Effects of the human apolipoprotein A-I promoter G-A mutation on postprandial lipoprotein metabolism[1–3]

Carmen Marín, José López-Miranda, Purificación Gómez, Elier Paz, Pablo Pérez-Martínez, Francisco Fuentes, José Antonio Jiménez-Perepérez, José María Ordovás, and Francisco Pérez-Jiménez

ABSTRACT

Background: There is considerable interindividual variability in the postprandial lipid response to a fat-rich meal, and genetic factors have been considered to account for some of these effects. We previously showed that the G-A mutation 5′ to the apolipoprotein (apo) A-I gene was significantly associated with the LDL-cholesterol response to diet.

Objective: We evaluated whether this effect is mediated by mechanisms involving postprandial lipoprotein metabolism.

Design: Twenty-eight *G/G* and 23 *G/A* healthy male subjects, homozygotes for the apo *E3* allele, were subjected to a vitamin A fat-loading test. Blood was drawn at time 0 and every hour for 11 h.

Results: There was a significant postprandial decrease in plasma cholesterol, LDL cholesterol, and apo B in *G/G* subjects but not in *G/A* subjects. A greater postprandial response in large triacylglycerol-rich lipoproteins (TRLs) and a smaller postprandial response in large TRL apo A-IV was observed in *G/A* than in *G/G* subjects. Retinyl palmitate in large and small TRL concentrations was similar for both genotypes. No significant genotype effects were detected for triacylglycerol concentrations in plasma, small TRL fraction, and apo A-I and HDL-cholesterol concentrations.

Conclusion: Our data suggest that the G-A mutation affects the LDL-cholesterol response to diet by mechanisms involving postprandial lipoprotein cholesterol metabolism. *Am J Clin Nutr* 2002;76:319–25.

KEY WORDS Postprandial lipemia, apolipoprotein A-I, G-A mutation, triacylglycerols, retinyl palmitate, coronary artery disease, HDL cholesterol, LDL cholesterol

INTRODUCTION

Elevated fasting concentrations of LDL cholesterol and reduced concentrations of HDL cholesterol are risk factors for coronary artery disease, the major cause of death and disability in most industrialized countries (1, 2). Members of these societies, by eating regular fat-rich meals, are predominantly in a postprandial state throughout the day. In these individuals, the fed state, and its effects on lipoprotein metabolism, may be more representative of their physiologic status than is the fasting state. Since the Zilversmit proposal in 1979 (3) about the important role of triacylglycerol-rich lipoproteins (TRLs) in the development of atherosclerosis, considerable knowledge about postprandial lipemia has been accumulated, and some fasting dyslipidemic conditions, as well as myocardial infarction, have been associated with abnormal postprandial lipoprotein patterns (4–13). In persons with a moderate elevation of fasting triacylglycerols, an impaired postprandial response to a fat load constitutes an early biological expression of a paternal history of premature coronary heart disease (14). The basic mechanisms involved during alimentary lipemia are relatively well known, and the effects of different nutrients on the variability of the postprandial response are under active investigation (15–27). However, less is known about the dramatic interindividual variability observed during postprandial lipemia. Some evidence suggests that the genetic variability at the apolipoprotein (apo) E gene locus might affect cholesterol absorption and the postprandial lipemic response (28–32). Other studies indicate that several other gene loci may also be involved in determining this variability (33–36).

A common variant due to a G-to-A transition was described 76 base pairs upstream from the apo A-I gene transcription start site (37). Some studies reported that subjects with the *A* allele, which occurs at a frequency of 0.15–0.20 in white populations, have higher concentrations of HDL cholesterol than do subjects who are homozygous for the most common *G* allele (38). However, the magnitude and the sex distribution of this effect have differed among studies (38–42). In a previous study (43), we examined the effect of this mutation on the responses of HDL and LDL cholesterol to changes in the amount of dietary fat. That study showed that subjects carrying the *A* allele had a significantly greater

[1] From the Unidad de Lipidos y Arteriosclerosis, Hospital Universitario Reina Sofía, Cordoba, Spain (CM, JL-M, PG, EP, PP-M, FF, JAJ-P, and FP-J), and the Lipid Metabolism Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston (JMO).

[2] Supported by grants from the Plan Nacional I+D+I, Spanish Ministry of Science and Technology (SAF96/0060, to FP-J), the Spanish Ministry of Health (FIS 96/1540 and 98/1531, to JL-M), the Fundación Cultural "Hospital Reina Sofía-Cajasur" (to CM and PG), the Consejería de Salud, the Servicio Andaluz de Salud (PAI 96/54, 97/58, 98/126, 98/132, 99/116, and 99/165), the Consejería de Agricultura y Pesca de la Junta de Andalucía (to FP-J), the Agencia Española de Cooperación Internacional (to EP), the NIH (HL 54776, to JMO), and the Patrimonio Comunal Olivarero.

[3] Address reprint requests to F Perez-Jiménez, Unidad de Lipidos y Arteriosclerosis, Hospital Universitario Reina Sofía, Avda, Menéndez Pidal, s/n, 14004 Córdoba, Spain. E-mail: fperez@hrs.sas.junta-andalucia.es.

Received April 12, 2001.

Accepted for publication July 31, 2001.

TABLE 1
Baseline characteristics of the study subjects according to *APOA1* genotype[1]

	G/G (*n* = 28)	*G/A* (*n* = 23)
Age (y)	22.9 ± 5.9	22.3 ± 5.2
BMI (kg/m^2)	24.5 ± 2.4	23.8 ± 2.9
Cholesterol (mmol/L)	4 ± 0.5	3.8 ± 0.8
Triacylglycerols (mmol/L)	0.9 ± 0.4	0.8 ± 0.3
LDL cholesterol (mmol/L)	2.3 ± 0.5	2.3 ± 0.7
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.2 ± 0.2
Apolipoprotein B (g/L)	0.6 ± 0.2	0.6 ± 0.2
Apolipoprotein A-I (g/L)	10.1 ± 0.2	1 ± 0.2
Large TRL triacylglycerol (mmol/L)	0.23 ± 0.16	0.16 ± 0.12

[1] $\bar{x}$ ± SD. TRL, triacylglycerol-rich lipoprotein. There were no significant differences between the 2 groups (ANOVA).

increase in LDL cholesterol after a high-fat meal than did subjects who were homozygous for the common *G* allele (44). The present study was therefore designed to evaluate whether the difference in dietary response between the 2 allele groups was mediated by mechanisms involving postprandial lipoprotein metabolism in subjects homozygous for the apo *E3* allele.

SUBJECTS AND METHODS

Human subjects

Fifty-one healthy men aged 18–49 y, 28 who were homozygous for the most common allele (*G/G*) and 23 who were carriers of allele *A* (*G/A*), were studied. All the subjects were students at the University of Cordoba, and all responded to an advertisement. None of the subjects had diabetes or liver, renal, or thyroid disease. All the subjects were selected to have the apo *E3/E3* genotype to avoid allele effects of this gene locus on postprandial lipemia (31). None of the subjects were taking medication or vitamins known to affect plasma lipids. The subjects' fasting plasma lipid, lipoprotein, and apolipoprotein concentrations; ages; and body mass indexes (in kg/m^2) are shown in **Table 1**. All studies were carried out in the Research Unit at the Reina Sofía University Hospital, and the experimental protocol was approved by the hospital's Human Investigation Review Committee.

Vitamin A fat-loading test

After a 12-h fast, the subjects were given a fatty meal enriched with 60 000 U vitamin A/m^2 body surface area. The meal consisted of 2 cups whole milk, eggs, bread, bacon, cream, walnuts, and butter. The meal provided 1 g fat and 7 mg cholesterol/kg body wt. It 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 6th hour, and every 2.5 h until the 11th 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 1500 × g for 15 min at 4 °C. The chylomicron fraction of TRL (large TRL) was isolated from 4 mL plasma overlayered with 0.15 mol NaCl/L, 1 mmol EDTA/L (pH 7.4, density < 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, and 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 of TRL (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 in plasma and lipoprotein fractions were assayed by enzymatic procedures (45, 46). Apo A-I and apo B were determined by turbidimetry (47). 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 et al (48). The LDL cholesterol was obtained as the difference between the 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.

Retinyl palmitate assay

The RP content of large and small TRL fractions was assayed with a method previously described (49). 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 with the use of isotonic sodium chloride solution. Retinyl acetate (40 ng in 200 μL mobile phase buffer) was added to each tube as an internal standard. Five hundred milliliters methanol was added, followed by 500 μL mobile phase buffer, for a total volume of 1.7 mL. The mobile phase buffer was prepared fresh daily by combining 90 mL hexane, 15 mL *n*-butyl chloride, 5 mL acetonitrile, and 0.01 mL acetic acid (82:13:5 by volume with 0.01 mL acetic acid). The tubes were thoroughly mixed after each step. The final mixture was centrifuged at 350 × g for 15 min at room temperature, and the upper layer was carefully removed by aspiration and placed in individual autosampler vials. The autoinjector was programmed to deliver 100 μL/injection and a new sample every 10 min in a custom-prepackaged silica column SupelcoSil LC-SI (5 mm, 25 cm × 4.6 mm inner diameter) provided by Supelco Inc (Bellefonte, PA). The flow was maintained at a constant rate of 2 mL/min, and the peaks were detected at 330 nm. The peaks of RP and retinyl acetate were identified by comparing their retention times with a purified standard (Sigma, St Louis), 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 (50). All operations were performed in subdued light.

Determination of apo B-48 and apo B-100

Apo B-48 and apo B-100 were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described by Karpe and Hamsten (51). 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 power supply (Amersham Pharmacia Biotech Inc, Piscataway, NJ) 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. Apo B-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 apo B-100 was applied to 4 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 ≥30 min and stained in 0.2% Coomassie G-250:40% methanol:10% acetic acid for ≥4 h. Destaining was done in 12% methanol:7% acetic acid with 4 changes of destaining solution for 24 h. Gels were scanned with a videodensitometer scanner (TDI, Madrid) connected to a personal computer for integration of the signals. Background intensity was calculated after scanning an empty lane. The CV for the SDS-PAGE was 7.3% for apo B-48 and 5.1% for apo B-100.

Apo A-IV measurement

Apo A-IV was measured in total plasma and in both large and small TRLs in postprandial samples obtained at 0, 1, 3, 5, 8.30, and 11 h with the use of an enzyme-linked immunosorbent assay. Briefly, polystyrene microtiter plates (Nuc, Naperville, IL) were coated with affinity-purified polyclonal apo A-IV antibody (10 μg/mL) in phosphate-buffered saline (PBS) 0.1 mol/L (pH 7.4), 100 μL/well. The plates were covered with acetate plate sealers (ICN Biomedicals, Asse-Relegem, Belgium) and incubated overnight at room temperature. The next day, the solution containing the unbound antibody was removed and the remaining binding sites in the plate were blocked with the use of 0.5% bovine serum albumin (radioimmunoassay grade BSA; Sigma) and 0.1% NaN_3 in PBS (1-h incubation). Plates were then washed 3 times with PBS containing 0.5% Tween-20 (PBST).

Control and plasma samples were diluted (1:5000) in PBS-BSA. Large and small TRL samples were diluted 1:500 and 1:100, respectively. Twofold serial dilutions were performed for the plasma standard (standard curve: 333.3–10.4 μ/L). Controls were prepared in the laboratory by pooling plasma from different individuals. Multiple aliquots were stored at −70 °C. Controls were calibred against a primary standard determined by amino acid analysis.

Aliquots (100 μL) of standards, controls, and plasma samples were added to designated wells in the microtiter plate. Aliquots were diluted and thoroughly mixed immediately before addition. Controls and samples were run in duplicate wells in each plate. After a 2-h incubation at 37 °C, the content of the plate was discarded and the plate was washed 3 times with PBST.

The goat-immunopurified apo A-IV antibody conjugated to peroxidase was diluted in PBS-BSA at 1:5000, and 100 μL was added to each well. The plate was sealed and incubated at 37 °C for 2 h. After this incubation, the plate was washed 5 times with PBST. The substrate used for the enzymatic color reaction is *o*-phenylene diamine and hydrogen peroxide in 0.1 mol/L citrate buffer. This solution was added to each well (100 μL) and incubated for 30 min at room temperature and then read at 410 nm on a microtiter plate reader (Dynatech MR 600; Albertville, Minnesota).

DNA amplification and genotyping

DNA was extracted from 10 mL EDTA-containing blood. Amplification of a 432–base pair region of *APOA1* was done by polymerase chain reaction (PCR) with 250 ng genomic DNA and 0.2 μmol of each oligonucleotide primer (P1, 5′-AGGGACAGA GCTGATCCTTGAACTCTTAAG-3′, and P2, 5′-TTAGGGGAC ACCTACCCGTCAGGAAGAGCA-3′) in 50 μL. DNA was denatured at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1.5 min, and extension at 72 °C for 2 min. The PCR product (10 μL) was digested with 5 U restriction enzyme *Msp*I (GIBCO BRL, Paisley, Scotland) in a total volume of 35 μL. Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized after silver staining. Samples containing the *A* allele were amplified a second time to verify the genotype.

Amplification of a region of 266 base pairs of *APOE* was done by PCR with 250 ng genomic DNA and 0.2 mmol of each oligonucleotide primer (E1, 5′-GAACAACTGACCCCGGTGGCGGAG-3′, and E2, 5′-TCGCGGGCCCCGGCCTGGTACACTGCCA-3′) and 10% dimethyl sulfoxide in 50 μL. DNA was denatured at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 63 °C for 1.5 min, and extension at 72 °C for 2 min. Twenty microliters of PCR product was digested with 10 U of restriction enzyme *Cfo*I (BRL) in a total volume of 35 μL. Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

Amplification of a region of *APOA4* was done by PCR with 250 ng genomic DNA and 0.2 μmol of each oligonucleotide primer (P1, 5′-GCCCCTGGTGCAGCAGATGGAACAGCTCAGG-3′, and P2, 5′-CATCTGCACCTGCTCCTGCTGCTGCTCCAG-3′) in 50 μL. DNA was denatured at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for 1 min, and extension at 70 °C for 2 min. The PCR product (10 μL) was digested with 5 U restriction enzyme *Hin*fI (Promega, Madison, WI) in a total volume of 35 μL. Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized after silver staining. Samples containing the *A* allele were amplified a second time to verify the genotype.

Statistical analysis

Several variables were calculated to characterize the postprandial responses of plasma triacylglycerols, large TRLs, and small TRLs 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 highest concentration. Data were tested for statistical significance between genotypes by analysis of variance (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 the time in the postprandial study; the effect of time alone or the change in the variable after the 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 subjects with a different genotype. When statistical significance was found, the Tukey's post hoc comparison test was used to identify

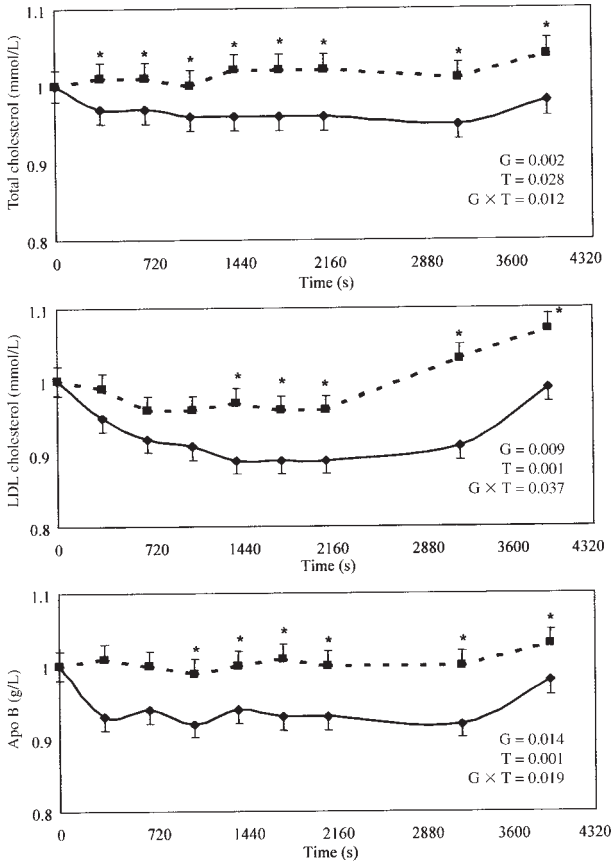

FIGURE 1. Postprandial normalized plasma total cholesterol, LDL-cholesterol, and apolipoprotein (apo) B responses in *G/G* ($n = 28$; ◆) and *G/A* ($n = 23$; ■) subjects. For each group, the concentrations at each time point were averaged and adjusted to the baseline concentration. Multivariate ANOVA for repeated measures: G, genotype effect; T, time effect; G × T, genotype-by-time interaction. *Significantly different from *G/G*, $P < 0.05$ (Tukey's test for normally distributed variables or Kruskal-Wallis for nonparametric variables).

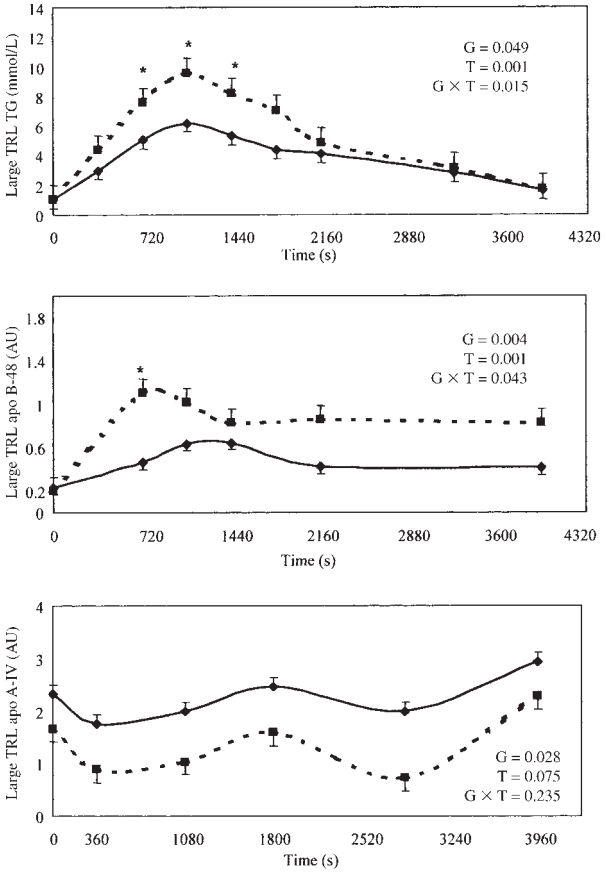

FIGURE 2. Postprandial normalized plasma triacylglycerol (TG), apolipoprotein (apo) B-48, and apo A-IV responses in large TG-rich lipoproteins (TRLs) in *G/G* ($n = 28$; ◆) and *G/A* ($n = 23$; ■) subjects. For each group, the concentrations at each time point were averaged and adjusted to baseline TG in the top panel. Multivariate ANOVA for repeated measures: G, genotype effect; T, time effect; G × T, genotype-by-time interaction, AU, arbitrary units. *Significantly different from *G/G*, $P < 0.05$ (Tukey's test).

group differences. A P value < 0.05 was considered significant. Stepwise multiple regression analyses were carried out using small and large TRL–apo B-48, large TRL–apo A-IV, total cholesterol, LDL cholesterol, and apo B concentrations, and the AUC was defined as dependent variables and age, body mass index, *APOA1* genotypes, HDL cholesterol, basal cholesterol, and triacylglycerol values as independent variables. Discrete variables were divided into classes for analysis. All data presented in the text and tables are expressed as means ± SDs. SPSS 7.5 for WINDOWS (SPSS Inc, Chicago) was used for the statisical comparisons.

RESULTS

The baseline characteristics of the study subjects are shown in Table 1. No significant differences for any of the variables analyzed were detected between subjects heterozygous for the *A* allele ($n = 23$) and those homozygous for the *G* allele ($n = 28$).

Plasma cholesterol responses after the fat-loading test are shown in **Figure 1**. In *G/G* subjects, the plasma cholesterol concentrations decreased significantly at time points 1–11 h compared with baseline concentrations; in *G/A* subjects, plasma cholesterol concentrations showed no significant change between 1 and 9 h but increased significantly from time 0 at the last time point (11 h). The *G/A* subjects had a significantly higher postprandial total cholesterol response than did the *G/G* subjects, as shown by ANOVA for repeated measures ($P = 0.002$) and by a higher AUC ($P < 0.002$).

In *G/G* subjects, plasma LDL-cholesterol concentrations decreased significantly at time points 1–9 h from baseline and returned to baseline concentrations at the end of the experimental period (Figure 1). In *G/A* subjects, plasma LDL-cholesterol concentrations increased significantly at 11 h. In *G/G* subjects, plasma apo B concentrations (Figure 1) decreased significantly at the 1–9-h time points from baseline; no significant changes in postprandial apo B were observed in *G/A* subjects. The *G/A* subjects had a greater postprandial response in LDL cholesterol ($P < 0.009$) and apo B ($P < 0.014$), as shown by ANOVA for repeated measures, and a higher AUC ($P < 0.006$; and $P < 0.008$, respectively) than did *G/G* subjects.

Triacylglycerol concentrations in large TRL particles (**Figure 2**) remained significantly elevated over baseline in *G/A* and *G/G* subjects during the entire period. A significant genotype effect was also observed by ANOVA for repeated measures (interaction

TABLE 2
Area under the postprandial curve in subjects according to *APOA1* genotype[1]

	G/G (*n* = 28)	*G/A* (*n* = 23)
Total cholesterol (mmol · s/L)	−1.491 ± 2.15	0.961 ± 2.83[2]
Triacylglycerols (mmol · s/L)	8.220 ± 5.73	8.934 ± 6.51
LDL cholesterol (mmol · s/L)	−1.884 ± 1.82	0.045 ± 2.66[3]
HDL cholesteol (mmol · s/L)	−0.083 ± 1.14	−0.300 ±1.27
Apo B (g/L s)	−0.373 ± 0.45	0.229 ± 0.99[4]
Apo A-I (g/L s)	−0.435 ± 0.62	−0.187 ± 0.91
Large TRL TG (mmol · s/L)	5.097 ± 3.64	5.417 ± 3.94
Small TRL TG (mmol · s/L)	0.822 ± 1.21	0.974 ± 1.48
Large TRL RP (ng · s/L)	46.202 ± 40.90	42.204 ± 50.29
Small TRL RP (ng · s/L)	16.688 ± 11.79	17.058 ± 17.01
Large TRL apo B-48 (AU)	147 ± 91	423 ± 419[5]
Small TRL apo B-48 (AU)	291 ± 322	122 ± 202
Large TRL apo B-100 (AU)	432 ± 628	337 ± 280
Small TRL apo B-100 (AU)	12.115 ± 18.311	12.882 ± 11.402

[1] $\bar{x}$ ± SD. TRL, triacylglycerol-rich lipoprotein; RP, retinyl palmitate; AU, arbitrary units.
[2–5] Significantly different from *G/G*: [2] $P = 0.002$, [3] $P = 0.006$, [4] $P = 0.008$, [5] $P = 0.007$.

between genotype and time), with a significantly greater postprandial response ($P = 0.022$) in the large TRL triacylglycerol concentrations in *G/A* than in *G/G* subjects. Triacylglycerols in the small TRL fraction increased over baseline during the first 6 h of the postprandial period for both *G/A* and *G/G* subjects. No significant genotype effects were observed by ANOVA for repeated measures in triacylglycerol concentrations in the small TRL fraction.

RP and apo B-48 were used as markers for intestinal lipoprotein production. RP concentrations in large and small TRLs were similar for both genotypes. No significant genotype effect was observed by ANOVA for repeated measures in postprandial large and small TRL-RP (**Table 2**). Large apo B-48 responses in *G/G* and *G/A* subjects are shown in Figure 2. Apo B-48 concentrations in large TRLs and small TRLs were significantly elevated over baseline in *G/G* and *G/A* subjects during the entire period. A significant genotype effect was also observed with ANOVA for repeated measures: *G/A* subjects had a significantly greater ($P = 0.007$) AUC in large apo B-48 than did *G/G* subjects (Table 2). There were no significant differences in the small TRL apo B-48 response between *G/G* and *G/A* subjects. No significant genotype effects were observed for apo B-100 in large or small TRL particles (Table 2). A significant genotype effect was also observed with ANOVA for repeated measures, showing a significantly greater postprandial decrease ($P = 0.028$) in large TRL apo A-IV in *G/A* subjects than in *G/G* subjects (Figure 2).

The possible linkage dysequilibrium between the G-A polymorphism and *APOA4* was analyzed. We did not observe any linkage dysequilibrium between the *APOA1* G-A polymorphism and the *APOA4* polymorphism.

Multiple regression analysis (**Table 3**) showed that the G-A mutation at *APOA1* was the only significant ($P = 0.003$) predictor of the variability in LDL-cholesterol postprandial response (AUC) in our study population, accounting for 18% of the variance. In addition, the G-A mutation ($P = 0.008$) was a significant predictor for the apo B postprandial response, accounting for the 14.1% of the variance. When total cholesterol postprandial response (AUC) was used as a dependent variable with the same variables in the model as for LDL cholesterol, only the *APOA1* genotype entered the model as a predictor ($P = 0.006$), accounting for the 15.2% of the variance. When large TRL apo B-48 and large TRL apo A-IV were introduced in the model as dependent variables, the G-A mutation was a significant predictor ($P < 0.004$ and $P < 0.042$, respectively), accounting for the 17.8% and 11.8% of the variance, respectively.

DISCUSSION

Our results show that carriers of the *A* allele mutation in the promoter region of *APOA1* (−76 base pairs *G/A* genotype) have a greater increase in large TRLs and a smaller decrease in LDL cholesterol and apo B after the consumption of a fatty meal than do those with the *G/G* genotype. These responses are accompanied by a greater postprandial decrease in the concentrations of apo A-IV carried in large TRLs.

Many factors—such as age, body mass index, tobacco, and alcohol consumption—influence the individual variability observed in postprandial lipid transport and affect the synthesis and catabolism of TRLs from the liver and the intestine. Our study found no significant differences among such factors that could influence the difference in postprandial lipemia between carriers of the mutation and homozygotes for the *G* allele. Furthermore,

TABLE 3
Multiple stepwise regression analyses[1]

Dependent variable	Independent variable	Variable in the model	β-Coefficient	Multiple R^2	*P*	Model R^2
Plasma TC AUC	*G-A* mutation	*G-A* mutation	0.468	0.148	0.009	0.197
	TC	BMI	0.237	0.049	0.115	
Plasma LDL cholesterol AUC	*G-A* mutation	*G-A* mutation	0.419	0.176	0.004	0.176
Plasma apo B AUC	*G-A* mutation	*G-A* mutation	0.399	0.141	0.011	0.233
	TC	Age	0.287	0.132	0.042	
	BMI	TC	−0.258	0.057	0.087	
Large TRL apo B-48 AUC	*G-A* mutation	*G-A* mutation	0.541	0.190	0.024	0.451
	TC	BMI	0.483	0.132	0.042	
	BMI	TC	−0.278	0.097	0.062	
	TG	TG	−0.189	0.031	0.277	

[1] We introduced as independent variables BMI, total triacylglycerol (TG), age, HDL cholesterol, and apolipoprotein (apo) A-IV and polymorphism. The *G/G* genotype was given the value of 1 and the *G/A* genotype the value of 2. TC, total cholesterol; TRL, triacylglycerol-rich lipoprotein; AUC, area under the curve.

the influence of several genetic factors, such as the genetic variants of apo B (51) and the isoform of apo E, on the absorption or clearance of dietary fat is well known (31, 52, 29). Carriers of the *E2* allele show a delayed clearance, and carriers of the *E4* allele show a faster process represented by the concentration of RP in the plasma (29).

Apo A-I is an apolipoprotein of intestinal origin that increases its synthesis after the consumption of a fatty meal. The gene for apo A-I is clustered with *APOC3* and *APOA4*, creating a genetic complex. Previous studies showed that the specific locus of *APOA1* could be responsible for 33% of the variability observed in the response to high-fat diets (53, 54). This fact may be relevant to postprandial metabolism. However, the mechanisms responsible for the effects of the genetic variants of *APOA1* on postprandial lipemia remain unknown. Calabresi et al (55) showed that carriers of the *APOA1* Milano mutation have greater postprandial lipemia. Our results show that carriers of the *A* allele have a longer postprandial lipemia than do homozygotes for the *G* allele, as well as a postprandial decrease in the concentrations of LDL cholesterol and apo B after a fat-rich meal.

Today, the mechanisms that determine the phenomena observed in our study are unknown, but we can consider several hypotheses. The different postprandial responses observed could be due to changes in fat absorption and cholesterol from the diet, as was previously shown with the apo E polymorphism (28–32). These differences could also be the result of less clearance of large triacylglycerol-enriched particles of intestinal origin, as indicated by the greater increase of apo B-48 and large TRL triacylglycerol concentrations. The lower concentrations of apo A-IV observed in the large TRLs of carriers of the *A* allele may determine this phenomenon. Goldberg et al (56) reported that in the presence of HDL, apo A-IV facilitates apo C-II catalysis of lipoprotein lipase by increasing transfer of apo C-II from HDL to substrate particles. The lipoprotein lipase activity is critical for normal clearance of postprandial TRL particles (57). Furthermore, apo A-IV itself increases catabolism of the triacylglycerol-enriched particles. Apo A-IV can modulate lipoprotein lipase and postprandial lipid metabolism. Thus, apo A-IV may regulate the clearance of TRLs of intestinal origin. Another possible explanation of our results is that they are not the direct consequence of the G-A mutation effect on *APOA1* but are caused by another functional mutation, closely connected to the previous one, in *APOC3* or *APOA4*. This would explain the lower content of apo A-IV in the large TRLs, but we did not observe any linkage dysequilibrium between the *APOA1* G-A polymorphism and *APOA4* polymorphisms.

In conclusion, carriers of the *G/A* genotype show a greater plasma postprandial response of large TRLs associated with a lower postprandial decrease in the concentrations of LDL cholesterol and apo B.

We appreciate the cooperation of Beatriz Pérez in the translation of the manuscript.

REFERENCES

1. Miller GJ, Miller NF. Plasma high density lipoprotein concentration and development of ischemic heart disease. Lancet 1975;1:16–20.
2. Castelli WP, Garrison RJ, Wilson PWF, Abbot RD, Kalousian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 1986;256:2835–8.
3. Zilversmit DB. Atherosclerosis: a postprandrial phenomenon. Circulation 1979;60:473–85.
4. Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 1988;29:469–79.
5. Ryu JE, Howard G, Craven TE, Bond MG, Hagaman AP, Crouse JR III. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. Stroke 1992;23:823–8.
6. Groot PHE, Van Stiphout W-AHJ, Krauss XH, et al. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb 1991;11:653–62.
7. Cavallero E, Dachet C, Neufcour D, Wirquin E, Mathe D, Jacotot B. Postprandial amplification of lipoprotein abnormalities in controlled type II diabetic subjects: relationship to postprandial lipemia and C-peptide/glucagon levels. Metabolism 1994;43:270–8.
8. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis 1994;106:83–97.
9. Uiterwaal CSPM, Grobbee DE, Witteman JCM, et al. Postprandial triglyceride response in young adult men and familial risk for coronary atherosclerosis. Ann Intern Med 1994;121:576–83.
10. Castro Cabezas M, Erkelens DW, Kock LAW, De Bruin TWA. Postprandial apolipoprotein B100 and B48 metabolism in familial combined hyperlipidemia before and after reduction of fasting plasma triglycerides. Eur J Clin Invest 1994;24:669–78.
11. Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipidemia, and triglycerides-rich remnant lipoproteins. Clin Chem 1995; 41:153–8.
12. Patsch JR, Miesenböck G, Hopferwieser T, et al. Relation of triglyceride metabolism and coronary artery disease: studies in the postprandial state. Arterioscler Thromb 1992;12:1336–45.
13. Hughes TA, Elam MB, Applegate WB, et al. Postprandial lipoprotein responses in hypertriglyceridemic subjects with and without cardiovascular disease. Metabolism 1995;44:1082–98.
14. Tiret L, Gerdes C, Murphy MJ, et al. Postprandial response to a fat tolerance test in young adults with a paternal history of premature coronary heart disease—the EARS II study (European Atherosclerosis Research Study). Eur J Clin Invest 2000;30:578–85.
15. Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. J Lipid Res 1998;29:925–36.
16. Ginsberg HN, Karmally W, Siddiqui M, et al. A dose-response study of the effects of dietary cholesterol on fasting and postprandial lipid and lipoprotein metabolism in healthy young men. Arterioscler Thromb 1994;14:576–86.
17. Grant KI, Marais MP, Dhansay MA. Sucrose in a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglycerides and cholesterol concentrations by decreasing triglyceride clearance. Am J Clin Nutr 1994;59:853–60.
18. Dubois C, Armand M, Azais-Braesco V, et al. Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr 1994;60: 374–82.
19. Dubois C, Armand M, Mekki N, et al. Effects of increasing amounts of dietary cholesterol on postprandial lipemia and lipoproteins in human subjects. J Lipid Res 1994;35:1993–2007.
20. Sandström B, Hansen LT, Sorensen A. Pea fiber lowers fasting and postprandial blood triglyceride concentrations in humans. J Nutr 1994;124:2386–96.
21. Muesing RA, Griffin P, Mitchell P. Corn oil and beef tallow elicit different postprandial responses in triglyceride and cholesterol, but similar changes in constituents of high-density lipoprotein. J Am Coll Nutr 1995;14:53–60.
22. Barr SI, Kottke BA, Mao SJ. Postprandial distribution of apolipoproteins C-II and C-III in normal subjects and patients with mild hyper-

triglyceridemia: comparison of meals containing corn oil and medium-chain triglycerides oil. Metabolism 1985;34:983–92.
23. Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n−3 fatty acids. J Lipid Res 1988;29:1451–60.
24. Weintraub MS, Zechner R, Brown A, Eisenberg S, Breslow JL. Dietary polyunsaturated fats of the ω-6 and ω-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. J Clin Invest 1988;82: 1884–93.
25. De Bruin TWA, Brouwer CB, Van Linde-Sibenius Trip M, Jansen H, Erkelens DW. Different postprandial metabolism of olive oil and soybean oil: a possible mechanism of the high-density lipoprotein conserving effect of olive oil. Am J Clin Nutr 1993;58:477–83.
26. Lichtenstein AH, Ausman LM, Carrasco W, et al. Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program step 2 diet. Arterioscler Thromb 1993;13:1533–42.
27. Salomaa V, Rasi V, Pekkanen J, et al. The effects of saturated fat and n−6 polyunsaturated fat on postprandial lipemia and hemostatic activity. Atherosclerosis 1993;103:1–11.
28. Weintraub MS, Eisenberg S, Breslow JL. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 1987;80:1571–7.
29. Brown AJ, Roberts DCK. The effect of fasting triacylglyceride concentration and apolipoprotein E polymorphism on postprandial lipemia. Arterioscler Thromb 1991;11:1737–44.
30. Nikkilä M, Solakivi T, Lehtimäki T, Koivula T, Laippala P, Astrom B. Postprandial plasma lipoprotein changes in relation to apolipoprotein E phenotypes and low density lipoprotein size in men with and without coronary artery disease. Atherosclerosis 1994;106:149–57.
31. Boerwinkle E, Brown S, Sharrett AR, Heiss G, Patsch W. Apolipoprotein E polymorphism influences postprandial retinyl palmitate but not triglyceride concentrations. Am J Hum Genet 1994;54:341–60.
32. Bjorkegren J, Karpe F, Milne RW, Hamsten A. Differences in apolipoprotein and lipid composition between human chylomicron remnants and very low density lipoproteins isolated from fasting and postprandial plasma. J Lipid Res 1998;39:1412–20.
33. Dallongeville J, Tiret L, Visvikis S, et al. Effect of apo E phenotype on plasma postprandial triglyceride levels in young male adults with and without a familial history of myocardial infarction: the EARS II study. European Atherosclerosis Research Study. Atherosclerosis 1999;145:381–8.
34. Ostos MA, López-Miranda J, Ordovas M, et al. Dietary fat clearance is modulated by genetic variation in apolipoprotein A-IV gene locus. J Lipid Res 1998;39:2493–500.
35. Ostos MA, López-Miranda J, Marín C, et al. The apolipoprotein A-IV 360His polymorphism determines the dietary fat clearance in normal subjects. Atherosclerosis 2000;153:209–17.
36. López-Miranda J, Ordovas LM, Ostos MA, et al. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein B. Arterioscler Thromb Vasc Biol 1997;17:1765–73.
37. Sigurdsson G Jr, Gudnason V, Sigurdsson G, Humphries SE. Interaction between a polymorphism of the apo A-I promoter region and smoking determines plasma levels of HDL and apo A-I. Arterioscler Thromb 1992;12:1017–22.
38. Jeenah M, Kessling A, Miller N, Humphries SE. G to A substitution in the promoter region of the apolipoprotein A-I gene is associated with elevated serum apolipoprotein A-I and high density lipoprotein cholesterol concentrations. Mol Biol Med 1990;7:233–41.
39. Blangero J, MacCluer JW, Kammerer CM, Mott GE, Dyer TD, McGill HD Jr. Genetic analyisis of apolipoprotein A-I in two dietary environments. Am J Hum Genet 1990;47:414–28.
40. Paul-Hayase H, Rosseneu M, Van Bervliet JP, Deslypere JP, Humphries SE. Polymorphism in the apolipoprotein (apo) AI-CIII-AIV gene cluster: detection of genetic variation determining plasma apo A-I, apo C-III and apo A-IV concentrations. Hum Genet 1992;88: 439–46.
41. Xu C-F, Angelico F, Del Ben M, Humphries SE. Role of genetic variation at the apo AI-CIII-AIV gene cluster in determining plasma apo A-I levels in boys and girls. Genet Epidemiol 1993;10: 113–22.
42. Smith JD, Brinton EA, Breslow JL. Polymorphism in the human apolipoprotein A-I gene promoter region. Association of the minor allele with decreased production rate in vivo and promoter activity in vitro. J Clin Invest 1992;89:1796–800.
43. López Miranda J, Jansen S, Ordovás JM, et al. Influence of the SstI polymorphism at the apolipoprotein C-III gene locus on the plasma low-density-lipoprotein-cholesterol response to dietary monounsaturated fat. Am J Clin Nutr 1997;66:97–103.
44. Mata P, López-Miranda J, Pocovi M, et al. Human apolipoprotein A-I gene promoter mutation influences plasma low density lipoprotein cholesterol response to dietary fat saturation. Atherosclerosis 1998; 137:367–76.
45. Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973;19:476–82.
46. Allain CC, Poon LS, Chang CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20:470–5.
47. Riepponen P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-1 and B in serum. Scand J Clin Lab Invest 1987;47:739–44.
48. Warnick R, Benderson J, Albers JJ. Dextran sulfate-Mg precipitation procedure for quantitation of high density lipoprotein cholesterol. Clin Chem 1982;28:1379–88.
49. Ruotolo G, Zhang H, Bentsianov V, Le N-A. Protocol for the study of the metabolism of retinyl esters in plasma lipoproteins during postprandial lipemia. J Lipid Res 1992;33:1541–9.
50. De Ruyter MGM, De Leeheer AP. Simultaneous determination of retinol and retinyl esters in serum or plasma by reversed-phase high performance liquid chromatography. Clin Chem 1978;24: 1920–3.
51. Karpe F, Hamsten A. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. J Lipid Res 1994;35:1311–7.
52. Brenninkmeijer BJ, Stuyt PMJ, Demacker PNM, Stalenhoef AFH, and Van't Laar A. Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype. J Lipid Res 1987;28:361–70.
53. Xu C-F, Boerwinkle E, Tikkanen MJ, Huttunen JK, Humphries SE. Genetic variation at the apolipoprotein gene loci contribute to response of plasma lipids to dietary change. Genet Epidemiol 1990; 7:261–75.
54. Pagani F, Sodoli A, Giudici GA, Barenghi L, Vergani C, Baralle FE. Human apolipoprotein A-I gene promoter polymorphism: association with hyperalphalipoproteinemia. J Lipid Res 1990;31: 1371–7.
55. Calabresi L, Cassinotti M, Qianfranceshi G, et al. Increased postprandial lipemia in apo A-I Milano carriers. Arterioscler Thromb 1993;13:521–8.
56. Goldberg IJ, Scheraldi CA, Yacoub LK, Saxena U, Bisgaier CL. Lipoprotein apo C-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. J Biol Chem 1990;265:4266–72.
57. Mero N, Suurinkeroinen L, Syvänne M, Knudsen P, Yki-järvinen H, Taskinen M-R. Delayed clearance of postprandial large TG-rich particles in normolipidemic carriers of LPL Asn291Ser gene variant. J Lipid Res 1999;40:1663–70.