinberg equilibrium (*p*=0.38).

The mean age was 51.7±3.1 years (range: 27-63) and the mean BMI 37.1±2.1 kg/m^2 (range: 32.8-38.7). Gender distribution was 260 females (71.8%) and 102 males (28.2%). Mean values of age (TT; 52.0±4.0 years *vs.* TC±CC; 51.6±3.1 years: ns), as well as proportions of gender (TT 28.5% males *vs.* 71.5% females *vs.* TC±CC; 27.3% males *vs.* 72.7% females), were similar between both genotype groups. Patients reached the dietary recommendations as indicated in the method section, with an increase in dietary intake of monounsaturated fatty acids and ω-6 polyunsaturated fatty acids (PUFA). A decrease in energy, carbohydrates, total fat, and protein intake was observed in both genotype groups (Table I). Moreover, after 12 weeks of the study, the physical activity was similar.

Adiposity Changes and Blood Pressure

Adiposity parameters and blood pressure were not significantly associated with the *APOA5* gene variant *rs662799* (Table II). After dietary intervention and in both genotype groups (TT *vs.* TC±CC), BMI (delta: -1.3±0.2 kg/m^2 *vs.* -1.2±0.1 kg/m^2; *p*=0.51), weight (delta: -4.4±2.0 kg *vs.* -4.1±1.7 kg; *p*=0.61), fat mass (delta: -3.8±0.8 kg *vs.* -3.9±1.0 kg; *p*=0.50), waist circumference (delta: -4.1±1.2 cm *vs.* -4.8±1.1 cm; *p*=0.49) and systolic blood pressure (delta: -5.0±2.0 mmHg *vs.* -3.9±2.0 mmHg; *p*=0.32) improved. This statistically significant change was similar in both groups.

Biochemical Parameters

Only triglyceride levels were higher in patients with the C allele Delta in basal levels: -14.4±3.1 mg/dl; *p*=0.03 and in post-treatment levels -20.7±3.2 mg/dl; *p*=0.01. The remaining biochem-

ical parameters were similar in both genotypes of *rs662799* (Table III).

After 12 weeks, the dietary intervention significantly improved triglyceride levels, insulin levels, and homeostasis model assessment (HOMA-IR) in non-C-allele carriers. After dietary intervention (TT *vs.* TC±CC); insulin levels (delta: -3.5±0.2 UI/L *vs.* - 1.2±0.6 UI/L; *p*=0.03), HOMA-IR (delta: -1.6±0.1 units *vs.* -0.3±0.2 units; *p*=0.01) and triglyceride levels (delta: -18.8±4.1 mg/dl *vs.* -3.7±3.0 mg/dl; *p*=0.02) decreased in non-C allele carriers.

Adipokine Levels

Table IV reports changes in serum adipokines and the ratio of adiponectin/leptin. After dietary and in both genotypes (TT *vs.* TC±CC), serum adiponectin (delta: 20.1±2.1 ng/dl *vs.* 21.9±2.0 ng/dl; *p*=0.39) increased in a significant way. Patients with both genotypes showed a significant decrease in leptin levels (delta: -12.1±5.0 ng/dl *vs.* -17.0±4.3 ng/dl; *p*=0.39). Additionally, the adiponectin/leptin ratio improved in both genotypes (delta: 0.37±0.1 *vs.* 0.35±0.2 ng/dl; *p*=0.43). Serum resistin levels did not change after dietary intervention.

Discussion

We found a significant interaction between the C allele of the genetic variant of *APOA5* gene (*rs662799*) and biochemical response after a hypocaloric diet with the Mediterranean pattern enriched in ω-6 PUFA on the changes of serum concentration of triglycerides, insulin, and HOMA-IR. In C-allele carriers, the dietary intervention did not produce significantly lower triglyceride, insulin, and HOMA-IR levels.

First of all, the observed low prevalence of the C allele in our study has already been demonstrated in previous studies[11,12,22-24], both in patients with obesity and healthy subjects, in a range below 10%. Our data support the idea that *rs662799* variant at *APOA5* locus may modulate the response of lipid profile secondary to different treatments. Additionally, it is also necessary to take into account that this SNP (*rs662799*) is associated with high levels of triglycerides[24], ischemic stroke[25] and ischemic heart disease[26]. This relationship with cardiovascular events and a worse lipid profile could be due to the impaired ribosomal translation efficiency secondary to the presence of the C allele. This alteration reduces the levels of ApoA5[27] producing an impaired interaction with lipoprotein lipase activity, increasing triglyceride levels[28].

In the literature, few studies[13-16] have evaluated the interaction of this genetic variant with dietary modifications. For instance, Jang et al[13] showed that dietary treatment replacing 1/3 refined rice intake with legumes and increased vegetable intakes reduced triglyceride levels in TT subjects with a 12-week dietary intervention without effect in C-allele carriers. The improvement in triglyceride levels in this study was similar to ours, between 15 and 20%, despite the fact that obese patients were not evaluated in this study. In another short-term intervention trial[14], healthy young subjects received a high carbohydrate/low-fat diet for six days; proportions of carbohydrates, proteins, and fats were 7%, 15%, and 15%, respectively. The response was similar, with a smaller decrease in triglyceride levels in patients carrying the C allele. On the other hand, some studies[15,16] have evaluated the effect of this genetic variant on the modification of the lipid profile after hypocaloric diets with a Mediterranean diet pattern in obese patients, with similar results of previous-mentioned interventions. In an intervention trial of 12 weeks with a hypocaloric Mediterranean diet in patients with obesity[15], an improvement in HOMA-IR, insulin levels and triglycerides after a significant weight loss was reported in non-C allele carriers with a lack of effect in C allele carriers. This dietary intervention consisted of a caloric restriction of 500 calories each day with the next macronutrient distribution (50% from carbohydrates, 30% from lipids, and 20% from proteins). The percentage of fats was 55% from monounsaturated fats, 30% from saturated fats, and 15% from polyunsaturated fats. In the second study with a hypocaloric diet with a Mediterranean pattern[16], the caloric restriction was higher than the previous one (a decrease of 700 calories each day) and the distribution of macronutrients was similar but with a higher percentage of monounsaturated fats reached 60%. Despite the greater caloric restriction and the longer duration of the study (9 months), the metabolic responses were similar in both studies, with a better improvement in triglyceride, insulin, and HOMA-IR levels in the non-carriers of the C allele. In our current study, the effect of caloric restriction and the increase in ω-6 PUFA with different foods in the context of a Mediterranean Diet was similar to that found in the two previously mentioned studies[15,16]. Possibly, the benefits found in previous studies and

the current one may be secondary to weight loss itself and also to the Mediterranean diet. The inclusion in the diet of foods such as olive oil, fish, and lean meats with large amounts of fiber, polyphenols, monounsaturated fats, polyunsaturated fats, minerals, and vitamins, can also explain the improvement in the profile lipid as described by Sanchez-Moreno et al[22] in a Mediterranean population with a cross-sectional study. In a recent cross-sectional study, Hubacek et al[23] showed that triglyceride concentrations were higher in subjects with the C allele, and energy intake was higher in C allele carriers than non-C allele carriers. The hypothesis to increase the amount of ω-6 PUFA in our intervention compared to previous studies[15,16], is due to the previous findings in Lai et al's study[29]. The authors reported that higher ω-6 PUFA intake increased fasting triglycerides in C-allele carriers with a more atherogenic lipid profile, and therefore we assessed whether the increase in ω-6 PUFA in the context of a Mediterranean hypocaloric diet enriched with nuts and vegetable oils modified the expected metabolic response. Jang et al[30] demonstrated that C-allele carriers had higher triglyceride area under the curve after a fat load enriched in ω-6 PUFA. In subjects carrying the C allele, there is less ApoA5 protein available and less sequestration of triglycerides, with subsequent increase of triglycerides levels, which can be increased in particles enriched in ω-6 PUFA and not in particles enriched in w-3 PUFA[31]. Therefore, this worse postprandial lipid response observed could be due to an impairment of the postprandial suppression of hormone-sensitive lipase by insulin in C-allele carriers secondary to an insulin resistance state in these subjects. This situation could be implied in the lack of insulin and HOMA-IR response in patients carrying the C allele in our study after dietary intervention[32]. All these findings are important as this genetic variant can change response to drugs or different foods. For example, subjects with dyslipemia and with TT genotype have been reported to benefit more from statin treatment compared with C allele carriers[33]. Recently, Choi et al[34] have reported an association between the C allele of this genetic variant and high processed and red meat consumption with the incidence of metabolic syndrome.

Limitations and Strengths

The present study has several limitations and strengths. First, the finding may not be generalized to other populations or ethnicity because our study is conducted in Caucasian patients with obesity. The second limitation is that dietary intake was based on self-reports obtained from patients with a potential bias. Third, there is a small sample size of C allele carriers. Fourth, our study is a 12-week dietary intervention, and this type of intervention might be more important than those observed in long-term dietary interventions. Fifth, circulating levels of ApoA5 have not been determined. Despite these limitations, the study boasts several strengths. It is the first of its kind to explore the impact of a ω-6-PUFA enriched hypocaloric diet with a Mediterranean pattern, addressing a significant knowledge gap. Furthermore, it is a prospective interventional study, allowing for the examination of causality.-

Conclusions

In summary, our data demonstrate that the minor C allele of the *APOA5* gene (*rs662799*) produces a worse response in triglyceride levels, insulin levels, and HOMA-IR after a ω-6 PUFA enriched hypocaloric diet with the Mediterranean pattern. These findings could be relevant within the context of future development of personalized dietary recommendations in the topic area of precision nutrition, taking into account different genetic variants and different dietary approaches[35,36].

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

The protocol was approved by the Ethics Committee "Comité De Ética De La Investigación Con Medicamentos Área De Salud Valladolid" (May 2017 code of registration PI-5-2017) following the principles of the Declaration of Helsinki.

Informed Consent

All volunteers signed the written informed consent to participate in the study.

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None.

Authors' Contribution

Daniel de Luis wrote the article and made a statistical analysis. Olatz Izaola made an anthropometric evaluation. David Primo made a biochemical evaluation.

Data Availability

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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