

An Apolipoprotein A-II Polymorphism (-265T/C, rs5082) Regulates Postprandial Response to a Saturated Fat Overload in Healthy Men^{1,2}

Javier Delgado-Lista,³ Francisco Perez-Jimenez,³ Toshiko Tanaka,⁴ Pablo Perez-Martinez,³ Yolanda Jimenez-Gomez,³ Carmen Marin,³ Juan Ruano,³ Laurence Parnell,⁴ Jose Maria Ordovas,⁴ and Jose Lopez-Miranda^{3*}

³Lipids and Atherosclerosis Research Unit, Reina Sofia University Hospital, Córdoba, and Ciber Fisiopatología Obesidad y Nutrición (CB06/03) Instituto de Salud Carlos III, 14004 Madrid, Spain and ⁴Jean Mayer-USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111-1524

Abstract

Apolipoprotein (Apo) A-II is an apolipoprotein with an unknown role in lipid metabolism. It has been suggested that the presence of the less frequent allele of a single nucleotide polymorphism (Apo A-II -265T/C, rs5082) reduces the transcription rate of Apo A-II and enhances VLDL postprandial clearance in middle-aged men. To further investigate the role of Apo A-II -265T/C on lipid metabolism, we studied 88 normolipidemic young men. The participants were given a fatty meal containing 1 g fat and 7 mg cholesterol/kg weight and capsules containing 60,000 IU vitamin A (retinyl palmitate, 15.15 mg RE) per square meter body surface area. Postprandial lipemia was assessed during the 11 h following the meal. Total cholesterol (Chol) and triacylglycerols (TG) in plasma and TG-rich lipoproteins (TRL) (large TRL and small TRL) were measured, as well as HDL, Apo A-I, Apo B, Apo B-48, and Apo B-100. Postprandial responses were higher in the TT group than in carriers of the minor allele (CC/TC) for total TG in plasma (21.37% of change of area under curve, $P = 0.014$), large TRL-TG (24.75% change, $P = 0.017$) and small TRL-Chol (26.63% change, $P = 0.003$). Our work shows that carriers of the minor allele for Apo A-II -265T/C (CC/TC) have a lower postprandial response compared with TT homozygotes. This finding may partially explain the role of Apo A-II in lipid metabolism and can identify a population with a decreased risk of cardiovascular disease, as corresponds to the lower level of postprandial hypertriglyceridemia. *J. Nutr.* 137: 2024–2028, 2007.

Introduction

Apolipoprotein (Apo)⁵ A-II is a major apolipoprotein that can be found in 2 forms in plasma. One creates dimers of 77 amino acids, whereas the other is linked to the HDL surface by a β -octyl glucoside molecule, accounting for up to the 20% of its protein content (1). Apo A-II has been widely studied in mice, where it influences the regulation of several key enzymes in lipoprotein metabolism, including hepatic lipase (2), cholesterol ester transfer protein (3), phospholipid transfer protein (4), and

lecithin-cholesterol acyltransferase (5). Moreover, recent studies of rodents correlated Apo A-II levels with plasma concentrations of glucose, free fatty acids, and insulin, as well as with delayed clearance of triacylglycerol (TG)-rich lipoproteins (TRL) and increased BMI (4,6–8). It has also been reported that its presence in HDL impairs cellular cholesterol mobilization compared with HDL which only contains Apo A-I (9,10). On the basis of the current evidence, therefore, Apo A-II has been regarded as proatherogenic. However, nearly all the clinical data available come from animal studies. Data on the functions of Apo A-II in humans are scarce and controversial. An indirect approach to evaluating its function is to look for genetic variations in its promoter region (chromosome 1, 1q23) that affect the apoprotein transcription rate and concentration in plasma and look for further influence on lipid metabolism. On this basis, several single nucleotide polymorphism (SNP) have been reported, but only MSP-I and -265T/C have been associated with plasma lipid concentration. MSP-1 was analyzed in a study of 1102 persons from the Pacific island of Kosrae, in which several other candidate genes for increased cardiovascular risk were also tested. The carriers of the less frequent MSP-1 allele had higher TG

¹ Supported by Consejería de Innovación, proyectos de Investigación de Excelencia Junta de Andalucía (AGR 05/00922 to P.P.-M. and P06-CTS-01425 to J.L.-M.) and by the Ministerio de Educación y Ciencia AGL-2006-01979/ALI (to J.L.-M.). The Lipids and Atherosclerosis Unit at Reina Sofia University Hospital is a partner of Ciber Fisiopatología Obesidad y Nutrición (CB06/03), Instituto de Salud Carlos III, Spain.

² Author disclosures: J. Delgado-Lista, F. Perez-Jimenez, T. Tanaka, P. Perez-Martinez, Y. Jimenez-Gomez, C. Marin, J. Ruano, L. Parnell, J. M. Ordovas, and J. Lopez-Miranda, no conflicts of interest.

⁵ Abbreviations used: Apo, apolipoprotein; AUC, area under the curve; Chol, total cholesterol; FAM, 6 carboxy-fluorescein; SNP, single nucleotide polymorphism; TG, triacylglycerol; TRL, triacylglycerol-rich lipoprotein.

* To whom correspondence should be addressed. E-mail: jlopezmir@uco.es.

levels and, interestingly, reduced blood pressure (11). -265T/C SNP was studied in an elegant design by Van t' Hooft et al. (12), who chose this SNP for study because of its location in a regulatory element (called element D) of the promoter region of Apo A-II, where it binds several nuclear factors. They showed that the presence of the minor allele of -265T/C SNP decreased Apo A-II production and reduced postprandial metabolism in middle-aged men (12). Specifically, it was noted that subjects homozygous for the C allele had a lower apoB-100 concentration of large TRL 6 h after oral fat load than subjects who were homozygous for the most frequent allele (T allele). Surprisingly, TG and other postprandial particles such as Apo B-48 or chylomicron remnants were unaffected by the SNP in that study. Methodological issues, such as the fact that only the first 6 h following the meal were studied, could have limited their results. To further investigate the possible relationship of Apo A-II -265T/C SNP and postprandial lipid metabolism in this study, we conducted vitamin A fat loading tests on a group of healthy young men.

Materials and Methods

Subjects

Between 1992 and 2003, a total of 88 saturated rich fatty meals were given to healthy persons enrolled in 2 studies performed by our unit. All the tests were performed using exactly the same methodology, as described below. Other results of these studies have been published elsewhere (13–23). The participants were healthy nonsmoking male students at the University of Cordoba aged 18–33 y. None of them had diabetes or liver, renal, or thyroid disease. None of them were taking medication or vitamins known to affect plasma lipids, as described in our previous studies (13–27). Anthropometric measures (weight, height, and BMI) and blood pressure were assessed and all subjects were encouraged to maintain their regular lifestyles and regular levels of physical activity. All volunteers had plasma cholesterol and TG concentrations <2.26 mmol/L. Potential subjects were excluded if they had a chronic illness, were extremely physically active, or had a family history of premature cardiovascular disease.

The studies in which these participants were enrolled were approved by the Ethics Committee for Clinical Investigations of the Reina Sofía University Hospital.

Study design

We included only young normolipemic Apo E3/E3 males to avoid the possible effects of Apo E isoforms or gender. Eighty-eight healthy Apo E3/E3 males (52 with the -265CC/TC genotype and 36 with the -265TT genotype) were included in this study.

After a 12-h overnight fast, subjects were given a fatty meal enriched with 60,000 IU vitamin A (retinyl palmitate, 15.15 mg RE) per square meter body surface area in the form of capsules (Auxina A masiva, Chiesi Labs). Subjects were given 1 g of fat and 7 mg of cholesterol/kg body weight. The meal contained 60% of its energy in the form of fat (35% SFA, 19% monosaturated fatty acids, 6.3% PUFA), 15% as protein, and 25% as carbohydrates, and was consumed in 20 min. After the meal, the subjects fasted for 11 h but were allowed to drink water. Blood samples were drawn before the meal, every hour until 6 h, and every 2 h and 30 min until 11 h.

Biochemical determinations

DNA amplification and genotyping. Genotyping of the Apo A-II -265T/C (T→C change at amino acid position 265, rs 5082) was carried out by the TaqMan assay (28,29). Each probe consisted of an oligonucleotide with a 5' reporter dye and a 3' quencher. The reporter dyes used were 6 carboxy-fluorescein (FAM) and an internal amplification control (VIC). The 3' quencher was a nonfluorescent quencher (Applied Biosystems). The primer and probe sequences used were as follows: forward primer, 5'-CCAGAGAAATAACTTGGAACTCCTGCTT-3'; reverse primer, 5'-GGTACAGCTCTTAGTGGAGGCTAA-3'; A allele probe, 5'-FAM-

TTGGACTTGAGTGCAACA-3'; and G allele probe, 5'-VIC-CTTGGACTTGAATGCAACA-3'. PCR was performed in a 10- μ L final volume. The reaction mixture contained 5 μ L TAQMAN 2 \times Universal PCR Master mix (Applied Biosystems), 200 nmol/L FAM-labeled probe, 150 nmol/L VIC-labeled probe, 900 nmol/L reverse primer, and 900 nmol/L forward primer (all from Epoch Biosciences), and 2–20 ng genomic DNA. The thermal cycler program included 1 cycle at 50°C for 2 min to activate uracil-N-glycosylase (Trevigen), which was added to prevent carryover contamination; 1 cycle at 95°C for 10 min to activate the AmpliTaq Gold Polymerase (Applied Biosystems); and then 40 cycles at 95°C for 15 s for denaturing and at 62°C for 60 s for annealing/ extending. Allelic discrimination was performed on the post-PCR product. Fluorescence data were collected by a 7900 Sequence Detection system (Applied Biosystems) on the samples for 5 s and analyzed with the use of Sequence Detection system software, version 6.0 (PerkinElmer/ Applied Biosystems), which could be visualized in graph form (28).

Lipoprotein separation. 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 1500 \times g; 15 min at 4°C. The chylomicron fraction of TRL (large TRL) was isolated from 4 mL of plasma overlaid with 0.15 mol/L NaCl, 1 mmol/L EDTA (pH 7.4, $d < 1.006$ kg/L) by a single ultracentrifugation (36,200 \times g; 30 min, 4°C) in a 50-type rotor (Beckman Instruments). Large TRL, contained in the top layer, were removed by aspiration after cutting the tubes and the infranatant was centrifuged at a density of 1.019 kg/L for 24 h at 183,000 \times 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 biochemical determinations were performed.

Lipid analysis. Total cholesterol (Chol) and TG in plasma and lipoprotein fractions were assayed by enzymatic procedures (30,31). Apo A-I and Apo B were determined by turbidimetry (32). HDL cholesterol was measured by analyzing the supernatant obtained following precipitation of a plasma aliquot with dextran sulfate-Mg²⁺, as described by Warnick et al. (33). LDL cholesterol levels were estimated using the Friedewald formula based on the Chol, TG, and HDL cholesterol concentrations (34).

Statistical analysis

Several variables were calculated to characterize the postprandial responses of plasma TG, large TRL, and small TRL to the test meal. The area under the curve (AUC) was defined as the area between the plasma concentration vs. time curve. These areas were calculated by a computer program using the trapezoidal rule. Due to the small number of CC subjects, we stratified the data into the carriers of the mutant allele (CC/TC) vs. the homozygotes for the wild allele (TT). Data were tested for significance between genotypes by a univariate ANOVA test (CC/TC vs. TT) adjusted for age and BMI, and between genotypes and time by ANOVA for repeated measures (adjusted for age and BMI). 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 change 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), which is indicative of the magnitude of the postprandial response in each group of subjects. Post hoc tests (Bonferroni's correction) were performed when the genotype effect was significant. Hardy Weinberg Equilibrium was tested with a goodness-of-fit (chi-squared) based test. $P < 0.05$ was considered significant. All data presented in the text and tables are expressed as means \pm SE unless otherwise specified. SPSS 12.0 for Windows was used for the statistical comparisons.

Results

The lipid variables measured and BMI did not differ between homozygotes for the T allele (TT, $n = 36$) and carriers of the C allele (TC $n = 40$, CC $n = 12$) (Table 1). The sample showed

TABLE 1 Baseline anthropometrics and plasma lipids in healthy young men, according to Apo A-II -265T/C polymorphism¹

Variable	CC/TC	TT	P ²
n	12/40	36	–
Weight, kg	76.4 ± 9.6	78.5 ± 12.3	0.37
Height, cm	173.9 ± 6.0	176.3 ± 6.3	0.07
BMI	25.3 ± 3.1	25.3 ± 4.2	0.95
Chol, mmol/L	3.9 ± 0.6	4.0 ± 0.7	0.41
TG, mmol/L	0.8 ± 0.3	1.0 ± 0.4	0.09
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.2	0.43
LDL cholesterol, mmol/L	2.3 ± 0.5	2.4 ± 0.6	0.48
Apo A-I, mmol/L	1.1 ± 0.2	1.1 ± 0.2	0.79
Apo B, mmol/L	0.6 ± 0.2	0.7 ± 0.2	0.24

¹ Values are means ± SD.

² P-values from univariate ANOVA (using BMI and age as covariates).

equilibrium according to the Hardy Weinberg test (chi-squared value = 0.02; $P = 0.87$).

The fat-loading test raised postprandial plasma TG, large TRL-TG, and small TRL-TG concentrations compared with baseline (all $P < 0.001$). The carriers of the C allele had lower

postprandial increases in plasma total TG ($P = 0.019$; Fig. 1A) and large TRL-TG ($P = 0.013$; Fig. 1B) than homozygotes for the T allele but not in small TRL-TG ($P = 0.936$). Plasma TG differed between groups at 1, 2, 3, 4, 5, and 6 h after the meal and in large TRL-TG at 1, 2, 3, 5, and 6 h (all $P < 0.05$). Plasma Chol and large TRL-Chol were unaffected by the polymorphism, but there was a lower postprandial increase in plasma small TRL-Chol in the CC/TC group than the TT group ($P = 0.003$; Fig. 1C). They differed at every time point from baseline to 11 h ($P < 0.05$). Plasma HDL cholesterol, Apo A-I, Apo B, Apo B48, and Apo B100 did not differ between the CC/TC and TT groups.

When we evaluated the AUC of the individual lipid variables measured, TG were lower in the CC/TC than the TT group, confirming the results of the ANOVA (21.37% of change; $P = 0.014$) (Table 2). The carriers of the minor allele (CC/TC) also had lower AUC for large TRL-TG (24.75% change; $P = 0.017$) and large TRL-Chol (26.63% change; $P = 0.003$) than TT homozygotes. Plasma Chol, HDL cholesterol, Apo A-I, total Apo B, apoB48, and B100 subfractions did not differ between the 2 groups.

Discussion

Our results show that carriers of the minor allele for the Apo A-II -265 T → C polymorphism (CC/TC) display a lesser degree of postprandial lipemia than TT homozygotes, in that the rise in total TG and chylomicron TG is lower in healthy young men with the E3/E3 genotype.

Most clinical studies of the functions of Apo A-II have been conducted on rodents, in which species Apo A-II levels correlate with the amount of fat intake and levels of free fatty acids, glucose, and insulin (4–8), while it has also been observed that Apo A-II-rich HDL stimulate monocyte migration (35). Moreover, mice that overexpress Apo A-II are at higher risk of obesity, have higher levels of TG and free fatty acids, and show changes in glucose tolerance (4,6–8). However, some studies, in which human Apo A-II was given to mice, found no differences in antioxidant power relative to Apo A-I (36,37).

TABLE 2 Postprandial AUC of lipid fractions in plasma of healthy young men, according to Apo A-II -265T/C¹

Measure	CC/TC	TT	P ²
n	12/40	36	–
	<i>min·mmol/L</i>		
TG	913.3 ± 63.4	1161.7 ± 75.5	0.01
Chol	2826.0 ± 61.7	2915.6 ± 73.4	0.35
HDL cholesterol	868.5 ± 26.4	828.2 ± 31.5	0.33
Apo A	678.9 ± 15.7	678.2 ± 18.7	0.98
Apo B	417.4 ± 13.4	449.8 ± 15.9	0.12
Large TRL-TG	355.8 ± 31.2	472.9 ± 36.8	0.02
Large TRL-Chol	161.5 ± 7.7	154.2 ± 9.1	0.54
Small TRL-TG	321.7 ± 26.1	328.2 ± 30.8	0.87
Small TRL-Chol	185.1 ± 14.1	252.3 ± 16.6	0.01
Large TRL-Apo B48	7.3 ± 1.0	6.5 ± 0.9	0.26
Large TRL-Apo B100	10.7 ± 1.3	10.6 ± 1.3	0.96
Small TRL-Apo B48	3.5 ± 0.5	4.0 ± 0.6	0.53
Small TRL-Apo B100	180.0 ± 18.2	151.0 ± 22.5	0.76

¹ Values are means ± SE.

² P-values from univariate ANOVA (using BMI and age as covariates).

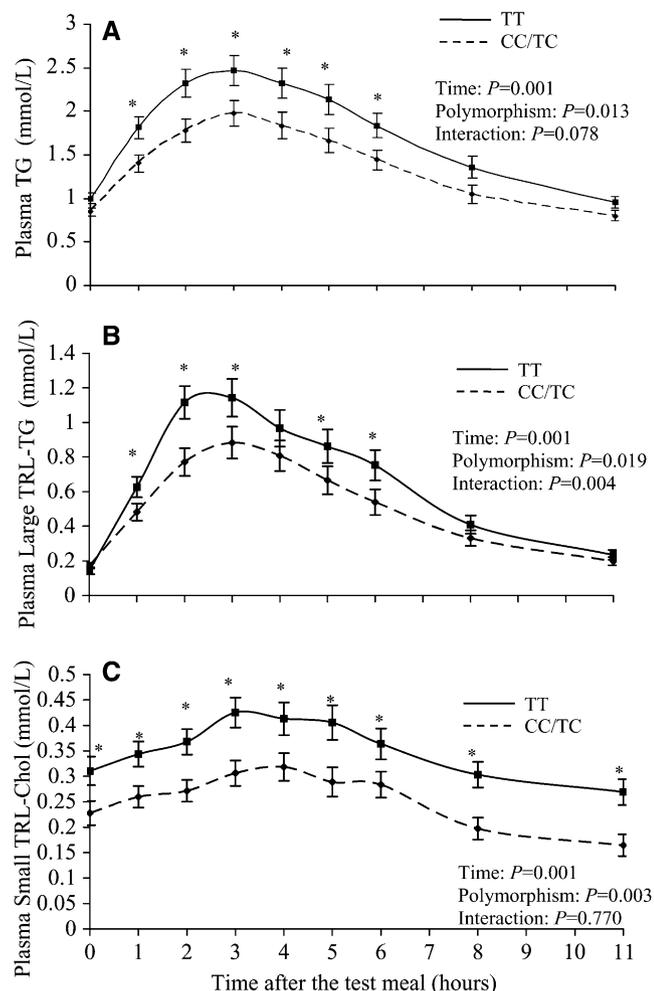


FIGURE 1 Postprandial plasma curves for TG (A), large TRL-TG (B), and small TRL-Chol (C) in healthy young men according to Apo A-II -265T/C polymorphism. Values are means ± SEM, $n = 36$ TT and 52 CC/TC; adjusted for BMI and age.

However, few human studies have been performed. A relationship between Apo A-II levels and TG has been found in families at high risk of cardiovascular disease (38), although the serious deficit in Apo A-II is not related to a change in the lipid profile or cardiovascular risk (39). It is known that values of this apoprotein rise during the postprandial phase as well as after the intake of diets rich in SFA, but their function in human beings is still unknown (40,41). A proatherogenic effect counteracting the effect of Apo A-I on HDL has been suggested on the basis of studies that have shown HDL that contain Apo A-I and A-II have poorer rates of tissue cholesterol clearance than those that contain Apo A-I alone (3,10,42–44). At the biochemical level, Apo A-II has a higher affinity for HDL than Apo A-I due to its greater degree of hydrophobicity (41). A rise in Apo A-II, such as takes place in the postprandial state, produces a partial substitution of HDL Apo A-I by Apo A-II, accelerating its catabolism (41,44). Moreover, the reduction in HDL Apo A-I reduces LCAT activity and the size of HDL particles (45).

Variations in the region of the gene in which the polymorphism we have studied is found (1q23) have previously been related to the prevalence of certain diseases such as diabetes mellitus and combined family hyperlipemia (1,46–48). Although few clinical studies have looked at the effect of this polymorphism on lipid metabolism, Van t' Hooft et al. (12) found a higher clearance rate of large TRL-Apo B100 (at 6 h after ingestion) in carriers of the minor allele (CC/TC), who also had less visceral fat than the TT group. The authors found no differences in TG (neither total plasma nor TRL TG) during the postprandial phase. However, the design of the study could have limited the statistical power, avoiding the detection of differences. In our study, which made a detailed analysis of the postprandial phase, we found lower levels of both total TG and TRL TG. These findings were corroborated both by ANOVA for repeated measures and by the AUC of the total period, as a reduced level of cholesterol in small TRL.

Although we do not know the precise mechanism through which this polymorphism is capable of influencing lipid metabolism, it is known that overexpression of Apo A-II raises plasma fatty acid levels (7,38,49), making its peripheral use difficult, prolonging its stay in the bloodstream, and encouraging its accumulation in fatty tissue (12). The presence of the minor allele (CC/TC) of this polymorphism has also been described as provoking a reduction in the transcription activity of the Apo A-II gene promoter (12), which suggests that carriers of this minor allele produce less Apo A-II in response to its stimulating factors, among which is the postprandial situation.

In summary, we found that a common variant of the Apo A-II gene that had a frequency of occurrence of ~40% in Caucasian series (50) and of 36.36% in our sample influences the postprandial response following a meal rich in saturated fats and may be responsible for a phenotype that carries a lower risk of cardiovascular disease, as corresponds to the lower level of postprandial hypertriglyceridemia.

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