The influence of the apolipoprotein E gene promoter (−219G/T) polymorphism on postprandial lipoprotein metabolism in young normolipemic males

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Abstract The apolipoprotein E (apoE) gene promoter (−219G/T) polymorphism has been associated with increased risk of myocardial infarction, premature coronary heart disease, and decreased plasma apoE concentrations. We examined whether the −219G/T polymorphism could modify the postprandial response of triacylglycerol-rich lipoproteins (TRLs). Fifty-one healthy apoE 3/3 male volunteers (14GG, 29GT, and 8TT) were given a vitamin A fat-loading test consisting of 1 g of fat/kg body weight and 60,000 IU of vitamin A per m^2 of body surface area. Blood samples were taken at time 0 and every hour until the sixth hour, and every 2 hours and 30 minutes until the eleventh hour. Cholesterol, triacylglycerols (TGs), and apoE were determined in plasma; and cholesterol, TG, apoB-100, apoB-48, and retinyl palmitate (RP) were analyzed in lipoprotein fractions. Postprandial lipemia data revealed that subjects with the −219TT genotype had a higher postprandial response of large TRL-cholesterol ($P < 0.03$), large TRL-triacylglycerols ($P < 0.001$), large TRL-RP ($P < 0.004$), and small TRL-apoB-48 ($P < 0.03$) than carriers of the −219G allele. Moreover, the −219TT subjects had the lowest postprandial levels of serum apoE ($P < 0.05$). In conclusion, the −219G/T polymorphism may influence TRL metabolism during the postprandial period, thus prolonging postprandial lipemia in subjects with the TT genotype.— Moreno, J. A., J. López-Miranda, C. Marín, P. Gómez, P. Pérez-Martínez, F. Fuentes, R. A. Fernández de la Puebla, J. A. Paniagua, J. M. Ordovas, and F. Pérez-Jiménez. **The influence of the apolipoprotein E gene promoter (−219G/T) polymorphism on postprandial lipoprotein metabolism in young normolipemic males. *J. Lipid Res.* 2003.** 44: **2059–2064.**

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Apolipoprotein E (apoE) is a structural component of several lipoproteins, including triacylglycerol-rich lipoproteins (TRLs) and plays an important role in lipid metabolism through the cellular uptake of lipoprotein particles by lipoprotein receptors in the liver and other tissues (1, 2).

apoE is a polymorphic protein with three common isoforms (apoE2, apoE3, and apoE4) that contribute to susceptibility to atherosclerotic cardiovascular disease (3). Several matched case control studies have shown a modest but significant increased prevalence of the *apoE4* allele in coronary heart disease (CHD) patients from various populations (4, 5). Nevertheless, even though the *apoE4* allele appears to be a significant genetic risk factor for CHD, individuals with the *apoE4* allele do not inevitably develop this disorder. This fact suggests that other genetic or environmental risk factors may interact with the *apoE* gene in determining CHD risk. It was recently proposed that together with the qualitative modification of the apoE structure due to the *apoE* alleles, quantitative variations of *apoE* mRNA expression play a major role in determining risk (6).

In accordance with this hypothesis, polymorphisms in the proximal promoter region of the *apoE* gene have recently been described at positions −491 A/T, −427 T/C, and −219 G/T (7, 8). In particular, there is experimental evidence, in vitro and in vivo, indicating that the *apoE* gene promoter (−219G/T) polymorphism produces variations in the transcriptional activity of the gene. Specifically, the −219G allele showed a higher transcriptional activity than −219T. This effect was probably due to the differential binding of nuclear proteins, as indicated by electrophoretic mobility shift assays (7). A study of a European population, including control individuals and multi-infarct patients, has shown that the *apoE* gene promoter (−219G/T) polymorphism is also associated with differential plasma apoE levels (9), illustrating the fact that this

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polymorphism influences *apoE* expression in vivo. Furthermore, the −219T allele is associated with an increased risk of myocardial infarction (MI) (9) and premature CHD (10).

As a result of eating fat-rich meals, subjects in most industrialized countries are predominantly in a postprandial state throughout the day. Since 1979, when Zilversmit (11) proposed that TRL played a role in atherosclerosis, many research teams have shown the important role of postprandial lipoprotein particles in the development of CHD (12–15). In the postprandial state, the hepatic uptake of TRL, determined by the interaction of the LDL receptor and the low-density lipoprotein receptor-related protein with apoE, is one of the main components of this TRL metabolism.

Thus, a postprandial study design would be the most suitable procedure for investigating the functional impact of variation in the *apoE* gene promoter on the catabolism of TRL. A strong linkage disequilibrium between the −219T and *apoE4* allele has been described, and *apoE* genotypes have been implicated in a variable postprandial lipid response (16). Consequently, we have studied the effect of the *apoE* gene promoter (−219G/T) polymorphism in apoE 3/3 subjects, to the exclusion of other apoE isoforms. The aim of this study was to determine whether the *apoE* gene promoter (−219G/T) polymorphism could modify the postprandial response of TRL in young normolipemic apoE 3/3 males in order to explain the increased risk of MI and premature CHD associated with homozygote carriers of the T allele.

MATERIALS AND METHODS

Human subjects

Fifty-one healthy apoE 3/3 male volunteers (8 with the −219TT genotype, 29 with the −219GT genotype, and 14 with the −219GG genotype) were studied. All subjects were students at the University of Cordoba, and all responded to an advertisement. They ranged in age from 18 to 49 years. None of them had diabetes or liver, renal, or thyroid disease. None of the subjects were taking medication or vitamins known to affect plasma lipids, as described in our previous studies (17, 18). The subjects' fasting plasma lipids, lipoproteins, apolipoprotein concentrations, age, and body mass indexes are shown in **Table 1**. All studies were carried out in the research unit at the Hospital Reina Sofia University . The experimental protocol was approved by the Human Investigation Review Committee at the hospital.

TABLE 1. Plasma lipids and apolipoproteins according to the −219G/T *apoE* promoter polymorphism

	GG (n = 14)	GT (n = 29)	TT (n = 8)	P^a
Age (years)	23.3 ± 8.0	22.9 ± 4.1	21.5 ± 4.5	0.79
Body mass index (kg/m^2)	25.8 ± 2.9	24.6 ± 3.6	26.5 ± 3.9	0.36
Cholesterol (mmol/l)	3.99 ± 0.5	4.05 ± 0.7	3.77 ± 0.5	0.61
Triacylglycerols (mmol/l)	0.90 ± 0.4	0.90 ± 0.4	1.02 ± 0.4	0.78
LDL-cholesterol (mmol/l)	2.35 ± 0.6	2.52 ± 0.6	2.27 ± 0.5	0.50
HDL-cholesterol (mmol/l)	1.29 ± 0.3	1.18 ± 0.3	1.14 ± 0.4	0.43
apoB (g/l)	0.67 ± 0.2	0.68 ± 0.2	0.66 ± 0.2	0.97
apoA-I (g/l)	0.99 ± 0.2	0.99 ± 0.2	0.89 ± 0.1	0.45
apoE (g/l)	0.12 ± 0.04	0.11 ± 0.03	0.09 ± 0.02	0.19

apoB, apolipoprotein B. Values are given as mean ± SD.
[a] Data were tested for statistical significance between genotypes by analysis of variance (ANOVA).

Vitamin A 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 fatty meal consisted of two cups of whole milk, eggs, bread, bacon, cream, walnuts, and butter. Subjects were given 1 g of fat and 7 mg of cholesterol per kg of body weight. The meal contained 65% of energy as fat, 15% as protein, and 25% as carbohydrates and was eaten in 20 min. After the meal, the subjects consumed no energy for 11 h, but were allowed to drink water. Blood samples were drawn before the meal, every hour until the sixth hour, and every 2.5 h 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 blood 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 overlayered with 0.15 mol/l NaCl and 1 mmol/l EDTA (pH 7.4, d < 1.006 kg/l) by a single ultracentrifugal spin (36,200 *g*, for 30 min at 4°C) in a type 50 rotor (Beckman Instruments, Fullerton, CA). Chylomicrons contained in the top layer were removed by aspiration after cutting the tubes. The infranatant fluid was centrifuged at a density of 1.019 kg/l 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. All operations were done in subdued light. Large and small TRL fractions were stored at −70°C until assayed for retinyl palmitate (RP).

Lipid analysis

Cholesterol and triacylglycerols (TGs) in plasma and lipoprotein fractions were assayed by enzymatic procedures (19, 20). apoA-I and apoB were determined by turbidimetry (21). The serum apoE concentration was determined using a kit from Wako Chemicals. HDL-cholesterol was measured by analyzing the supernatant fluid obtained after precipitation of a plasma aliquot with dextran sulfate and Mg^{2+} as described by Warnick, Benderson, and Albers (22). LDL-cholesterol was obtained as the difference between HDL-cholesterol and the cholesterol from the bottom part of the tube after ultracentrifugation (183,000 *g* for 24 h at 4°C) at a density of 1.019 kg/l.

RP assay

The RP content of large and small TRL fractions was assayed using a method previously described (23). 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 (24).

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 and Hamsten (25). 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 apoB-48 and 5.1% for apoB-100.

DNA amplification and genotyping

Genomic DNA extraction and *apoE* E2/E3/E4 (26) and −219G/T (7, 8) genotypes were determined as previously described. Digested DNA was separated by electrophoresis on an

8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining. Samples containing the T allele of the −219G/T polymorphism were amplified a second time to verify the genotype.

Statistical analysis

Several variables were calculated to characterize the postprandial responses of the plasma TGs large TRL and small TRL to the test meal. The area under the curve (AUC) was defined as the area between the plasma concentration versus the time curve and a line drawn parallel to the horizontal axis through the 0 h concentration. Because the latter time points for plasma apoE were lower than the 0 h level, the apoE AUC was calculated as the area between the plasma apoE concentration and the horizontal axis. These areas were 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 highest concentration. Data were tested for statistical significance between genotypes by ANOVA and the Kruskal-Wallis test, and between genotypes and time by ANOVA for repeated measures. In this analysis, we studied the statistical effects of the genotype alone, independent of time, in the postprandial study, the effect of time alone or change in the variable after ingesting fatty food over the entire lipemic period, and the effect of the interaction of both factors (genotype and time), which is indicative of the magnitude of the postprandial response in each group of subjects. When statistical significance was found, the Tukey post-hoc comparison test was used to identify group differences. A probability value of less than 0.05 was considered significant. All data presented in the text and tables are expressed as means ± SD. SPSS 7.5 for WINDOWS (SPSS Inc., Chicago, IL) was used for the statistical comparisons.

RESULTS

The baseline characteristics of the subjects are shown in Table 1. Significant differences were not observed for any of the variables analyzed in the basal state among subjects homozygous for the G allele (GG, n = 14), subjects heterozygous for the T allele (GT, n = 29), and subjects homozygous for the T allele (TT, n = 8) of the −219G/T polymorphism located in the *apoE* gene promoter region.

The postprandial response of triglycerides, RP, and cholesterol in large TRL is shown in **Fig. 1**. The fat-loading test significantly increased triglycerides ($P < 0.001$), RP ($P < 0.009$), and cholesterol ($P < 0.001$) in both large and small TRL, with respect to baseline levels, indicating an increase of these parameters in the different groups of subjects during the postprandial period.

The analysis of the interaction between genotype and time showed that the subjects homozygous for the T allele have a higher postprandial response of triglycerides ($P < 0.001$), RP ($P < 0.005$), and cholesterol ($P < 0.05$) in large TRL than carriers of the G allele (Fig. 1). Furthermore, the AUC of triglycerides ($P < 0.05$), RP ($P < 0.05$), and cholesterol ($P < 0.05$) in large TRL was greater in subjects homozygous for the T allele when compared with carriers of the G allele (**Table 2**).

apoB-100 and apoB-48 in large and small TRLs were also analyzed. Significant differences were not found for

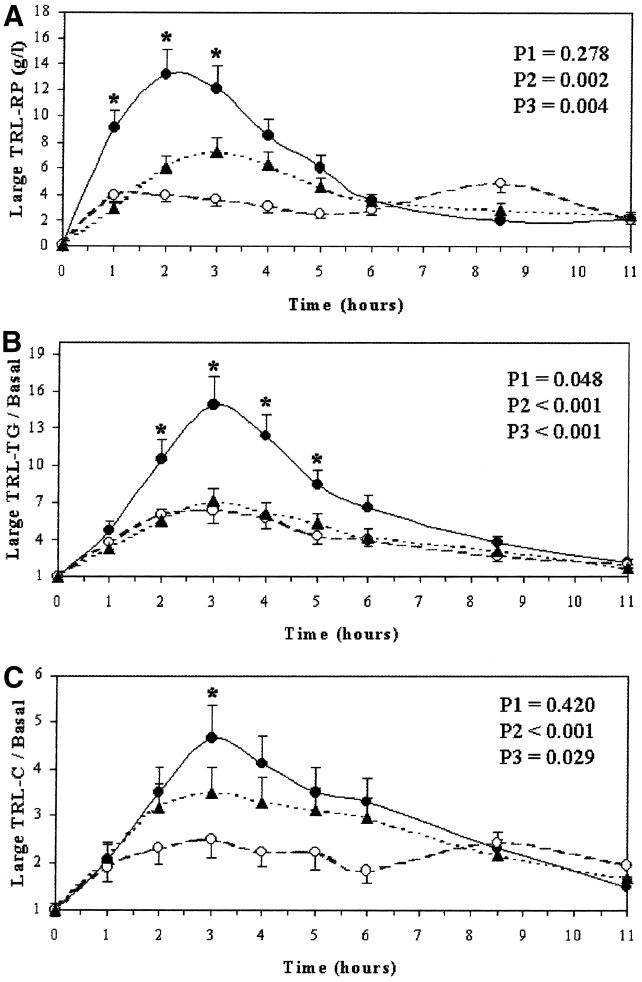

Fig. 1. Line plots of postprandial large triacylglycerol-rich lipoprotein (TRL)-retinyl palmitate (A), large TRL-triacylglycerol (TG) (B), and large TRL-cholesterol (C) response in TT subjects (n = 8, continuous line, black circles), GT subjects (n = 29, discontinuous line, black triangles), and GG subjects (n = 14, discontinuous line, white circles). The levels at each time point were averaged and adjusted to baseline TG (B) and cholesterol (C) for each group. P1: genotype effect; P2: time effect; P3: genotype by time interaction. ANOVA for repeated measures. * Indicates statistically significant differences among genotypes at that specific time point. $P < 0.05$ using the Tukey test. Error bars indicate mean ± SD.

the apoB-100 in large or small TRL or for the apoB-48 in large TRL among the three genotypes studied (Table 2). However, a significant effect of the interaction between genotype and time was observed for the apoB-48 levels in small TRL ($P < 0.05$) (**Fig. 2A**). Subjects homozygous for the T allele showed a higher postprandial response of apoB-48 levels in the small TRL compared with carriers of the G allele. The intake of fat significantly increased levels of apoB-48 in the small TRL ($P < 0.001$) with respect to baseline levels (Fig. 2A). Moreover, the AUC of apoB-48 in small TRL ($P < 0.05$) was greater in subjects homozygous for the T allele when compared with carriers of the G allele (Table 2).

When we studied the serum apoE levels, a gradual effect was observed, whereby the subjects with the TT geno-

TABLE 2. Area under the postprandial curve according to the −219G/T *apoE* promoter polymorphism

	GG (n = 14)	GT (n = 29)	TT (n = 8)	P^a
Total cholesterol (mmol·s/l)	0.75 ± 0.12	0.74 ± 0.14	0.71 ± 0.10	0.87
Triacylglycerols (mmol·s/l)	0.31 ± 0.11	0.31 ± 0.15	0.36 ± 0.16	0.67
LDL-cholesterol (mmol·s/l)	0.41 ± 0.11	0.44 ± 0.14	0.41 ± 0.10	0.74
HDL-cholesterol (mmol·s/l)	0.23 ± 0.05	0.21 ± 0.05	0.21 ± 0.06	0.26
apoB (g·s/l)	0.12 ± 0.03	0.12 ± 0.04	0.12 ± 0.04	0.54
Large TRL-TG (mmol·s/l)	0.10 ± 0.04	0.12 ± 0.07	0.20 ± 0.09	0.031[b]
Small TRL-TG (mmol·s/l)	0.09 ± 0.05	0.08 ± 0.04	0.09 ± 0.06	0.70
Large TRL-C (mmol·s/l)	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.042[b]
Small TRL-C (mmol·s/l)	0.05 ± 0.03	0.03 ± 0.02	0.05 ± 0.04	0.81
Large TRL-RP (ng·s/ml)	21.15 ± 12.12	21.84 ± 16.15	51.78 ± 16.01	0.042[b]
Small TRL-RP (ng·s/ml)	12.30 ± 7.29	11.44 ± 9.47	11.45 ± 8.61	0.97
Large TRL-apoB48 (AU)	319 ± 313	418 ± 322	515 ± 333	0.59
Small TRL-apoB48 (AU)	227 ± 249	482 ± 325	782 ± 384	0.046[b]
Large TRL-apoB100 (AU)	985 ± 942	615 ± 628	537 ± 217	0.41
Small TRL-apoB100 (AU)	30904 ± 24742	25370 ± 15986	21231 ± 18462	0.70
Serum apoE (g·s/l)	0.023 ± 0.007	0.018 ± 0.005	0.016 ± 0.002	0.048[b]

TRL, triacylglycerol-rich lipoprotein; RP, retinyl palmitate; AU, arbitrary unit. Values are given as mean ± SD × 10^5, except for apoB48 and apoB100 (mean ± SD) in both Large and Small TRL.

[a] Data were tested for statistical significance between genotypes by ANOVA.

[b] $P < 0.05$.

type showed the lowest postprandial levels and subjects with the GG genotype had the highest levels ($P < 0.05$) (Fig. 2B). Furthermore, the AUC of serum apoE levels ($P < 0.05$) was greater in subjects homozygous for the T allele when compared with carriers of the G allele (Table 2). However, when we studied the correlation between baseline plasma apoE levels and the postprandial response (AUC for triglycerides, RP, cholesterol, apoB-48, and apoB-100 in small or large TRL), no significant differences were observed.

DISCUSSION

Our results demonstrate that subjects homozygous for the T allele of the −219G/T polymorphism located in the *apoE* gene promoter region have a higher postprandial response of cholesterol, triglycerides, and RP in chylomicrons, and of apoB-48 in chylomicron remnants. Furthermore, we have found that the presence of this polymorphism determines serum apoE levels during the postprandial period.

Several studies have demonstrated that the presence of polymorphisms located in the AI-CIII-AIV complex (17, 18) and in other gene loci determines variations in the postprandial response. Moreover, the effects of variations in the *apoE* gene on the absorption and clearance of dietary fats are well known (16). For example, clearance of postprandial particles is delayed in carriers of the E2 allele compared with carriers of the E4 allele. However, in recent years, it has been suggested that in addition to the qualitative effect induced by the *apoE* gene polymorphisms, the quantitative changes in gene expression could also be an important factor in the variability of the postprandial state. This hypothesis is backed by the recent discovery of a new polymorphism, −219G/T, located in the *apoE* gene promoter region that determines levels of gene expression.

The influence of this polymorphism on postprandial lipemic response in healthy subjects remains unknown. In our study, we have observed that subjects homozygous for the T allele have a higher postprandial response, with elevated levels of intestinal TRL, as demonstrated by a higher AUC and higher levels of apoB-48 and RP. Additionally, we have found a lower AUC and lower serum apoE levels in

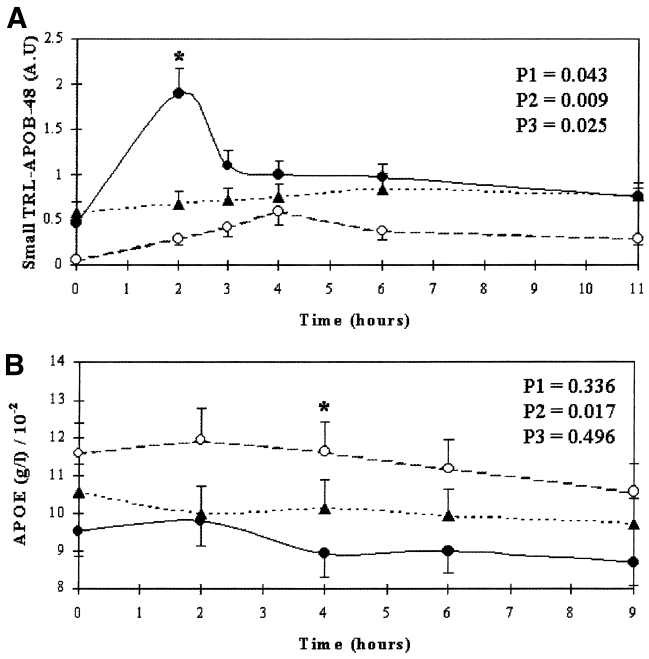

Fig. 2. Line plots of postprandial apolipoprotein B-48 (apoB-48) in small TRL (A) and plasma apoE (B) response in TT (n = 8, continuous line, black circles), GT (n = 29, discontinuous line, black triangles), and GG subjects (n = 14, discontinuous line, white circles). P1: genotype effect; P2: time effect; P3: genotype by time interaction. ANOVA for repeated measures. * Indicates statistically significant differences among genotypes at that specific time point. $P < 0.05$ using the Tukey test. Error bars indicate mean ± SD.

subjects with the TT genotype, with significant differences observed at 4 h, coinciding with the highest peak of postprandial lipemia. These differences are in accordance with the results obtained in other studies that demonstrated that *apoE* gene expression was modified by the −219G/T polymorphism (7). This can be explained by the fact that the presence of a G instead of a T significantly increases promoter activity by as much as 169%, as compared with the controls. Furthermore, a study with a European population that included controls and multi-infarct patients has shown that subjects homozygous for the T allele had significantly lower baseline plasma levels of apoE than other subjects (9).

The mechanisms via which the −219T allele of the apoE gene enhances atherothrombosis are yet to be elucidated. In the study by Lambert et al. (9), the −219G/T polymorphism did not modify baseline plasma lipid or lipoprotein concentrations, as in our study. The authors speculated that the −219TT genotype could increase the risk of MI at a local level by modifying the macrophage apoE expression, but experimental data to corroborate this hypothesis do not exist. Unlike Lambert et al., we designed a postprandial study to investigate the functional impact of the −219G/T polymorphism because of the important role of apoE in the uptake of TRL that occurs in the postprandial state. Several studies have shown that the apoE in the TRL interacts with different receptors in the liver, inducing the uptake of these particles from the bloodstream and their hepatic metabolism (1, 2). It is probable that lower apoE plasma levels observed in subjects homozygous for the T allele are also associated with lower apoE-TRL levels, thus reducing clearance by hepatic receptors. This phenomenon could explain the higher postprandial response observed in our study, as well as the increased risk of MI and premature CHD (9, 10) associated with homozygous carriers of the T allele, owing to the importance of triglyceride-rich postprandial particles as a risk factor for atherosclerosis (26, 27).

A limitation of our study is that it includes a relatively small number of subjects. However, we wanted to include in our study only young normolipemic apoE 3/3 males in order to avoid the variable postprandial lipid response of the other apoE isoforms or gender. Therefore, only 51 healthy apoE 3/3 male volunteers were selected from over 100 subjects.

In conclusion, our data suggest that the –219G/T polymorphism located in the *apoE* gene promoter region may influence TRL metabolism during the postprandial period, thus prolonging postprandial lipemia in subjects with the TT genotype.

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