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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