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ORIGINAL ARTICLE

Postprandial Lipemia is Modified by the Presence of the *APOB*-516C/T Polymorphism in a Healthy Caucasian Population

Pablo Pérez-Martínez · Francisco Pérez-Jiménez · José María Ordovás · Juan Antonio Moreno · Carmen Marín · Rafael Moreno · Yolanda Jiménez-Gómez · Juan Antonio Paniagua · José López-Miranda

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Abstract Apolipoprotein (apoB) plays a fundamental role in the transport and metabolism of plasma triacylglycerols (TAGs) and cholesterol. Several apoB polymorphic sites have been studied for their potential use as markers for coronary heart disease in the population. In view of the importance of apoB in postprandial metabolism, our objective was to determine whether the presence of the -516C/T polymorphism in the *APOB* gene promoter could influence postprandial lipoprotein metabolism in healthy subjects. Forty-seven volunteers who were homozygous for the E3 allele at the APOE gene were selected (30 homozygous for the common genotype (C/C) and 17 heterozygotes for the -516T allele (C/T). They were given a fat-rich meal containing 1 g fat and 7 mg cholesterol per kg body weight and vitamin A 60,000 IU/m^2 body surface. Fat accounted for 60% of calories, and protein and carbohydrates for 15 and 25% of energy, respectively. Blood samples were taken at time 0, every 1 h until 6 h, and every 2.5 h until 11 h. Total cholesterol and TAGs in plasma, and cholesterol, TAGs and retinyl palmitate in triacylglycerol-rich lipoproteins (large and small triacylglycerol-rich lipoproteins) were determined by ultracentrifugation. Individuals carrying the C/T genotype presented greater postprandial concentrations of TAGs in small triacylglycerol-rich lipoproteins than did carriers of the C/C genotype ($P = 0.022$). Moreover, C/T individuals presented higher concentrations of plasma TAGs during the postprandial period than did C/C subjects ($P = 0.039$). No other statistically significant genotype-related differences for other parameters were observed. These results suggest that the presence of the genotype C/T is associated with a higher postprandial response. Thus, the allele variability in the -516C/T polymorphism in the *APOB* gene promoter may partly explain the interindividual differences in postprandial lipemic response in healthy subjects.

Keywords *APOB* polymorphism · Nutrigenetics · Postprandial lipemia · Triacylglycerol-rich lipoproteins

Abbreviations

apoB	Apolipoprotein B
BMI	Body mass index
CHO	Carbohydrate
CHD	Coronary heart disease
LDL	Low-density lipoprotein
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
TAGs	Triacylglycerol
TRL	Triacylglycerol-rich lipoproteins

P. Pérez-Martínez (✉) · F. Pérez-Jiménez · J. A. Moreno · C. Marín · R. Moreno · Y. Jiménez-Gómez · J. A. Paniagua · J. López-Miranda
Unit of Lipids and Atherosclerosis, Hospital Universitario Reina Sofia, Avda. Menéndez Pidal, s/n, 14004 Cordoba, Spain
e-mail: pablopermar@yahoo.es

J. M. Ordovás
Nutrition and Genomics Laboratory, JM-US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA

Introduction

The postprandial state constitutes the normal metabolic situation of human beings throughout the day in

developed societies, as the clearance of triacylglycerol (TAG)-rich lipoproteins (TRL) of intestinal or hepatic origin last for a period of 6–8 h. Determination of the postprandial response is a complex process, which makes it all the more challenging to assess the cardiovascular risk associated with postprandial lipemia than during fasting conditions. Previous studies have suggested that postprandial small TRL may predict the onset of coronary heart disease (CHD), and evidence supporting an association between postprandial lipemia and atherosclerosis has been provided by clinical trials [1–6]. Individual variability in the postprandial lipemic response is usually greater than that observed in the fasting state, and appears to be modulated by environmental and genetic factors [7, 8]. This concept is supported by studies showing that certain polymorphisms at candidate gene loci are associated with variability in postprandial clearance of lipoprotein [9].

Apolipoprotein (apoB) plays a fundamental role in the transport and metabolism of plasma TAG and cholesterol and it is synthesized primarily in hepatocytes and enterocytes. The human *APOB* gene is located on chromosome 2 and several *APOB* polymorphic sites have been studied for their potential use as markers for CHD in the population [10]. Moreover, some of them have been investigated for the inter-individual variability observed during postprandial lipemia (XbaI polymorphism, I/D polymorphism within the apoB signal peptide) [11–13]. Previously, a common functional polymorphism of the *APOB* promoter was described by van't Hooft et al. [14] involving a C to T change located at 516 base pairs upstream from the transcription star site. This polymorphism was found to induce significant increase in the transcription rate of the *APOB* gene and, consequently, in circulating levels of low-density lipoprotein (LDL) cholesterol. These findings are consistent with the fact that the *APOB* gene promoter plays a major role in cholesterol homeostasis [15]. Furthermore, we have demonstrated that healthy males carriers of the -516T allele, have a significant increase in insulin resistance after the consumption of diets with different fat content, but the difference is more pronounced after an SFA-enriched diet than following MUFA and CHO-rich diets [16].

In view of the insulin sensitivity of postprandial metabolism, it would be interesting to find out whether the presence of this polymorphism in the *APOB* gene could modulate postprandial lipemia, in the same way as it has already been observed in other genotypes.

Material and Methods

Subjects of the Study

Forty-seven healthy men were recruited from among 97 students at the University of Cordoba. The 47 subjects had a mean age (±SD) of 23 ± 4.12 years. Of these, 30 were homozygotes for the -516C allele (C/C) and 17 heterozygotes for the -516T allele (C/T). The distribution of genotypes was as expected from the Hardy–Weinberg equilibrium. Informed consent was obtained from all participants. All the subjects were selected to have the *APOE* 3/3 genotype to avoid the potential confounder from the presence of the other common APOE alleles on postprandial lipemia [17]. All subjects underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before inclusion in the study, and they did not show evidence of any chronic disease (hepatic, renal, thyroid, or cardiac dysfunction). None of the subjects were obese, nor did they perform unusually high levels of physical activity (e.g., sports training). In addition, none of the subjects had a family history of premature coronary artery disease or had taken medications or vitamin supplements in the 6 months prior to the study. Subjects were encouraged to maintain their regular physical activity and lifestyle and were asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits and alcohol consumption in the week before the fat loading test. Previous to the fat loading test, the volunteers consumed a saturated fatty acid diet period as a baseline regimen, as this Western-style diet is frequently consumed by Western countries, including most areas of Spain. All studies were carried out in the Research Unit at the Reina Sofia University Hospital, and the experimental protocol was approved by the Hospital's Human Investigation Review Committee.

Vitamin A and Fat Loading Test

After a 12-h fast, subjects were given a fatty meal enriched with 60,000 units of vitamin A per m^2 of body surface area. The amount of fat given was 1 g of fat and 7 mg of cholesterol/kg of body weight. The meal contained 65% of energy as fat, 15% protein and 25% carbohydrates and was eaten in 20 min. We calculated the amount of each ingredient fed as a function of individual weight, so that although all the subjects consumed the same types of food, the quantities involved were different. The foods were bread, whole milk, eggs and butter. After the meal, the subjects had

no energy intake for 11 h, but were allowed to drink water. Blood samples were drawn before the meal, every hour until the sixth hour and then every 2 h and 30 min until the eleventh hour.

Lipoprotein Separations

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. Plasma was separated from red cells by centrifugation at 1,500 *g* for 15 min at 4 °C. The chylomicron fraction of TRL (large TRL) was isolated from 4 mL of plasma obtained from EDTA tubes. Plasma was put in the bottom of a 13.4 mL polyallomer ultracentrifuge tube (UltraClear, Beckman Instruments, Palo Alto, CA, USA) and overlayered with a preservative solution consisting of NaCl (0.15 mol/L), sodium azide (0.05 g/L), chloramphenicol (0.05 g/L), gentamicin sulfate (40 mg/L) and EDTA (1 mmol/L), (pH 7.4, $d < 1.006$ kg/L) Ultracentrifugation was performed in a type TY65 rotor (Beckman Instruments, Fullerton, CA, USA) at 36,200×*g* and 4 °C for 30 min. Chylomicrons contained in the top layer were removed by aspiration, placed directly into individual vials and stored at –80 °C until assay for retinyl palmitate, biochemical determinations, apoB-48 and apoB-100. The infranatant fluid was centrifuged at a density of 1.019 kg/L and overlayered with the same preservative cocktail. The ultracentrifugation was performed for 24 h at 183,000 *g* in the same rotor. The nonchylomicron fraction (also referred to as small TRL) was removed from the top of the tube by aspiration, placed directly into individual vials and stored at –80 °C until assay for retinyl palmitate, biochemical determinations, apoB-48 and apoB-100. All operations were done under subdued light in order to prevent the degradation of retinyl palmitate (RP).

Lipid Analysis

Cholesterol and TAGs in plasma and lipoprotein fractions were assayed by enzymatic procedures [18, 19]. Apo A-I and apo B were determined by turbidimetry [20]. HDL cholesterol (HDL-C) was measured by analyzing the supernatant obtained following precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}, as described by Warnick et al. [21]. The LDL cholesterol (LDL-C) was obtained as the difference between the cholesterol from the bottom part of the tube after ultracentrifugation at a density of 1.019 kg/L.

Retinyl Palmitate Assay

The RP content of large and small TRL fractions was assayed using a method previously described [22]. Briefly, different volumes of the various fractions (100 µL for chylomicrons and 100–500 µL for remnant) were placed in 13 × 100 mm glass tubes. The total volume in each tube was adjusted, as necessary, to 500 µL using normal saline. Retinyl acetate (40 ng in 200 µL of mobile phase buffer) was added to each tube as internal standard. Five hundred milliliter of methanol was added followed by the addition of 500 µL of the mobile phase buffer for a total volume of 1.7 mL. The mobile phase buffer was prepared fresh on a daily basis by combining 90 mL of hexane, 15 mL *n*-butyl chloride, 5 mL acetonitrile, and 0.01 mL acetic acid (82:13:5 by volume with 0.01 mL of acetic acid). The tubes were thoroughly mixed after each step. The final mixture was centrifuged at 350 g for 15 min (room temperature) and the upper layer was carefully removed by aspiration and was injected into a high-performance liquid chromatography (HPLC) system. The autoinjector was programmed to deliver 100 µL per injection and a new sample every 10 min in a custom prepackaged silica column SupelcoSil LC-SI (5 mm, 25 cm × mm ID) provided by Supelco Inc. The flow was maintained at a constant rate of 2 mL/min and the peaks were detected at 330 nm. The peak of RP and retinyl acetate were identified by comparing its retention time with a purified standard (Sigma, St Louis, MO, USA) and the RP concentration in each sample was expressed as the ratio of the area under the RP peak to the area under the retinyl acetate peak [23]. All operations were performed in subdued light.

Determination of ApoB-48 and ApoB-100

ApoB-48 and apoB-100 were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Karpe et al. [24]. In brief, samples containing isolated lipoprotein fractions were delipidated in a methanol/diethyl ether solvent system and the protein pellet was dissolved in 100–500 µL of 0.15 mol/L sodium phosphate, 12.5% glycerol, 2% SDS, 5% mercaptoethanol, and 0.001% bromophenol blue (pH 6.8), at room temperature for 30 min followed by denaturation at 80 °C for 10 min. Electrophoresis was performed with a vertical Hoefer Mighty Small II electrophoresis apparatus connected to an EPS 400/500 (Pharmacia) power supply on 3–20% gradient polyacrylamide gels. The upper and lower electrophoresis buffers contained 25 mmol/L

Tris, 192 mmol/L Glycine, and 0.2% SDS adjusted to pH 8.5. ApoB-100 derived from LDL was used as a reference protein and for standard-curve dilutions. A dilution curve ranging from 0.10 to 2 mg of apo B-100 was applied to four of the gel lanes. Electrophoresis was run at 60 V for the first 20 min and then at 100 V for 2 h. Gels were fixed in 12% trichloroacetic acid for at least 30 min and stained in 0.2% Coomassie G-250/40% methanol:10% acetic acid for at least 4 h. Destaining was done in 12% methanol:7% acetic acid with four changes of destaining solution for 24 h. Gels were scanned with a videodensitometer scanner (TDI, Madrid, Spain) connected to a personal computer for integration of the signals. Background intensity was calculated after scanning an empty lane. The coefficient of variation for the SDS-PAGE was 7.3% for apo B-48 and 5.1% for apo B-100.

DNA Amplification and Genotyping

DNA extraction was performed by standard procedures. Genotyping for the –516C/T polymorphism was carried out using the restriction enzyme Ear I (Biolabs, New England, Inc.) as described previously [14].

Statistical Analysis

Several variables were calculated in order to characterize the postprandial responses of plasma TAGs, large TRL and small TRL to the test meal. The area under the curve (AUC) is defined as the area between the plasma concentration versus time curve and a line drawn parallel to the horizontal axis through the 0 h concentration. This area was calculated by a computer program using the trapezoidal rule. Other variables were the normalized peak concentration above baseline and the peak time, which was the average of the time of peak concentration and the time to the second greatest concentration. Data were tested for statistical significance between genotypes, and between genotypes and time by analysis of variance for repeated measures and the Kruskal–Wallis test. In this analysis, we studied the statistical effects of the genotype alone, independent of the time in the postprandial study, the effect of time alone or of changes in the variable after ingestion of fatty food over the entire lipemic period, and the effect of the interaction of both factors, genotype and time, indicative of the magnitude of the postprandial response in each group of individuals with a different genotype. When statistical significance was found, the Tukey's post-hoc comparison test was used to identify group differences. A probability value less than 0·05 was considered significant.

All data presented in text and tables are expressed as means ± SDs. SPSS 11.0 was used for the statistical comparisons.

Results

The basal characteristics by genotype are shown in Table 1. No differences for any of the variables examined were observed at baseline between genotype groups.

The postprandial response of plasma TAGs and TAGs in different lipoprotein fractions were analyzed. A significant effect of time in plasma for all variables measured except large TRL-RP was observed, with respect to baseline levels, indicating an increase of these parameters in the different groups of subjects during the postprandial period.

Individuals carrying the C/T genotype displayed greater postprandial concentrations of small-TRL TAGs than did carriers of the C/C genotype (P = 0.022) (Fig. 1). C/T individuals also presented higher concentrations of plasma TAGs during the postprandial period than C/C subjects (P = 0.039) (Fig. 1). No other statistically significant genotype-related differences for other parameters were observed.

The area under the postprandial curve in study participants according to the APOB -516C/T polymorphism was analyzed and no significant differences were observed between genotypes (Table 2).

Discussion

Our findings show that healthy male carriers of the T allele at the -516C/T polymorphism in the *APOB* gene

Table 1 Baseline characteristics according to the -516C/T polymorphism in the ApoB gene promoter

	Genotype		*P*
	C/C [n = 30]	C/T [n = 17]	
Age (year)	23.40 ± 4.10	22.60 ± 3.98	0.504
BMI (kg/m^2)	25.16 ± 3.62	24.70 ± 3.42	0.804
Total Cholesterol (mmol/L)	3.97 ± 0.6	3.90 ± 0.7	0.783
Triacylglycerol (mmol/L)	0.92 ± 0.3	0.87 ± 0.4	0.653
LDL-C (mmol/L)	2.36 ± 0.6	2.39 ± 0.6	0.858
HDL-C (mmol/L)	1.23 ± 0.2	1.19 ± 0.2	0.605
Apo B (g/L)	0.65 ± 0.1	0.66 ± 0.1	0.822
Apo A-I (g/L)	0.99 ± 0.2	0.95 ± 0.2	0.435
Large TRL-TAG (mmol/L)	0.20 ± 0.1	0.21 ± 0.1	0.921
Small TRL-TAG (mmol/L)	0.38 ± 0.1	0.36 ± 0.2	0.720

Large TRL-TAG large triacylglycerol-rich lipoprotein triacylglycerol

Data are mean ± SD. There were no significant differences between genotypes (ANOVA)

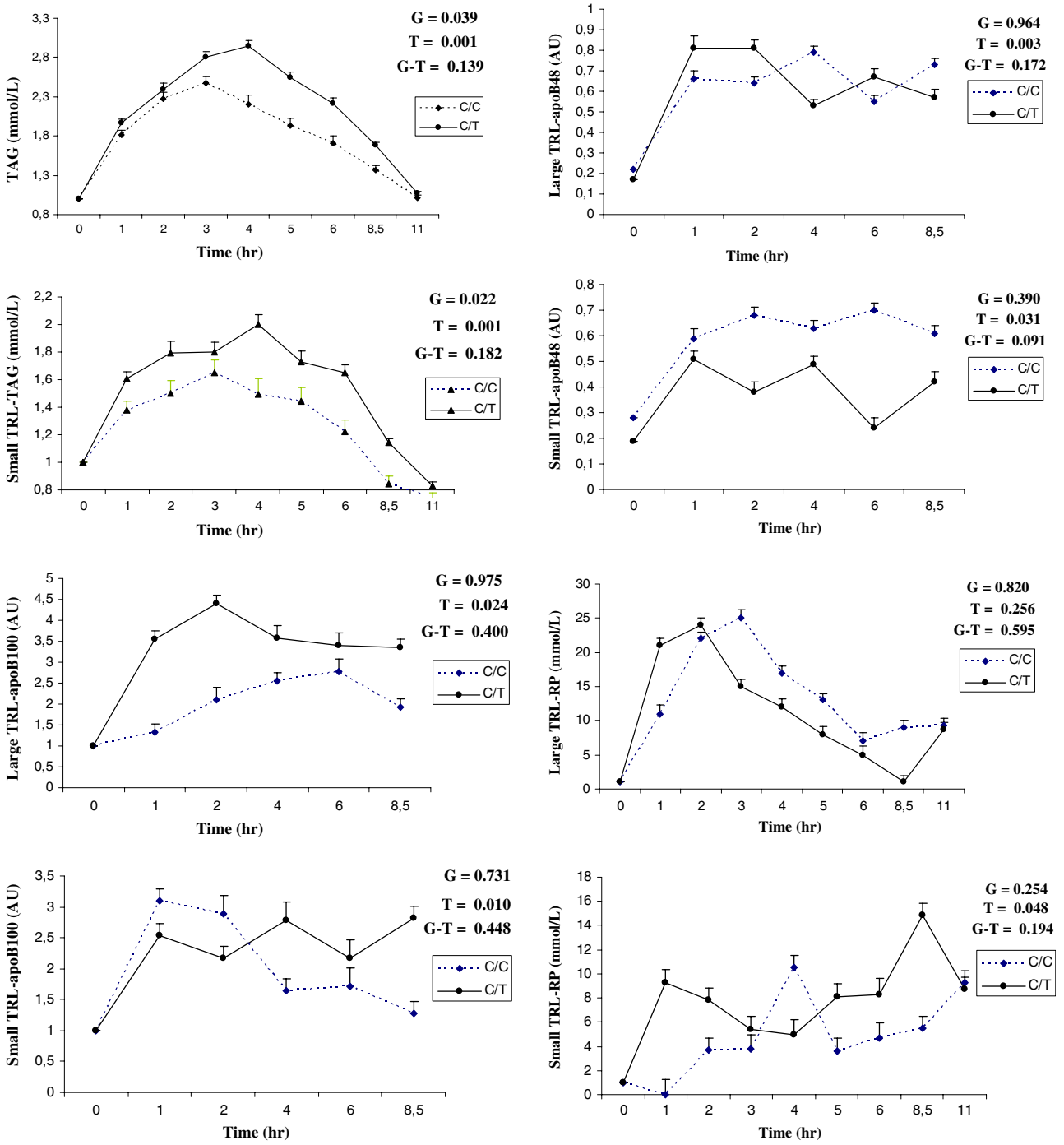

Fig. 1 Line plots of postprandial triacylglycerol plasma, small TRL-TAG, large TRL-apoB100, small TRL-apoB100, large TRL-apoB48, small TRL-apoB48, large TRL-RP, small TRL-RP in C/C subjects (n = 30, *dashed line*, *black triangles*), and C/T subjects (n = 17, *continuous line*, *black circles*). ANOVA for repeated measures

promoter have a greater postprandial response of TAGs in small TRL as compared with C/C individuals.

A growing body of experimental and clinical evidence suggests that TRL, and in particular small TRL, contribute to atherogenesis and consequently to cardiovascular disease progression and that high levels of small TRL of both hepatic and intestinal origin are associated with the progression of coronary atherosclerosis [25, 26]. A study by Phillips et al. found that neither LDL-C nor TG fasting levels correlate highly with lesion progression or clinical events [26]. Small TRL-TG concentrations in plasma reflect postprandial

Table 2 Area under the postprandial curve in study participants, according to the APOB -516C/T polymorphism

	Genotype	
	C/C	C/T
Large TRL Chol (mmol/L sg)	0.034 ± 0.011	0.039 ± 0.014
Small TRL Chol (mmol/L sg)	0.049 ± 0.024	0.049 ± 0.029
Large TRL RP (ng/mL sg)	827.53 ± 653.13	715.77 ± 521.26
Small TRL RP (ng/mL sg)	335.65 ± 229.31	311.29 ± 307.42
Large TRL Apo-B48 (AU)	408 ± 323	407 ± 348
Small TRL Apo-B48 (AU)	415 ± 442	245 ± 292
Large TRL Apo-B100 (AU)	600 ± 634	600 ± 735
Small TRL Apo-B100 (AU)	22075 ± 12471	24716 ± 22475

RP retinyl palmitate, *AU* arbitrary units, *sg* seconds

Values are mean ± SD. No significant differences were observed between genotypes

metabolism, and these did correlate with both lesion progression and cardiac events. Thus, smaller, partially catabolized TRL are believed to be more atherogenic or thrombogenic than larger, newly secreted TRLs, which makes our results of particular interest.

ApoB is required for the assembly secretion of chylomicrons in the intestine and very low-density lipoproteins (VLDL) in the liver, and it also acts as the ligand for LDL recognition by LDL receptors. Several studies have demonstrated an association between the C/T genotype and elevated plasma levels of LDL-C [27]. However, the influence of this polymorphism on postprandial lipemic response remains unknown. Our study is to our knowledge the first to examine the association between the -516C/T polymorphism in the *APOB* gene promoter and postprandial lipemic response in healthy subjects. We have observed a delayed postprandial clearance of TAGs in small TRL in subjects with the less common C/T genotype than in C/C individuals. A limitation of our study is that it includes a relatively small number of subjects. However, because the magnitude of postprandial lipemia is subject to considerable individual variability and is affected by several genetic factors [28, 29], our study sample was limited to healthy men with the Apo E3/E3 genotype, in order to avoid the variable postprandial lipid response of other apoE isoforms or gender [30].

Two mechanisms might explain the change observed in the catabolism of TRL in subjects with the -516C/T polymorphism. We have previously demonstrated that carriers of the -516T allele also have a significant increase in insulin resistance [16]. These data are in accordance with previous evidence demonstrating that small TRL concentrations are significantly higher in insulin-resistant healthy individuals with normal glucose tolerance [31]. In this context, a positive relation between insulin resistance and altered postprandial lipemia has repeatedly been reported [32, 33]. Consequently, insulin resistance increases the hepatic synthesis of lipid substrates. These effects potentially increase the plasma concentrations of small TRL and increase competition for hepatic uptake between small TRL and VLDL remnants [34]. Nevertheless, postprandial lipemia is not a uniform abnormality and its pathophysiology has not yet been fully clarified; it is possible that the response to dietary fat is a multifactorial phenomenon. Previous studies suggest that the relationship between plasma insulin and TRL levels is partly influenced by other polymorphisms in the *APOB* gene (i.e., apoB XbaI polymorphism [11]. Furthermore, in our study C/T subjects showed a higher postprandial response of plasma TAGs than did carriers of the C/C genotype. In this context, subjects who display an increase in plasma TAGs concentrations have smaller and denser LDL particles and an enhanced degree of postprandial lipemia [35]. Previous data also suggest that individuals carrying the C/T genotype present significant increases in the transcription rate of the *APOB* gene and, consequently, in circulating levels of LDL cholesterol, a delayed postprandial clearance of TAGs in small TRL, and an increase in insulin resistance. On the other hand, the presence of this polymorphism is independently associated with the presence of carotid atherosclerotic disease [36]. Although these results are preliminary, the fact that our study was carried out on healthy individuals in a controlled interventional trial could be the key to a better understanding the effect of this polymorphism on postprandial lipoprotein metabolism.

Furthermore, because all the study subjects were young and healthy, it is possible that the effects on postprandial lipemia response observed in these individuals will be magnified as the subjects become older or obese, as has previously been shown with other polymorphisms (LPL *Hin*dIII polymorphism) [37].

In conclusion, allele variability in the -516C/T polymorphism in the *APOB* gene promoter could partly explain the interindividual differences in the postprandial lipemic response in healthy subjects.

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