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A single nucleotide polymorphism of the apolipoprotein A–V gene −1131T > C modulates postprandial lipoprotein metabolism

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Abstract

The Apolipoprotein A–V (apoA–V) gene promoter polymorphism −1131T > C modulates triacylglycerol (TG) concentrations. We evaluate whether this polymorphism could be involved in the interindividual variability observed during postprandial lipemia.

Fifty-one healthy apo E3E3 male volunteers [12 with −1131CC/CT genotype, and 39 with −1131TT genotype] underwent a Vitamin A fat-load test consisting of 1 g of fat/kg body weight and 60,000 IU of Vitamin A. Blood samples were taken at time 0 and every hour until the 6th and every 2 h and 30 min until the 11th. Cholesterol (Chol) and TG were determined in plasma and Chol, TG, ApoB-100, ApoB-48, and retinyl palmitate (RP) were determined in lipoprotein fractions.

Data of postprandial lipemia revealed that subjects with the −1131CT/CC genotype had a higher postprandial response of total plasma TG ($p = 0.043$), large triacylglycerol-rich lipoproteins-TG (TRL-TG) ($p = 0.002$), large TRL-Chol ($p = 0.004$), small TRL-Chol ($p = 0.004$) and small TRL-RP ($p = 0.001$) than subjects with the −1131TT genotype.

The modifications observed in postprandial lipoprotein metabolism in subjects with the apoA–V −1131T > C polymorphism could be involved in the increased fasting plasma TG concentrations previously described in carriers of the C allele.

Keywords: ApoA–V; Postprandial lipemia; Triacylglycerol; ApoA–V −1131T > C polymorphism

1. Introduction

Subjects in western societies, by eating regular fat-rich meals, are predominantly in a postprandial state throughout the day. In these subjects, the fed state and its effects on lipoprotein metabolism, may be more representative of their physiological status than the fasting state. Since 1979 when Zilversmit proposed the important role of triacylglycerol-rich lipoproteins (TRL) in the development of atherosclerosis, both coronary artery disease and myocardial infarction have been associated with abnormal postprandial lipoprotein patterns [1]. The basic mechanisms involved during postprandial lipemia are relatively well known and the effects of different nutrients on the variability of the postprandial response are under active investigation. Less is known, however, about the dramatic interindividual variability observed during this period [2]. Thus, several studies have demonstrated that the presence of polymorphisms located in the AI–CIII–AIV complex and in other gene loci determine variation in the postprandial response [3–5].

Recently, a gene coding for apolipoprotein A–V (apoA–V) has been identified in the vicinity of AI–CIII–AIV cluster on human chromosome 11 [6]. Studies in knockout and trans-

Abbreviations: apoA–V, apolipoprotein A–V; TG, triacylglycerol; Chol, cholesterol; RP, retinyl palmitate; TRL, triacylglycerol-rich lipoproteins; CAD, coronary artery disease; BMI, body mass index; large-TRL, chylomicron fraction of TRL; small-TRL, non-chylomicron fraction of TRL; SDS, sodium dodecyl sulphate; AUC, area under the curve

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genic mice revealed that its expression pattern correlates negatively with plasma triacylglycerol (TG) concentrations. In apoA–V-knockout mice, TG increased four-fold [7,8] and expression of the human A–V genetic sequence in transgenic mice decreased serum TG concentrations by 50–70% [9]. These observations were verified in healthy subjects and in patients with familial combined hyperlipidemia [10]. Fascinatingly, this decrease in serum TG concentrations was associated with both diminished VLDL production and increased VLDL catabolism [11]. A polymorphism (−1131 T>C) in the apoA–V gene promoter region have been described [6]. This polymorphism was shown to vary significantly in allele frequency among ethnic groups. Thus, the frequency of the less common C allele is much higher in Japanese and Singaporean populations, than in whites [12,13]. Carriers of the −1131C allele have higher fasting [6,14–16] and postprandial [17] plasma TG concentrations, lower LDL particle size [18] and an increased risk of developing dyslipidemia [19] and coronary artery disease [20] in different racial groups. Furthermore, in a previous study the −1131 T>C polymorphism determined the TRL metabolism during the postprandial period in Korean men [17]. However, no other studies have confirmed this finding, and no similar studies were represented in Caucasian, supporting the need for more extensive studies.

There are differences in postprandial lipemic response between populations, probably related with different genetic background [21]. Thus, the purpose of this study was to determine whether this (−1131 T>C) polymorphism could modulate the postprandial response of TRL in young normolipemic Caucasian males, in order to explain the higher risk of coronary artery disease (CAD) associated to the −1131C allele.

2. Methods

2.1. Human subjects

We wanted to include only young normolipemic E3/E3 males in order to avoid the variable postprandial lipid response of other apoE isoforms or gender. Therefore, only 51 healthy apo E3/E3 males (12 with the −1131CC/CT genotype and 39 with the −1131TT genotype) were selected among over 100 volunteers. All subjects were students at the University of Cordoba and all responded to an advertisement. They ranged from 18 to 49 years of age. None of them had diabetes and none had liver, renal or thyroid disease. All subjects were selected to have the apo E 3/3 genotype to avoid allele effects of this gene locus on postprandial lipemia [22]. None of the subjects was taking medication or vitamins known to affect plasma lipids. Fasting plasma lipids, lipoproteins, apolipoproteins, age and body mass index (BMI) are shown in Table 1. All studies were carried out in the Research Unit at the Reina Sofia University Hospital. The experimental protocol was approved by the Human Investigation Review Committee at the Reina Sofia University Hospital.

Table 1
Baseline characteristics of plasma lipids and apolipoproteins according to the apoA–V gene promoter (−1131T/C) polymorphism

	TT ($n=39$)	TC/CC ($n=12$)	p[a]
Age (years)	22.9 ± 5.9	22.4 ± 2.3	0.76
BMI (kg/m^2)	24.7 ± 3.4	27.0 ± 3.9	0.06
Total Chol (mg/dL)	151.5 ± 24.0	162.6 ± 16.5	0.15
Total TG (mg/dL)	81.6 ± 36.4	86.9 ± 28.9	0.65
LDL-Chol (mg/dL)	90.9 ± 22.0	103.7 ± 20.4	0.10
HDL-Chol (mg/dL)	46.5 ± 10.9	46.0 ± 12.0	0.89
ApoB (g/L)	0.65 ± 0.18	0.70 ± 0.20	0.42
ApoA–I (g/L)	0.97 ± 0.17	0.97 ± 0.16	0.98

Values are given as mean ± S.D. BMI was used as a covariable.
[a] ANOVA.

2.2. 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. The amount of fat given was 1 g of fat and 7 mg of cholesterol per kg of body weight. The meal contained 65% of energy as fat, 15% protein and 25% carbohydrates and was eaten in 20 min. After the meal, the subjects ate 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 h and 30 min until the 11th hour.

2.3. 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 × 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, 1 mmol/L EDTA (pH 7.4, $d<1.006$ kg/L) by a single ultracentrifugal spin (20,000 rpm, 30 min, 4 °C) in a 50-type rotor (Beckman Instruments, Fullerton, CA). 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 45,000 rpm in the same rotor. The non-chylomicron fraction of TRL (also referred 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).

2.4. Lipid analysis

Cholesterol (Chol) and TG in plasma and lipoprotein fractions were assayed by enzymatic procedures [23,24]. ApoA-I and ApoB were determined by turbidimetry [25]. HDL (high density lipoprotein) Chol was measured by analyzing the supernatant obtained following precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}, as described by Warnick

et al. [26]. LDL-Chol was obtained as the difference between the Chol from the bottom part of the tube after ultracentrifugation at 1.019 kg/L [27].

2.5. RP assay

The RP content of large and small TRL fractions was assayed using a method previously described [28]. 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 [29].

2.6. 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 [30]. 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.

2.7. DNA amplification and genotyping

Genotyping for the apoA–V −1131 T > C was carried out using the protocol reported previously [6].

2.8. 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 versus time curve and a line drawn parallel to the horizontal axis through the 0 h concentration. These areas were calculated by a computer program using the trapezoidal rule. TG plasma concentrations were log transformed before statistical analyses. 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 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. BMI was used as a covariable in ANOVA for repeated measures analysis. A probability value less than 0.05 was considered significant. All data presented in the text and tables are expressed as mean ± S.D.s. SPSS 7.5 for WINDOWS (SPSS Inc., Chicago) was used for the statistical comparisons.

3. Results

Baseline characteristics of the subjects are shown in Table 1. No significant differences were observed for any of the lipid parameters studied and BMI between homozygotes for the T allele (TT, $n = 39$) and carriers for the C allele (TC and CC, $n = 12$) of the −1131 T > C apoA–V gene promoter region polymorphism at baseline. Since there was a trend toward higher BMI, we used BMI as a covariable in all statistical analyses.

The postprandial response of plasma TG, small TRL-RP, large TRL-TG and large and small TRL-Chol, is shown in Figs. 1 and 2. The fat-loading test significantly increased the large TRL-TG ($p = 0.013$), small TRL-RP ($p = 0.043$) and small TRL-Chol ($p = 0.018$) with respect to baseline concentrations, indicating an increase of these parameters

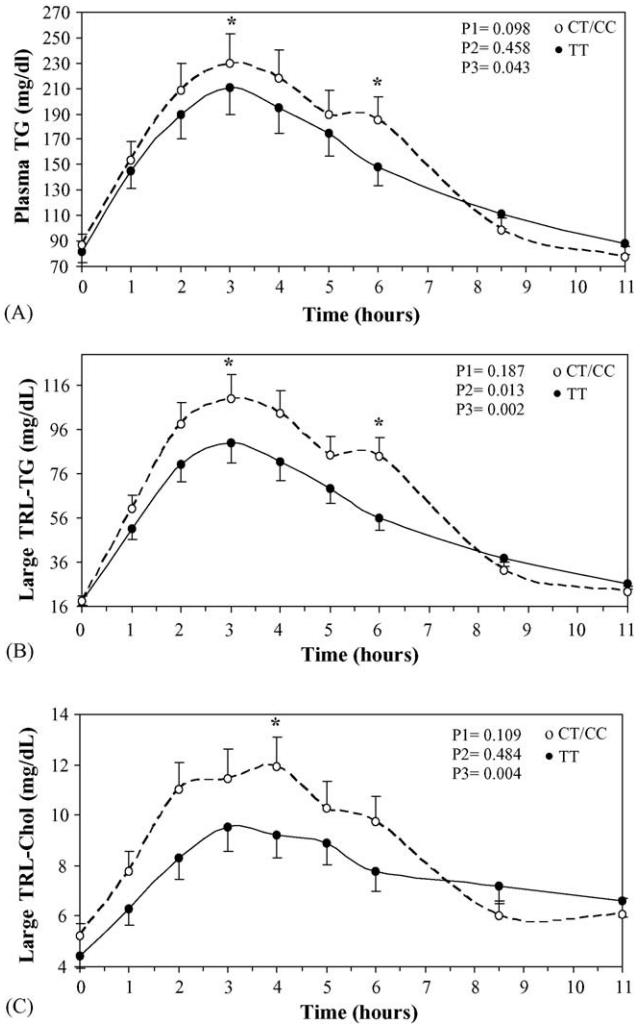

Fig. 1. Line plots of postprandial plasma TG (A) large TRL-TG (B) and large TRL-C (C) response in TT ($n = 39$, continuous line) and carriers of the C allele (TC and CC, $n = 12$, discontinuous line). P1: genotype effect; P2: time effect; P3: genotype by time interaction. ANOVA for repeated measures. $^{*}p < 0.05$ using Tukey's post hoc test. BMI was used as a covariable in ANOVA for repeated measures analysis.

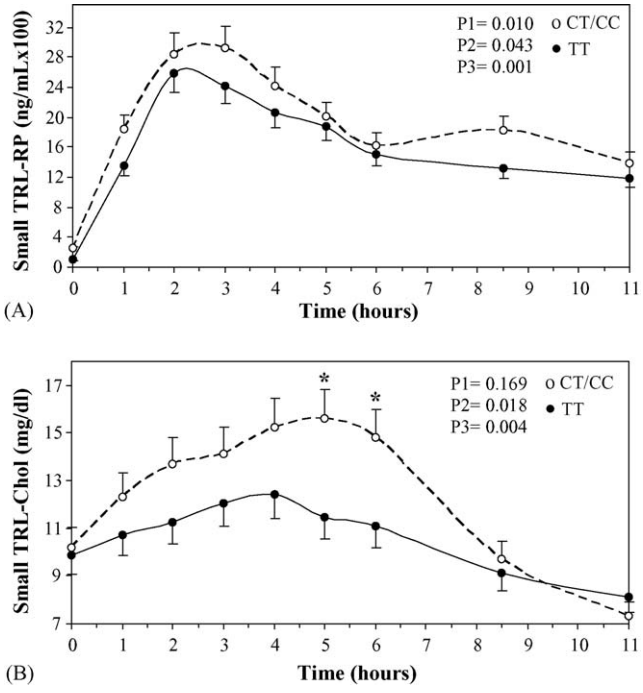

Fig. 2. Line plots of postprandial small TRL-RP (A) and small TRL-C (B) response in TT ($n=39$, continuous line) and carriers of the C allele (TC and CC, $n=12$, discontinuous line). P1: genotype effect; P2: time effect; P3: genotype by time interaction. ANOVA for repeated measures. $^{*}p<0.05$ using Tukey's post hoc test. BMI was used as a covariable in ANOVA for repeated measures analysis.

in the different groups of subjects during the postprandial period. Analysis of the interaction between genotype and time showed that carriers for the C allele have a higher postprandial response of total plasma TG ($p=0.043$), large TRL-Chol ($p=0.004$) and large TRL-TG ($p=0.002$) than subjects homozygous for the T allele. In addition, subjects carriers of the C allele have a higher postprandial response of small TRL-RP ($p=0.001$) and small TRL-Chol ($p=0.004$), than homozygous for the T allele. Furthermore, the area under the curve (AUC) of ApoB-100 ($p=0.02$) in small TRL, was greater in carriers of the C allele when compared to homozygotes for the T allele (Table 2).

No significant differences were observed in the other variables analyzed in our study.

4. Discussion

Our results reveal that carriers of the C allele for the apoA–V gene promoter (−1131 T > C) polymorphism show a higher postprandial response of plasma TG concentrations, RP, ApoB-100 and Chol in small TRL, as well as TG and Chol in large TRL.

Several studies have demonstrated that the presence of polymorphisms in the AI–CIII–AIV cluster and in other gene loci determine the variability of the postprandial lipoprotein response [4,31]. Recently, a gene coding for apoA–V was identified in this cluster. The apoA–V is emerging as a main candidate gene for modulating TG metabolism in humans [6].

Table 2
Increment of the area under the curve according to the (−1131 T/C) apoA–V promoter polymorphism

	TT ($n=39$)	TC/CC ($n=12$)	p
Large TRL-RP (mg/dL h)	40474 ± 32032	53291 ± 52241	0.33
Small TRL-RP (mg/dL h)	13503 ± 10339	31834 ± 28330	0.08
Plasma TG (mg/dL h)	638 ± 379	915 ± 416	0.04
Large TRL-TG (mg/dL h)	395 ± 240	678 ± 231	0.01
Small TRL-TG (mg/dL h)	81 ± 117	116 ± 20	0.41
Large TRL-Chol (mg/dL h)	20.98 ± 24.25	43.78 ± 34.01	0.03
Small TRL-Chol (mg/dL h)	6.69 ± 19.08	27.28 ± 21.37	0.02
ApoB (g/dL h)	7.71 ± 14.40	24.38 ± 33.05	0.82
LDL Chol (mg/dL h)	−36 ± 99	84 ± 55	0.17
HDL Chol (mg/dL h)	−13.88 ± 37.11	5.22 ± 39.22	0.15
Large TRL ApoB-100 (AU)	9.95 ± 9.85	20.83 ± 29.88	0.12
Small TRL ApoB-100 (AU)	144 ± 291	670 ± 930	0.02
Large TRL ApoB-48 (AU)	8.36 ± 9.44	3.37 ± 2.72	0.25
Small TRL ApoB-48 (AU)	6.22 ± 8.86	3.04 ± 4.47	0.44

TRL: triacylglycerol-rich lipoproteins; RP: retinyl palmitate; AU: arbitrary units. BMI was used as a covariable.

In our results, carriers of the −1131C allele polymorphism were associated with higher postprandial TG levels. Paradoxically, in the study of Masana et al. [32], these subjects showed a lower postprandial TG excursion reflected by a lower TG-AUC. However, their results cannot be considered conclusive because the subjects did not receive a standardised diet with the same amount of fat per kg body weight. This may have led to differences in the amount of fat ingested by the different groups. Moreover, the authors did not characterize the lipoproteins involved in their findings. Our study, in which a standard amount of fat was given to all subjects, showed that carriers of the C allele present a higher postprandial TG-AUC.

Our data confirm and extend the recent publication of Jang et al. [17], who showed that the −1131 T > C polymorphism determined the TRL metabolism during the postprandial period, in Korean men. Thus, our study show a markedly higher postprandial responses in both large and small TRL for the −1131C allele. Many research teams have shown the important role of these postprandial particles in the development of CHD [33]. This fact could be related with the higher risk of CAD associated to the −1131C allele [20].

There are several mechanisms to explain why this polymorphism may be responsible for our findings. The apoA–V −1131 T > C polymorphism was found to be associated with significant differences in ApoA–V plasma concentration. Thus, the presence of the C allele was associated with lower plasma ApoA–V concentration [34]. In a previous study, the importance of ApoA–V plasma levels on TG metabolism was shown. TG concentrations increased dramatically with the suppression of apoA–V expression, while overexpression of this apoprotein had the opposite effect [6]. In addition, VLDL that lack ApoA–V do not bind as well as normal or ApoA–V enriched VLDL to the LDL receptor and this may explain the

slower small TRL removal observed in mice lacking apoA–V [35]. The higher levels of plasma ApoA–V could be related with an enhanced LPL activity, increasing the conversion of TRL and stimulating remnant formation and hepatic clearance. This phenomenon could explain the higher postprandial response observed in carriers of the C allele. Thus, ApoA–V induced both an increase in lipolysis, through the increase in the LPL activity, and an elevated removal of VLDL particles [36]. Nevertheless, the level of ApoA–V is low. This raises the question of whether ApoA–V could really have any influence on the turnover of the VLDL pool lipid metabolism. However, genetic analysis of polymorphisms in the human apoA–V gene demonstrated a strong relationship between the genetics of apoA–V and plasma TG concentration [16]. In the hepatocyte, ApoA–V is involved in modifying the lipidation of ApoB-100, thus influencing the assembly of VLDL. An intracellular role for ApoA–V would explain its large effect on plasma TG levels despite its low abundance in plasma [37]. We have not observed significant differences in RP or ApoB-48 in intestinal TRL, also called large-TRL. RP is a marker of the TRL of intestinal origin and ApoB indicates the particle number. Thus, our results show that the effect of ApoA–V on LPL decreases the hydrolysis of TG in intestinal TRL without changes the number of these particles. However, ApoA–V modifies the number of particles of hepatic origin, because we have observed changes in ApoB-100 in the small-TRL fraction, which also contains large VLDL. This hypothesis is in agreement with the hepatic role of ApoA–V, since ApoA–V stimulates the hepatic secretion of VLDL particles, increasing the plasma number of these particles.

In conclusion, the modifications observed in postprandial lipoprotein metabolism in subjects with apoA–V −1131 T > C polymorphism could be involved in the higher risk of coronary artery disease observed in carriers of the −1131C allele.

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