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The −250G/A polymorphism in the hepatic lipase gene promoter influences the postprandial lipemic response in healthy men

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Abstract *Background and aim:* The −250G/A promoter polymorphism of the hepatic lipase gene has been associated with changes in the activity of the enzyme. We investigated whether this polymorphism modifies the postprandial response of triacylglycerol-rich lipoproteins (TRL) in young normolipemic males.
Methods and results: Fifty-one healthy apolipoprotein (apo) E3/E3 male volunteers (30 G/G and 21 carriers of the A allele) underwent a vitamin A fat-loading test and blood samples were drawn every hour until the 6th, and every 2 h and 30 min until the 11th. Total plasma cholesterol and triacylglycerols (TG), as well as cholesterol, TG and retinyl palmitate (RP) in TRL, isolated by ultracentrifugation, were determined.

Carriers of the A allele showed a higher response ($P = 0.008$), a higher area under the curve (AUC; $P = 0.022$) and a lower RP peak time ($P = 0.029$) in small TRL during the postprandial response, as well as a lower peak time in total plasma TG levels ($P = 0.034$) and large TRL-TG ($P = 0.033$) than subjects who were homozygous for the G allele.
Conclusion: Our data indicate that the presence of the A allele in the −250G/A promoter polymorphism of the hepatic lipase gene is associated with a higher postprandial lipemic response.

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Introduction

Human hepatic lipase (HL) is a lipolytic enzyme synthesized in the liver [1–3], which is secreted from hepatocytes and bound to the hepatic sinusoidal endothelial surface. It plays an important role in the metabolism of high-density lipoprotein (HDL), and transforms large triacylglycerol-rich HDL_2 into small, dense HDL_3 [4]. HL also acts as a ligand for lipoproteins during the uptake of these by hepatic cell-surface receptors [5–7], and is involved in the reverse transport of cholesterol [8,9]. Furthermore, this enzyme takes part in the formation of small, dense low-density lipoprotein (LDL) particles [10]. Therefore, HL activity may be involved in cardiovascular disease (CVD) because of its essential functions in the metabolism of lipoproteins.

HL activity appears to be associated with several different factors, including intra-abdominal fat [11], ethnic background [12,13], sex-steroid hormones [14], age [15,16] and various hepatic lipase gene promoter polymorphisms [12,17]. The 5′ flanking region of the HL gene contains four polymorphic sites: G-250A, C-514T, T-710C and A-763G [17], and all of them are in almost complete linkage disequilibrium. The frequency of rare alleles varies from 0.15 to 0.29 in white populations, 0.45 to 0.53 in African-Americans, and 0.47 in Japanese-Americans. The presence of these alleles have been associated with low HL activity and with high TG levels [18,19], high HDL cholesterol (HDL-C) levels [13,19,20], buoyant LDL particles [13], and coronary heart disease [20].

Postprandial lipemia is characterized by an increase in triacylglycerol-rich lipoproteins (TRL) (chylomicrons, very low density lipoproteins (VLDL) and their remnants). Several studies have demonstrated that the inhibition of HL activity produces an impairment of the uptake of chylomicron remnants by the liver [21,22]. The −514C/T variant in the HL gene promoter has been found to influence fasting and postprandial lipoprotein containing both apo C-III and apo B (LpC-III:B) levels in the European Atherosclerosis Research Study II (EARSII) population in such a way that carriers of the −514T had higher levels of apo CIII:B [19]. However, our group has demonstrated that the T allele of the −514C/T polymorphism in the promoter region of the hepatic lipase gene is associated with a lower postprandial lipemic response [23] in young normolipemic males. This last results contrasts with the existing knowledge that the T allele is associated with lower levels of hepatic lipase activity [13] and with the effects of this enzyme on TRL metabolism. Although a linkage disequilibrium exists between the −514C/T and −250G/A polymorphisms, in the present study we observed that this linkage is not complete since some subjects with the common −250G allele had the rare −514T haplotype (30 subjects with the −250G/G polymorphism, of which 23 were C/C and seven were carriers of the T allele of the −514C/T polymorphism). For this reason, we wanted to investigate the effect of the −250G/A promoter polymorphism of the hepatic lipase gene on the postprandial response of TRL in the same population used in our previous study [23].

Methods

Population

Fifty-one healthy male students were included in this study. Thirty were homozygous for the most common allele (G/G) and 21 were carriers of the A allele (18 GA and 3 AA). Informed consent was obtained from all participants. Volunteers showed no evidence of any chronic disease (hepatic, renal, thyroid or cardiac dysfunction). All volunteers were selected to have the apo E3/E3 genotype in order to avoid the allele effects of this gene locus on postprandial lipemia [24]. None of the subjects was taking medication or vitamins known to affect plasma lipids. The 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 hospital's Human Investigation Review Committee.

Table 1 Plasma lipids and apolipoproteins according to the −250G/A HL promoter polymorphism

	GG (30)	GA/AA (21)	*P*
Age (years)	22.5 ± 3.3	21.0 ± 1.9	0.060
BMI (kg/m^2)	25.0 ± 2.9	25.6 ± 4.4	0.580
C (mmol/L)	4.0 ± 0.65	3.9 ± 0.6	0.479
LDL-C (mmol/L)	2.5 ± 0.6	2.4 ± 0.6	0.375
HDL-C (mmol/L)	1.20 ± 0.3	1.22 ± 0.3	0.573
TGs (mmol/L)	0.90 ± 0.3	0.99 ± 0.4	0.408
Apo A-I (g/L)	0.97 ± 0.2	0.98 ± 0.2	0.874
Apo B (g/L)	0.69 ± 0.2	0.64 ± 0.1	0.271

Values are given as mean ± SD. BMI, body mass index; C, cholesterol; TGs, triacylglycerols; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol *P*: ANOVA. There were no significant differences between the two groups.

Vitamin A fat-loading test

After a 12-h fast, volunteers were given a fatty meal enriched with 60,000 U of vitamin A/m^2 of body surface area. The fatty meal consisted of two cups of whole milk, eggs, bread, bacon, cream, walnuts and butter. The meal provided 1 g of fat per kg of body weight and 7 mg of cholesterol per kg of body weight. It contained 65% of energy as fat, 15% as protein and 25% as carbohydrates, and was eaten in 20 min. After the meal, the volunteers consumed no energy for 11 h but were allowed to drink water. Blood samples were drawn before the meal and every hour until the 6th and every 2 h and 30 min until the 11th.

Lipoprotein separations

Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) to give a final concentration of 0.1%. Plasma was separated from red blood cells by centrifugation at 1500 × *g* for 15 min at 4 °C. The large TRL (Sf (flotation rate) > 400) were isolated from 4 ml of plasma over-layered with 0.15 mol/L NaCl and 1 mmol/L EDTA (pH 7.4, density < 1.006 kg/L) by a single ultracentrifugal spin (20,000 rpm, 30 min, 4 °C) in a type 50 rotor (Beckman Instruments, Fullerton, CA, USA). Large TRL, which were contained in the top layer, were removed by aspiration and the infranatant fluid was centrifuged at a density of 1.019 kg/L for 24 h at 45,000 rpm in the same rotor. The small TRL (Sf 12−400) were 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 RP.

Lipid analysis

Cholesterol and TG in plasma and lipoprotein fractions were assayed by enzymatic procedures [25,26], apo A-I and apo B were determined by turbidimetry [27], and HDL-C was measured by analyzing the supernatant fluid obtained after precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}, as described by Warnick et al. [28]. LDL cholesterol (LDL-C) was obtained as the difference between the HDL-C and the cholesterol from the bottom part of the tube after ultracentrifugation (45,000 rpm, 24 h, 4 °C) at a density of 1.019 kg/L.

Retinyl palmitate assay

The RP content of large and small TRL fractions was assayed using a method described previously [29]. Briefly, different volumes of the various fractions (100 μl for large TRL and 100−500 μl for small TRL) were placed in 13 × 100-mm glass tubes. The total volume in each tube was adjusted to 500 μl using normal saline. Retinyl acetate (40 ng in 200 μl of mobile phase buffer) was added to each tube as an internal standard, then 500 μl of methanol was added followed by 500 μl of the mobile phase buffer, for a total volume of 1.7 ml. The mobile phase buffer was prepared fresh daily by combining 90 ml of hexane, 15 ml *n*-butyl chloride, 5 ml acetonitrile, and 0.01 ml acetic acid (82:13:5 by volume with 0.01 ml of acetic acid). The tubes were mixed thoroughly after each step. The final mixture was centrifuged at 350 × *g* for 15 min at room temperature, and the upper layer was then carefully removed by aspiration and placed into individual autosampler vials. The autoinjector was programmed to deliver 100 μl per 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, USA). 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 retention time with a purified standard (Sigma, St Louis, MO, USA). 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 [30]. 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 et al. [31]. In brief, samples containing isolated lipoprotein fractions were delipidated in a methanol/diethyl ether solvent system. 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, USA) 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 of apo

B-100 was applied to four 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 at least 30 min and stained in 0.2% Coomassie G-250/40% methanol/10% acetic acid for at least 4 h. Destaining was performed in 12% methanol/7% acetic acid with four changes of destaining solution for 24 h. 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 apo B-48 and 5.1% for apo B-100.

DNA amplification and genotyping

DNA was extracted from 10 ml of EDTA-containing blood. The genotype at position −250 of the HL gene was done by polymerase chain reaction (PCR) with the use of the primer pairs (P1; 5′-CCT ACCCCGACCTTTGGCAG-3′, and P2, 5′-GGGGTC CAGGCTTTCTTGG-3′). Amplification was carried out in a 10 μl reaction. DNA was denatured at 94 °C for 2 min followed by 35 cycles of denaturation at 92 °C for 15 s, annealing at 64 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. The PCR product (5 μl) was digested with 10 U restriction enzyme *Dra*I (BRL, MD, USA) in a total volume of 35 μl. Digested DNA was separated by electrophoresis on an 8% non-denaturing 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 bp of the apo E gene was done by PCR with 250 ng of genomic DNA and 0.2 mmol each of 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. The 20 μl of PCR product was digested with 10 U restriction enzyme *Cfo*I (BRL, MD, USA) in a total volume of 35 μl. Digested DNA was separated by electrophoresis on an 8% non-denaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

Statistical analysis

Several variables were calculated to characterize the postprandial responses of lipid parameters to the test meal. The 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. Another variable included the peak time, which was the average of the highest peak concentration time 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 time alone or the change in the variable after ingesting fatty food over the entire lipemic period (represented as P1); the effect of genotype (represented as P2), was analyzed independently of the time in the postprandial study. We also studied 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 with a different genotype (represented as P3). When statistical significance was found, Tukey's post hoc comparison test was used to identify group differences. A probability value of less than 0.05 was considered significant. Stepwise multiple regression analyses were carried out using the AUC of the small TRL-RP and total plasma TG levels peak times as dependent variables, and HL genotypes, age, BMI, and total TG, total cholesterol and HDL-C at baseline as independent variables. Discrete variables were divided into classes for analysis. All data presented in the text and tables are expressed as mean ± SD. SSPS 8 for Windows (SSPS Inc., Chicago, IL, USA) was used for the statistical comparisons.

Results

The baseline characteristics of the volunteers are shown in Table 1. No significant differences were found at baseline for any of the lipid parameters studied between volunteers carriers of the A allele ($n = 21$) and those homozygous for the G allele ($n = 30$).

Postprandial responses of RP in the different lipoprotein fractions are shown in Fig. 1. The intake of fatty food increased large TRL-RP ($P = 0.001$) and small TRL-RP ($P = 0.001$), indicating an increase in these parameters in the different groups of volunteers during the postprandial period. When we measured the interaction between genotype and time, we observed that carriers of the A allele showed a higher postprandial response in small

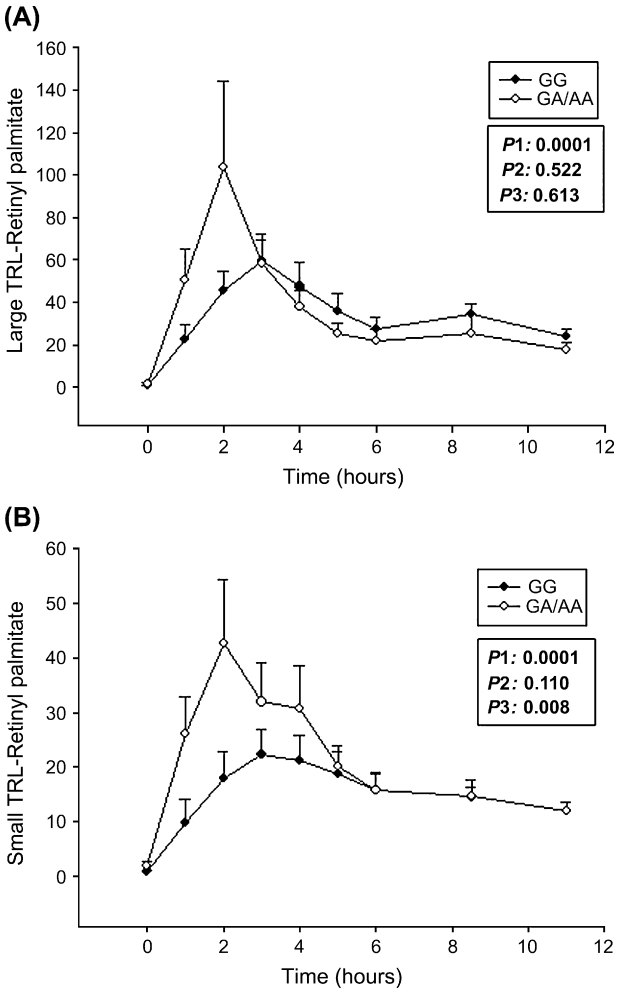

Figure 1 Line plots of postprandial large TRL-RP (A) and small TRL-RP (B) response in GG ($n = 30$, continuous line, black diamonds) and GA/AA volunteers ($n = 21$, discontinuous line, black squares). *P*1: time effect; *P*2: genotype effect; *P*3: genotype by time interaction. ANOVA for repeated measures.

TRL-RP ($P = 0.008$) than those homozygous for the G allele (Fig. 1B). The AUC of the small TRL-RP ($P = 0.022$) was greater in the volunteers that were carriers of the A allele than in those homozygous for the G allele (Table 2). No significant effects of genotype were observed in terms of the AUC for the rest of the analyzed parameters.

The peak time for different lipid parameters was measured. Volunteer carriers of the A allele showed a lower peak time in total plasma TG levels ($P = 0.034$), large TRL-TG ($P = 0.033$) and small TRL-RP ($P = 0.029$) than volunteers that were homozygous for the G allele (Table 3). No significant effect of genotype was observed when the remaining lipid parameters were studied.

Multiple regression analysis (Table 4) justified 48% of the variability in the AUC of the small TRL-RP and 11.5% of the peak time for total plasma TG levels. The −250G/A polymorphism in the promoter region of the HL gene was a significant predictor of the AUC for the small TRL-RP ($P = 0.001$) and total plasma TG peak time ($P = 0.047$) in our study population.

Discussion

Our results show that healthy male carriers of the A allele in the −250G/A polymorphism of the HL gene had a lower peak time, a greater AUC and a higher response of RP in small TRL, as well as a lower peak time in total plasma TG levels and large TRL-TG than volunteers homozygous for the G allele, after consumption of a fatty meal.

In the present study no significant associations were found between the −250G/A polymorphism and plasma HDL-C levels. A possible explanation is that the subjects included in our study were young, healthy, and normolipidemic. In this population, the effect of the isolated polymorphism could be cushioned by multiple genes that regulate HDL. In support of this theory, Ko et al. [32] only found significant associations between the −514C/T and −250G/A polymorphisms of the HL gene promoter and HDL-C levels in obese men. HL has also been associated with apo B levels [33]. However, Carlson et al. [34] did not show an increase in apo B with a deficiency in HL activity; similarly, we failed to demonstrate an association between apo B levels and the −250G/A polymorphism.

The postprandial state is associated with endothelial dysfunction during fat ingestion [35] and much attention has been paid to postprandial lipid levels as independent cardiovascular risk factors [36]. Many factors such as age, BMI, smoking and alcohol consumption are likely to account for the individual variability observed in postprandial metabolism. Furthermore, the influence of several genetic factors, such as the genetic variants in the different apolipoproteins, also modifies postprandial lipid metabolism [37−39]. Several studies have demonstrated the direct atherogenicity of TRL and their remnants [40,41]. Indeed, high remnant lipoprotein levels are associated with the presence, severity and progression of atherosclerosis [42], and these particles have been identified as independent predictors of future coronary events in patients with coronary artery disease [43]. Because HL is involved in the metabolism of postprandial particles, it may play an anti-atherogenic role. The influence of this enzyme on postprandial lipids could be direct or indirect. HL is

Table 2 Area under the curve in volunteers according to the −250G/A HL promoter polymorphism

	GG (30)	GA/AA (21)	*P*
Total cholesterol (nmol s/L)	0.75 ± 0.1	0.71 ± 0.1	0.380
Triacylglycerols (nmol s/L)	0.32 ± 0.1	0.31 ± 0.1	0.834
LDL cholesterol (nmol s/L)	0.44 ± 0.15	0.40 ± 0.09	0.401
HDL cholesterol (nmol s/L)	0.20 ± 0.05	0.22 ± 0.05	0.309
Apo A-1 (g/L s)	0.17 ± 0.03	0.17 ± 0.02	0.877
Apo B (g/L s)	0.13 ± 0.04	0.11 ± 0.03	0.216
Large TRL-C (nmol/s L)	0.036 ± 0.013	0.037 ± 0.014	0.839
Small TRL-C (nmol/s L)	0.054 ± 0.028	0.045 ± 0.023	0.233
Large TRL-TG (nmol/s L)	0.13 ± 0.084	0.12 ± 0.055	0.621
Small TRL-TG (nmol s/L)	0.09 ± 0.05	0.08 ± 0.04	0.284
Large TRL-RP (ng/ml s)	7.3 ± 7.7	9.0 ± 6.6	0.446
Small TRL-RP (ng/ml s)	2.5 ± 1.7	4.0 ± 2.9[a]	0.022
Large TRL apo B48 (A.U.)	431 ± 355	337 ± 226	0.407
Small TRL apo B48 (A.U.)	327 ± 488	432 ± 372	0.517
Large TRL apo B100 (A.U.)	727 ± 605	624 ± 658	0.672
Small TRL apo B100 (A.U.)	27600 ± 18356	22446 ± 18007	0.433

TRL, triacyglycerol rich lipoproteins; C, cholesterol; TG, triacylglycerol; RP, retinyl palmitate; A.U., arbitrary units. *P*: ANOVA for repeated measures.
[a] $P < 0.05$ vs. GG.

involved in the hydrolysis of phospholipids and the TG of chylomicron remnants. Hydrolysis of chylomicron remnant phospholipids by HL leads to the unmasking of apo E, and thereby enhances binding to apo E-binding receptors [44]. Moreover, HL may also act as a ligand for the binding of chylomicron remnants in the liver [5]. Several studies have showed that a genetic deficiency of HL [45] or inhibition of HL activity [21] is associated with impaired clearance of lipoprotein remnants. The −250G/A, −514C/T, −710T/C and −763A/G polymorphisms in the promoter region of the HL gene account for approximately 20−32% of the variance of HL activity [13]. It has generally been assumed that this association is due to an effect of ≥1 of the promoter polymorphisms on the rate of transcription of the HL gene. In the present study we showed that carriers of the A allele in the −250G/A polymorphism had a higher postprandial response in small TRL-RP. RP is a TRL marker of intestinal origin that remains linked to these particles until being taken up by liver. Therefore, this data suggests that in metabolic conditions in which HL activity is decreased, lipoprotein particles tend to accumulate. The enhancement of hepatic uptake of apo B-containing lipoproteins has been observed to be independent of lipolytic activity and did not require apo E [5,46,47]. In this respect, we observed that after the intake of fatty food postprandial total plasma TG concentrations were not significantly different between carriers of the A allele and those homozygous for the G allele. Furthermore, in carriers of the A allele the lower peak time in total plasma TG levels, large TRL-TG and small TRL-RP demonstrated a premature increase in these parameters during the postprandial state. A possible explanation for these findings could be that the lipoprotein remnants may induce new HL synthesis [48]. Although the −514C/T and −250G/A polymorphisms are associated with low HL activity, we have found that their postprandial effects are different. Our previous

Table 3 Peak time according to the −250G/A promoter polymorphism

Lipoprotein fractions	Peak time (min)		*P*
	GG (30)	GA/AA (21)	
Total TG	251 ± 70	210 ± 62[a]	0.034
Large TRL-TG	254 ± 70	211 ± 66[a]	0.033
Small TRL-TG	215 ± 62	198 ± 57	0.323
Large TRL-RP	342 ± 209	275 ± 209	0.268
Small TRL-RP	379 ± 181	261 ± 189[a]	0.029

TG, triacylglycerol; TRL, triacylglycerol rich lipoproteins; RP, retinyl palmitate. *P*: ANOVA for repeated measures.
[a] $P < 0.05$ vs. GG.

Table 4 Multiple stepwise regression analyses

Dependent variable	Independent variable	β-Coefficient	*R*	R^2	*P*
Small TRL-RP AUC	G250A	0.431			0.001
	BMI	0.329			0.012
	TC	0.334	0.694	0.482	0.009
	HDL-C	−0.417			0.005
	TG	−0.518			0.001
Total TG peak time	G250A	−0.272	0.393	0.115	0.047
	TC	0.258			0.059

TRL, triacylglycerol rich lipoproteins; RP, retinyl palmitate; TC, total cholesterol; TG, triacylglycerol; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; AUC, area under the curve.

study [23] demonstrated that carriers of the T allele to the −514C/T polymorphism had lower postprandial levels of apo B, total plasma TG, small TRL-TG, large TRL-TG and small TRL-cholesterol when compared to subjects that were homozygous for the C allele. However, in the present study we have observed that the effect of the −250G/A polymorphism took place mainly on small TRL-RP. Since RP is an intestinal lipoprotein particle marker, our results showed that the −250G/A polymorphism in the promoter of the HL gene was associated with a higher postprandial response in terms of TRL of intestinal origin.

Several models of nutrition have been proposed for the prevention and treatment of cardiovascular disease. Nevertheless, there exists great inter-individual variability in terms of dietary response, which indicates the importance of genetic components. These types of studies could assist in the future development of specific dietary recommendations for each individual on the basis of their genetic profile, and could thus lead to an improvement in health and the prevention of nutrigenetic-based diseases.

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References

[1] Olivecrona G, Olivecrona T. Triglyceride lipases and atherosclerosis. Curr Opin Lipidol 1995;6:291−305.
[2] Bensadoun A, Berryman DE. Genetics and molecular biology of hepatic lipase. Curr Opin Lipidol 1996;7:77−81.
[3] Cohen JC, Vega GL, Grundy SM. Hepatic lipase: new insights from genetic and metabolic studies. Curr Opin Lipidol 1999;10:259−67.
[4] Demant T, Carlson LA, Holmquist L, Karpe F, Nilsson-Ehle P, Packard CJ, et al. Lipoprotein metabolism in hepatic lipase deficiency: Studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. J Lipid Res 1988;29:1603−11.
[5] Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, et al. Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). J Lipid Res 1996;37:926−36.
[6] Komaromy M, Azhar S, Cooper AD. Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. Characterization of low density lipoprotein and chylomicron remnant uptake and selective uptake of high density lipoprotein-cholesteryl ester. J Biol Chem 1996; 271:16906−14.
[7] Santamarina-Fojo S, Haudenschild C, Amar M. The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. Curr Opin Lipidol 1998;9:211−9.
[8] Barrans A, Collet X, Barbaras R, Jarpard B, Manent J, Vieu C, et al. Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2: a study comparing liver perfusion to in vitro incubation with lipases. J Biol Chem 1994;269: 11572−7.
[9] Clay MA, Newnham HH, Forte TM, Barter PI. Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo A-I from HDL and subsequent formation of discoidal HDL. Biochim Biophys Acta 1992;1124:52−8.
[10] Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. Arterioscler Thromb 1993;13:147−53.
[11] Despres JP, Ferland M, Moorjani S, Nadeau A, Tremblay A, Lupien PJ, et al. Role of hepatic-triglyceride lipase activity in the association between intra-abdominal fat and plasma HDL cholesterol in obese women. Arteriosclerosis 1989;9: 485−92.
[12] Vega GL, Clark LT, Tang A, Marcovina S, Grundy SM, Cohen JC. Hepatic lipase activity is lower in African American men than in white American men: effects of

5′ flanking polymorphism in the hepatic lipase gene (LIPC). J Lipid Res 1998;39:228—32.
[13] Zambon A, Deeb SS, Hokanson JE, Brown BG, Brunzell JD. Common variants in the promoter of the hepatic lipase gene are associated with lower levels of hepatic lipase activity, buoyant LDL, and higher HDL2 cholesterol. Arterioscler Thromb Vasc Biol 1998;18:1723—9.
[14] Colvin PL Jr, Auerbach BJ, Case LD, Hazzard WR, Applebaum-Bowden D. A dose-response relationship between sex hormone-induced change in hepatic triglyceride lipase and high-density lipoprotein cholesterol in postmenopausal women. Metabolism 1991;40:1052—6.
[15] Huttunen JK, Ehnholm C, Kekki M, Nikkila EA. Post heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and patients with hypertriglyceridemia: correlations to sex, age, and various parameters of triglyceride metabolism. Clin Sci Mol Med 1976;50:249—60.
[16] Willians PT, Krauss RM. Associations of age, adiposity, menopause and alcohol intake with low-density lipoprotein subclasses. Arterioscler Thromb Vasc Biol 1997;17: 1082—90.
[17] Guerra R, Wang J, Grundy SM, Cohen JC. A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. Proc Natl Acad Sci USA 1997;94:4532—7.
[18] Tahvanainen E, Syvänne M, Frick M, Murtomäki-Repo S, Antikainen M, Kesäniemi Y, et al. Association of variation in hepatic lipase activity with promoter variation in the hepatic lipase gene: the LOCAT study Investigators. J Clin Invest 1998;101:956—60.
[19] Jansen H, Chu G, Ehnholm C, Dallongeville J, Nicaud V, Talmud P. The T allele of the hepatic lipase promoter variant C-480T is associated with increased fasting lipids and HDL and increased preprandial and postprandial LPCIII:B: European Atherosclerosis Research Study (EARS) II. Arterioscl Thromb Vasc Biol 1999;19:303—8.
[20] Jansen H, Verhoeven A, Weeks L, Kastelein J, Halley D, van den Ouweland A, et al. Common C-to-T substitution at position −480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. Arterioscler Thromb Vasc Biol. 1997; 17:2837—42.
[21] Sultan F, Lagrange D, Jansen H, Griglio S. Inhibition of hepatic lipase activity impairs chylomicron remnant removal in rats. Biochim Biophys Acta 1990;1042:150—2.
[22] Shafi S, Brady SE, Bensadoun A, Havel RJ. Role of hepatic lipase in the uptake and processing of chylomicrons remnants in rat liver. J Lipid Res 1994;35: 709—20.
[23] Gomez P, Lopez-Miranda J, Marin C, Bellido C, Moreno JA, Moreno R, et al. Influence of the −514C/T polymorphism in the promoter of the hepatic lipase gene on postprandial lipoprotein metabolism. Atherosclerosis 2004;174:73—9. doi:10.1016/j.atherosclerosis.2003.12.038.
[24] 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.
[25] Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973;19: 476—82.
[26] Allain CC, Poon LS, Chang CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470—5.
[27] Riepponen P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-1 and B in serum. Scan J Clin Lab Invest 1987;47:739—44.
[28] Warnick GR, Benderson JM, Albers JJ. Dextran sulfate-Mg precipitation procedure for quantitation of high density lipoprotein cholesterol. Clin Chem 1982;28:1379—88.
[29] Ruotolo G, Zhang H, Bentsianow 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.
[30] De Ruyter MGM, De Leeheer AP. Simultaneous determination of retinal and retinyl esters in serum or plasma by reversed-phase high performance liquid chromatography. Clin Chem 1978;24:1920—3.
[31] 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.
[32] Ko YL, Hsu LA, Hsu KH, Ko YH, Lee YS. The interactive effects of hepatic lipase gene promoter polymorphisms with sex and obesity on high-density-lipoprotein cholesterol levels in Taiwanese-Chinese. Atherosclerosis 2004;172: 135—42. doi:10.1016/j.atherosclerosis.2003.09.013.
[33] Breckenridge WC, Little JA, Alaupovic P, Wang CS, Kuksis A, Kakis G, et al. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. Atherosclerosis 1982;45:161—79.
[34] Carlson LA, Holmquist L, Nilsson-Ehle P. Deficiency of hepatic lipase activity in post-heparin plasma in familial hyper-β-triglyceridemia. Acta Med Scand 1986;219: 435—47.
[35] Vogel RA, Corretti MC, Plonick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol 1997;79:350—4. doi:10.1016/s0002-9149(96)00760-6.
[36] Roche HM, Gibney MJ. The impact of post-prandial lipemia in accelerating atherothrombosis. J Cardiovasc Risk 2000; 7:317—24.
[37] Lopez-Miranda J, Ordovas JM, Ostos MA, Marin C, Jansen S, Salas J, et al. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein B gene locus. Arterioscler Thromb Vasc Biol 1997; 17:1765—73.
[38] Ostos MA, Lopez-Miranda J, Ordovas JM, Marin C, Blanco A, Castro P, et al. Dietary fat clearance is modulated by genetic variation in apolipoprotein A-IV gene locus. J Lipid Res 1998;39:2493—500.
[39] Marin C, Lopez-Miranda J, Gomez P, Paz E, Perez-Martinez P, Fuentes F, et al. Effects of the human apolipoprotein A-I promoter G-A mutation on postprandial lipoprotein metabolism. Am J Clin Nutr 2002;76:319—25.
[40] Cohn JS. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. Can J Cardiol 1998;14:18B—27B.
[41] Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med 1999;246:341—55.
[42] Hodis HN. Triglyceride-rich lipoprotein remnant particles and risk of atherosclerosis. Circulation 1999;99: 2852—4.
[43] Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, et al. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation 1999;99:2858—60.
[44] Chang S, Borensztajn J. Uptake of chylomicron remnants and hepatic lipase treated chylomicrons by a non-transformed murine hepatocyte cell line in culture. Biochim Biophys Acta 1995;1256:81—7.
[45] Breckenridge WC, Little JA, Alauporic P, Wang CS, Kuksis A, Kakis G, et al. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. Atherosclerosis 1982;45:161—79.

[46] Ji ZS, Dichek HL, Miranda RD, Mahley RW. Heparan sulfate proteoglycans participate in hepatic lipase and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. J Biol Chem 1997;272:31285−95.
[47] Huff MW, Miller DB, Wolfe BM, Connelly PW, Sawyez CG. Uptake of hypertriglyceridemic very low density lipoproteins and their remnants by HEPG2 cells: the role of lipoprotein lipase, hepatic triglyceride lipase, and cell surface proteoglycans. J Lipid Res 1997;38: 1318−33.
[48] Ebert DL, Warren RJ, Barter PJ, Mitchell A. Infusion of atherogenic lipoprotein particles increases hepatic lipase activity in the rabbit. J Lipid Res 1993;34:89−94.