Postprandial triacylglycerol metabolism is modified by the presence of genetic variation at the perilipin (PLIN) locus in 2 white populations

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ABSTRACT

Background: Several perilipin (PLIN) polymorphic sites have been studied for their potential use as markers for obesity and the metabolic syndrome.

Objective: We aimed to examine whether the presence of polymorphisms at the perilipin (PLIN) locus (PLIN1, 6209T→C; PLIN4, 11482G→A; PLIN5, 13041A→G; and PLIN6, 14995A→T) influence postprandial lipoprotein metabolism in 2 white populations.

Design: Eighty-eight healthy Spanish men and 271 healthy US subjects (men and women) underwent an oral-fat-load test in 2 independent studies. Blood samples were taken in the fasting state and during the postprandial phase at regular intervals. Total cholesterol and triacylglycerol and triacylglycerol in triacylglycerol-rich lipoproteins (TRL, large and small) were measured.

Results: Carriers of the minor C allele at the PLIN1 variant displayed lower postprandial concentrations of large-TRL triacylglycerol (Spanish subjects: P = 0.024; US subjects: P = 0.005) than did subjects carrying the T/T genotype. The same pattern was observed in the Spanish population at the PLIN4 locus (P = 0.015), and both SNPs were in strong linkage disequilibrium. In both populations, subjects carrying the minor C and A alleles at PLIN1 and PLIN4, respectively, had significantly lower postprandial concentrations of plasma triacylglycerol (P < 0.05) and lower concentrations of small-TRL triacylglycerol than did those who were homozygous for the major alleles at PLIN1 and PLIN4 (Spanish subjects: P = 0.020 and 0.008, respectively; US subjects: P = 0.021 and 0.035, respectively).

Conclusion: These 2 studies suggest that the presence of the minor C and A alleles at PLIN1 and PLIN4, respectively, are associated with a lower postprandial response that may result in lower atherogenic risk for these persons. Am J Clin Nutr 2008;87:744–52.

KEY WORDS Nutrigenetics, postprandial lipemia, perilipin, triacylglycerol-rich lipoproteins, single-nucleotide polymorphisms

INTRODUCTION

Current evidence supports the notion that the magnitude and extent of postprandial lipemia may be significant factors in the pathogenesis of coronary heart disease (1–6). Individual variability in the postprandial lipemic response is usually greater than that observed in the fasting state, and, like most other coronary heart disease risk factors, that variability appears to be modulated by environmental and genetic factors (7, 8). This concept is supported by studies showing associations between polymorphisms at candidate genes and both the postprandial lipoprotein response and interactions with dietary factors (9).

Perilipins are proteins localized at the surface of the lipid droplet in adipocytes, steroid-producing cells, and ruptured atherosclerotic plaques, and they play a key role in the cellular regulation of triacylglycerol deposition and mobilization (10, 11). In humans, the perilipin gene (PLIN) has been localized to chromosomal location 15q26.1 (12), within a region previously linked to obesity, hypertriglyceridemia, and diabetes (13, 14). We previously showed that polymorphisms in the PLIN locus

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were associated with obesity-related phenotypes in US and Spanish white women (15, 16). Moreover, we have shown that a particular haplotype was associated with a greater risk of obesity in Malays and Indians but not in Chinese (17). In view of the physiologic role of PLIN in the metabolism of adipocyte lipid droplets and of the link between postprandial plasma lipid metabolism and fatty acid deposition in the adipocyte, we hypothesized that genetic variation associated with PLIN activity or function in humans could be related to individual variability in postprandial triacylglycerol-rich lipoprotein (TRL) metabolism.

We first conducted an intervention study in a well-controlled group of healthy white subjects from Spain. Our primary aim was to examine the previously stated hypothesis regarding the association between PLIN single-nucleotide polymorphisms (SNPs) and postprandial lipoprotein metabolism. However, initial associations between SNPs at candidate genes and variability in postprandial lipid responses to a fat-load test quite often are not subsequently supported. Therefore, our secondary aim was to replicate the initial findings in a second population drawn from a multicenter, population-based study comprising a large group of US white subjects.

SUBJECTS AND METHODS

Spanish population and fat-loading test

Eighty-eight healthy male students at the University of Cordoba responded to an advertisement and were recruited for the study. They had a mean ± SD age of 23 ± 4 y. All subjects underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before inclusion in the study, and they did not show evidence of any chronic disease (eg, hepatic, renal, thyroid, or cardiac dysfunction). Plasma total cholesterol concentrations were <240 mg/dL, and plasma triacylglycerol concentrations were <150 mg/dL. None of the subjects had a body mass index (BMI; in kg/m²) > 30. Self-reported levels of physical activity were consistent with moderate-intensity exercise several times per week. Moreover, none of the participants had taken medications or vitamin supplements during the 6 mo before the study. Sudden changes in physical activity or lifestyle (eg, smoking or drinking) also may affect the postprandial response. Therefore, subjects were instructed to avoid such changes during the week before the fat challenge and to maintain a diary reflecting their daily activities. The metabolic study was carried out in the Research Unit of the Reina Sofia University Hospital.

Written informed consent was obtained from all participants. The experimental protocol and secondary analyses of the data were approved by the Human Investigation Review Committee at the Reina Sofia University Hospital and the Tufts-New England Medical Center institutional review boards.

After a 12-h fast, volunteers were given a fatty meal enriched with 60 000 units of vitamin A/m² body surface area. The amount of fat given, per kg body wt, was 1 g fat and 7 mg cholesterol. The meal contained 65% of energy as fat, 15% of energy as protein, and 25% of energy as carbohydrates; it was consumed in 20 min. We calculated the amount of each ingredient fed as a function of individual weight, so that, although all of the subjects consumed the same types of food, the quantities involved were different. The foods were bread, whole milk, eggs, and butter. After this meal, subjects fasted for 11 h, but they were allowed to drink water. Blood samples were drawn before the meal, every hour until hour 6, and every 2.5 h until hour 11.

US population and fat-loading test

The US study sample was a group of 98 men and 173 women selected from among those who participated in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study (Minneapolis, MN, and Salt Lake City, UT) and for whom there was a full data record for the postprandial triacylglycerol test. The detailed design and methods of the GOLDN study were described previously (18). Briefly, exclusion criteria included the following: recent history (6 mo) of myocardial infarction, coronary bypass surgery, coronary angioplasty, or percutaneous transluminal coronary angioplasty; self-report of a positive history of liver, kidney, pancreas, or gallbladder disease or a history of malabsorption of nutrients; current use of insulin or warfarin; or pregnancy or nonuse of contraception by women of childbearing potential. Subjects who reported current use of prescription or over-the-counter hypolipidemic drugs (or both) or dietary supplements known to influence lipid values (eg, fish oil, flaxseed oil, and niacin) were required to consult their physician for approval to discontinue these lipid-lowering agents for 4 wk before beginning the study. Criteria for inclusion in the subgroup analyses included a BMI < 25 to match the anthropometric characteristics of the participants in the primary study.

Participants were asked to fast for >12 h and to abstain from using alcohol for >24 h before visiting the clinic. The oral-fat-load test study consisted of a meal the composition of which was according to the protocol of Patsch et al (19). This high-fat meal was consumed after fasting laboratory measurements were collected. The meal was consumed within 15 min, and had 700 cal/m² body surface area (2.93 MJ/m² body surface area); 3% of calories were derived from protein, 14% from carbohydrates, and 83% from fat sources. The cholesterol content and the ratio of polyunsaturated to saturated fat were 240 mg and 0.06, respectively. The average person consumed 175 mL heavy whipping cream (39.5% fat) and 7.5 mL of chocolate- or strawberry-flavored syrup to increase palatability. Blood samples were drawn immediately before (time 0) and 3.5 and 6 h after consumption of this high-fat meal. For the duration of the 6-h study period, participants were allowed to drink water, but they did not ingest any other food or beverage. In addition, they abstained from exercise and strenuous physical work until the final blood sample was obtained. All meals were administered in the early morning, usually between 0630 and 0900.

Written informed consent was obtained from each participant at the screening visit. This protocol was approved by the institutional review boards at the University of Minnesota, the University of Utah, and Tufts University.

Laboratory methods in the Spanish population

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, Swedberg flotation units (Sf) > 400] was isolated from 4 mL plasma obtained from EDTA-coated tubes. Plasma was placed in the bottom of a 13.4-mL polyallomer ultracentrifuge tube (Ultraclear; Beckman Instruments, Palo Alto, CA) and overlaid with a preservative solution consisting of NaCl (0.15 mol/L), sodium azide (0.05 mol/L), and 0.15 mol/L sodium chloride.
chloramphenicol (0.05 g/L), gentamicin sulfate (40 mg/L), and EDTA (1 mmol/L) (pH 7.4; d < 1006 kg/L). Ultracentrifugation was performed in a Ty65 rotor (Beckman Instruments, Fullerton, CA) at 36 200 × g and 4 °C for 30 min. Chylomicrons contained in the top layer were removed by aspiration, placed directly into individual vials, and stored at −80 °C until they were assayed for retinyl palmitate (RP), biochemical determinations, and apolipoprotein (apo) B-48 and apoB-100. The infranatant fluid was centrifuged at a density of 1019 kg/L and overlaid with the same preservative cocktail. The ultracentrifugation was performed for 24 h at 18 3000 × g using the Beckman Ty65 rotor. The nonchylomicron fraction (also referred to as small TRL, Sf 20–400) was removed from the top of the tube by aspiration, placed directly into individual vials, and stored at −80 °C until it was assayed for RP, biochemical determinations, apoB-48, and apoB-100. All operations were done under subdued light to prevent the degradation of RP.

Cholesterol and triacylglycerol in plasma and lipoprotein fractions were assayed by enzymatic procedures (20–21). ApoA1 and apoB were measured by turbidimetry (22). HDL cholesterol was measured by analyzing the supernatant fluid obtained after precipitation of a plasma aliquot with dextran sulfate-Mg2+, as described by Warnick et al (23). LDL cholesterol was measured as the difference between the total cholesterol before ultracentrifugation and that at the bottom part of the tube after ultracentrifugation at a density of 1019 kg/L.

The RP content was assayed by using a previously described method (24). The peaks of RP and retinyl acetate were identified by comparing their retention times with that of a purified standard (Sigma Chemical Co, St Louis, MO), 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 (25). All operations were performed in subdued light.

ApoB-48 and apoB-100 were measured by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described by Karpe and Hamsten (26). Gels were scanned with a densitometer scanner (TDI, Madrid, Spain) connected to a personal computer for integration of the signals. Background intensity was calculated after scanning an empty lane. The CV for the sodium dodecyl sulfate–polyacrylamide gel electrophoresis was 7.3% for apoB-48 and 5.1% for apoB-100.

Laboratory methods in the US population

Triacylglycerol was measured by using the glycerol-blanked enzymatic method on the Roche COBAS FARa centrifugal analyzer (Roche Diagnostics Corporation, Indianapolis, IN). The GOLDN study measured nuclear magnetic resonance (NMR) chylomicrons and nonchylomicron fractions (total, large, medium, and small VLDL). This method uses signal amplitudes of the lipoprotein subclasses of difference sizes as its basis of quantification (27). Comparison of NMR and ultracentrifugation separation in this study population showed a high degree of correlation, which suggested that NMR is a valid alternative method for measuring TRL triacylglycerol (28). Blind duplicate samples from 5% of participants were sent to the laboratory for assessment of repeatability. For all lipid subfractions, the repeatability was >90%. All blood samples from each subject were stored until the completion of the subjects’ participation and then analyzed together.

Given that different methods were used to separate, isolate, and measure TRLs in the 2 studies (by ultracentrifugation in the Spanish population and by NMR spectroscopy in the US population), in this report we have precisely redefined the terminology. In the Spanish population, the chylomicrons and other large hepatic apoB-100 particles are referred to here as large TRLs (Sf > 400), and the nonchylomicron fraction is referred to as small TRLs (Sf 20–400). In the US population, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size as the basis of the quantification, without measuring which particles contain apoB-100. Thus, the chylomicron fraction of TRL is referred to as large TRL. In addition, in the US population, the term “small TRL” includes the nonchylomicron fractions grouped in 3 size categories: large (60–200 nm), medium (35–60 nm), and small (27–35 nm) VLDL.

**PLIN genotyping**

The genotyping study was carried out in the Nutrition and Genomics Laboratory, at the Jean Mayer–US Department of Agriculture Human Nutrition Research Center on Aging. DNA extraction was performed by standard procedures. Four polymorphisms at the PLIN locus were genotyped: PLIN1 6209T→C (intron 2), PLIN4 11482G→A (intron 6), PLIN5 13041A→G (exon 8, synonymous), and PLIN6 14995A→T (exon 9, untranslated region). Genotyping was carried out by using the 5′nuclease allelic discrimination Taqman assay with the ABI 7900HT system (Applied Biosystems, Foster City, CA). Standard good laboratory practices were undertaken to ensure the accuracy of genotypic data. Internal controls and repetitive experiments were used. The final success rate for PLIN genotyping in the study participants was 100%.

**Statistical analysis**

A chi-square test was used to determine whether the genotype distribution followed Hardy-Weinberg equilibrium. Pairwise linkage disequilibrium coefficients were estimated by using the LINKAGE program (version 5.1; Internet: http://linkage.rockefeller.edu/soft/list2.html#1). D and D′ coefficients were calculated. Normal distribution followed Hardy-Weinberg equilibrium. DNA was tested for statistical significance between genotypes by 1-factor analysis of variance, after adjustment for the covariates of sex, BMI, age, smoking, and alcohol consumption in the US population. In the present analysis, we 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. When statistical significance was found, the Tukey’s post hoc comparison test was used to identify group differences. The contrast statistic used when the sphericity assumption was not satisfied was Greenhouse-Geisser. For all analyses, P < 0.05 was considered to be significant. All data presented in text and tables are expressed as means ± SDs. SPSS software (version 14.0; SPSS Institute, Chicago, IL) was used for the statistical comparisons.
TABLE 1
Baseline characteristics according to the minor alleles C and A at the PLIN1 and PLIN4 polymorphisms in the Spanish population

<table>
<thead>
<tr>
<th></th>
<th>PLIN1 (6209T&gt;C)</th>
<th>PLIN4 (11482G&gt;A)</th>
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<tbody>
<tr>
<td></td>
<td>TT (n = 37)</td>
<td>TC+CC (n = 51)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>22.7 ± 5.7²</td>
<td>22.3 ± 2.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 3.4</td>
<td>25.7 ± 3.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.92 ± 0.6</td>
<td>3.96 ± 0.5</td>
</tr>
<tr>
<td>Triglycerol (mmol/L)</td>
<td>0.91 ± 0.3</td>
<td>0.86 ± 0.3</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.35 ± 0.5</td>
<td>2.36 ± 0.5</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.15 ± 0.1</td>
<td>1.23 ± 0.1</td>
</tr>
<tr>
<td>Large-TRL TAG (mmol/L)</td>
<td>0.16 ± 0.1</td>
<td>0.13 ± 0.1</td>
</tr>
<tr>
<td>Small-TRL TAG (mmol/L)</td>
<td>0.32 ± 0.2</td>
<td>0.28 ± 0.1</td>
</tr>
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</table>

¹ LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TRL, triacylglycerol-rich lipoprotein; TAG, triacylglycerol. Significance was evaluated with ANOVA.
² ± SD (all such values).

RESULTS
Biochemical and genotypic characteristics of study participants

The Spanish study sample comprises 88 healthy men. Of these: 37 were T/T, 40 T/C, and 11 C/C for PLIN1; 40 were G/G, 41 A/G, and 7 A/A for PLIN4; 38 were A/A, 31 A/G, and 19 G/G for PLIN5; and 12 were A/A, 39 A/T, and 37 T/T for PLIN6. In the US population, 116 were T/T, 124 T/C, and 31 C/C for PLIN1; 139 were G/G, 108 A/G, and 24 A/A for PLIN4; 103 were A/A, 138 A/G, and 30 G/G for PLIN5; and 116 were A/A, 121 A/T, and 34 T/T for PLIN6. Genotype distributions did not deviate from Hardy-Weinberg expectations for PLIN1 6209T→C, PLIN4 11482G→A, or PLIN6 14995A→T, whereas for PLIN5 13041A→G SNP, Hardy-Weinberg equilibrium was observed in the US but not in the Spanish population. The frequency of the C allele for PLIN1, the A allele for PLIN4, the G allele for PLIN5, and the T allele for PLIN6 was consistent with prior reports in other white populations. Strong pairwise linkage disequilibrium was found between the PLIN1 and the PLIN4 polymorphisms (Spanish subjects: D’ = 0.909, P < 0.0001; US subjects: D’ = 0.832, P < 0.001). The basal characteristics by genotype are shown in Table 1 and Table 2. There were no significant baseline differences in age, BMI, and fasting lipid variables by genotype in the Spanish group. However, there were significant baseline differences for triacylglycerol (P = 0.041) for PLIN4 in the US population.

PLIN genotypes and postprandial lipemic response

The Spanish subjects

Our data show that carriers of the minor alleles [T/C and C/C for PLIN1 (Figure 1) and G/A and A/A for PLIN4 (data not shown)] displayed lower postprandial concentrations of large-TRL (P = 0.024 and 0.015, respectively) and small-TRL (P = 0.020 and 0.008, respectively) triacylglycerol than did subjects carrying the T/T genotype for PLIN1 and the G/G genotype for PLIN4. Specific genotype comparisons showed that subjects carrying the C allele had significantly lower concentrations of plasma triacylglycerol during the postprandial period than did carriers of the T/T genotype for PLIN1 at hour 3 (P = 0.05), hour 4 (P = 0.015), and hour 5 (P = 0.025) (data not shown). The PLIN4 polymorphism behaved in a similar fashion at hours 3 (P = 0.05), 4 (P = 0.039), 5 (P = 0.05), and 11 (P = 0.045), and a similar trend was observed at hour 1 (P = 0.074) and hour 2 (P = 0.073) (data not shown). ApoB-100 and apoB-48 in large and

TABLE 2
Baseline characteristics according to the minor alleles C and A at the PLIN1 and PLIN4 polymorphisms in the Genetics of Lipid Lowering and Diet Network

<table>
<thead>
<tr>
<th></th>
<th>PLIN1 (6209T&gt;C)</th>
<th>PLIN4 (11482G&gt;A)</th>
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<tbody>
<tr>
<td></td>
<td>TT (n = 116)</td>
<td>TC+CC (n = 155)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43.8 ± 17²</td>
<td>40.7 ± 16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 1.83</td>
<td>22.3 ± 1.85</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.51 ± 0.9</td>
<td>4.60 ± 1.0</td>
</tr>
<tr>
<td>Triglycerol (mmol/L)</td>
<td>1.07 ± 0.6</td>
<td>0.98 ± 0.5</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.79 ± 0.7</td>
<td>2.79 ± 0.8</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.32 ± 0.3</td>
<td>1.39 ± 0.3</td>
</tr>
<tr>
<td>Large-TRL TAG (mmol/L)</td>
<td>0.05 ± 0.03</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Small-TRL TAG (mmol/L)</td>
<td>0.75 ± 0.5</td>
<td>0.66 ± 0.6</td>
</tr>
</tbody>
</table>

¹ LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TRL, triacylglycerol-rich lipoprotein; TAG, triacylglycerol. Significance was evaluated with ANOVA.
² ± SD (all such values).
small TRLs also were analyzed. We found no significant differences for apoB-100 and apoB-48 in large or small TRLs among the genotypes for PLIN1 (Figure 2) or PLIN4. The postprandial response of plasma HDL cholesterol and apoA1 were different in both groups. At the PLIN1 polymorphism, we observed a greater response for the C allele in apoA1 ($P = 0.007$) and a tendency for higher HDL-cholesterol ($P = 0.067$) concentrations than with the $T/T$ genotype (data not shown). Moreover, carriers of the A allele showed greater postprandial concentrations of HDL cholesterol and apoA1 than did carriers of the $G/G$ genotype for PLIN4 [HDL cholesterol, $P = 0.028$ (Figure 3A); apoA1, $P = 0.014$]. No other statistically significant genotype-related differences in other variables were observed. We also investigated the potential associations between PLIN5 and PLIN6 and relevant postprandial lipoproteins. There was no statistically significant association between any of the variables examined and these SNPs.

The postprandial AUCs in study participants according to the PLIN polymorphisms were analyzed; significant differences were observed between genotypes. The AUCs for large-TRL ($P = 0.05$) and small-TRL ($P = 0.033$) triacylglycerol were lower in persons carrying the $C$ allele than in those carrying the $T/T$ genotype for PLIN1 (Table 3). On the other hand, carriers of the $A$ allele for PLIN4 had lower total ($P = 0.039$), large-TRL ($P = 0.050$), and small-TRL ($P = 0.036$) triacylglycerol than did $G/G$ subjects (data not shown). These observations are consistent with the trend observed during the postprandial measurements, which suggested an association between the PLIN1 and PLIN4 polymorphisms with postprandial particles. No other statistically significant genotype-related differences in the AUC for other variables (ie, total cholesterol, RP, apoB-48, or apoB-100) were observed.

The US subjects

The postprandial response of plasma total cholesterol, triacylglycerol, and triacylglycerol in different lipoprotein fractions were analyzed. Because we did not observe any significant sex × genotype interaction, men and women were combined for subsequent analyses. After adjustment for sex, age, BMI, smoking, and alcohol consumption, we found that carriers of the $C$ allele at PLIN1 had lower postprandial concentrations of large-TRL triacylglycerol ($P = 0.018$) than did persons carrying the $T/T$ genotype (Figure 4). These results are in concordance with those observed in the Spanish population. In contrast, although a trend was observed, no significant differences were observed for large-TRL triacylglycerol at PLIN4 ($P = 0.193$) (data not shown).

To examine how postprandial lipemic response affects the distribution of lipoprotein subclasses, we analyzed the changes of small-TRL triacylglycerol fractions (total, large, medium, and small VLDL) among subjects with different genotypes in response to the fat-load test. Subjects carrying the minor alleles $C$ and $A$ at PLIN1 and PLIN4, respectively, had lower postprandial concentrations of total [($P = 0.021$, (Figure 4) and $P = 0.035$ (data not shown), respectively], medium ($P = 0.033$ and 0.05, respectively), and small ($P = 0.002$ and 0.014, respectively) VLDL than did carriers of the $T/T$ and $G/G$ genotypes, respectively (data not shown). No significant differences were found for large VLDL ($P = 0.206$) postprandial concentration according to the PLIN1 variant. However, carriers of the $A$ allele had a lower

![FIGURE 1](https://academic.oup.com/ajcn/article-abstract/87/3/744/4633380/744/PEREZ-MARTINEZ-ET-AL/748-748.png)
postprandial response for large VLDL (P/L1155 0.021) than did carriers of the G/G genotype at PLIN4.

Specific genotype comparisons showed that carriers of the alleles C for PLIN1 and A for PLIN4 had significantly lower concentrations of plasma triacylglycerol during the postprandial period than did carriers of the T/T and G/G genotypes at hour 3.5 (P/L1155 0.036 and 0.033, respectively) (data not shown). The postprandial response of plasma HDL cholesterol was different depending on the genotype. Carriers of the C allele had higher postprandial concentrations of HDL cholesterol than did carriers of the T/T genotype for PLIN1 (P/L1155 0.016; Figure 3B). A similar trend was observed for PLIN4, but we found no significant differences in postprandial concentrations of HDL cholesterol (P/L1155 0.158) (data not shown).

The AUCs for triacylglycerol according to the PLIN polymorphisms were analyzed. The AUC for the total triacylglycerol was lower in carriers of the A allele than in carriers of G/G for PLIN4 (P = 0.047), and a trend was also observed for PLIN1 (P = 0.092) (data not shown). No differences were observed in the AUC for total cholesterol. No other significant genotype-related differences were observed for other variables. We also investigated the potential associations between PLIN5 and PLIN6 and relevant postprandial lipoproteins. There was no significant association between any of the variables examined and these 2 SNPs.

**DISCUSSION**

Our findings show that white Spanish and US subjects carrying the minor alleles C and A (T/C and C/C for PLIN1 and G/A and A/A for PLIN4) had lower postprandial concentrations of
Glycerol (TAG) responses in large and small TRL than did carriers of TT and GG genotypes at PLIN1 and PLIN4, respectively. These findings were observed consistently in both populations.

Replication studies are used in scientific methodology to test for the robustness of the scientific findings, because replicated studies are more likely to represent “true” associations. In the current study, we first conducted an intervention study on a well-controlled group of healthy white subjects from Spain. The major findings of the primary study were externally replicated in a second population of healthy US whites.

The postprandial state is a dynamic, nonsteady condition, in which there is rapid remodeling of lipoproteins. This period may be relevant to individual cardiovascular disease risks worldwide, but its measurement and interpretation are both cumbersome and complex (29). Large and small TRLs have been investigated as potential markers of both lesion progression and cardiac events. The current evidence suggests that small TRLs are more atherogenic or thrombogenic than are large TRLs. This finding makes our results of particular interest, because we found that carriers of the minor alleles C and A had a lower postprandial response for both families of particles.

Perilipin A is the most common isoform in human adipose tissue (30, 31). Perilipin is a target of protein kinase A, and nonphosphorylated perilipin may act as a barrier to the hormone-sensitive lipase (HSL)–mediated lipolysis of triacylglycerol in lipid droplets (32–34). Thus, perilipin A functions to increase sensitive lipase (HSL)–mediated lipolysis of triacylglycerol in nonphosphorylated perilipin may act as a barrier to the hormone-sensitive lipase (HSL)–mediated lipolysis of triacylglycerol in lipid droplets (32–34). Thus, perilipin A functions to increase sensitive lipase (HSL)–mediated lipolysis of triacylglycerol in

![FIGURE 4. Line plots of mean (±SD) postprandial large-triaclylglycerol-rich lipoprotein (TRL) and small-TRL (represented as total VLDL) triacylglycerol (TAG) responses in TT subjects (●, n = 116) and carriers of the C allele (■, n = 155) at PLIN1 variant in the US population. P values are for the genotype × time interaction (ANOVA). *Significant genotype effect, P < 0.05.](https://academic.oup.com/ajcn/article-abstract/87/3/744/4633380/Downloaded-from)
women. Despite the different experimental designs, both studies consistently showed that PLIN4 is associated with the triacylglycerol postprandial state.

The study by Mottagui-Tabar et al. (37) suggested that subjects carrying the minor allele A at PLIN4 had a decrease in the content of perilipin in the adipocyte and an increase in the rate of lipolysis as compared with subjects who were GG homozygous. These data, together with previous evidence from animal models, suggest that perilipin is an important regulatory component of adipocyte lipolysis during the fasting state (38). However, no data on its role during the postabsorptive phase are available. We have found a consistently greater postprandial clearance of triacylglycerol in small TRLs in subjects with the minor A allele for PLIN4 than in GG subjects.

During the postprandial state, adipose tissue is the major site for the uptake of dietary triacylglycerol fatty acid, after lipoprotein lipase (LPL)–mediated hydrolysis of chylomicrons (39). LPL is produced mainly in adipose tissue and muscle, secreted by those tissues, and transported to the surfaces of capillary endothelial cells in these tissues. A previous study showed that LPL and HSL are counterregulated. During the overnight-fasted state, HSL is much more active than LPL; however, after a fat-rich meal, HSL activity is rapidly suppressed by dephosphorylation, whereas LPL is activated (40). It has been reported that a loss of functional perilipin proteins causes a dramatic attenuation of stimulated lipolytic activity as a result of impairment of protein kinase–mediated stimulation of HSL translocation (41). We hypothesize that the lower perilipin concentrations reported in association with the PLIN4 A allele (37) may induce a similar, although milder effect. Therefore, given the opposite regulation of HSL and LPL (40), a further decrease in the activity of HSL may result in a greater increase in the postprandial LPL activity and a greater TRL clearance, which would be consistent with the greater TRL clearance observed in subjects with the A allele at PLIN4 or the C allele at PLIN1 SNPs, both of which are in strong LD.

We have also observed that persons carrying the minor alleles C and A at PLIN1 and PLIN4, respectively, had a greater postprandial response of HDL cholesterol and apoA1 than did those carrying the T/T genotype for PLIN1 and the G/G genotype for PLIN4. During the postprandial state, cholesteryl ester transfer activity is increased, and large TRLs become preferred acceptors of cholesterol ester from HDL. The extent of postprandial cholesteryl ester transfer depends on plasma concentrations of acceptor lipoproteins (chylomicrons and VLDL), and enhanced postprandial transfer of cholesterol into triacylglycerol-rich, apoB-containing lipoproteins is associated with a reduction in HDL-cholesterol concentrations. Hepatic lipase activity is enhanced in the fed state, which favors the formation of smaller LDL and HDL. In the current study, data suggest that, during the postprandial period, there is an active exchange of lipids between circulating lipoproteins, and subjects with a delayed postprandial clearance of TRLs also had a lower postprandial concentration of antithromogenic particles.

In contrast, it has been suggested that higher concentrations of serum triacylglycerol could lead to insulin resistance (42). The enlarged pool of circulating TRLs could also increase plasma fatty acid concentrations by saturating peripheral removal mechanisms and thus could contribute to establishing an insulin-resistant state. Future studies should measure the free fatty acids to determine the effect of PLIN polymorphisms on insulin resistance. Although these results are preliminary, the fact that our study was carried out in 2 different populations supports the involvement of these polymorphisms in postprandial lipoprotein metabolism.

In summary, these 2 studies suggest that allele variability at the PLIN1 and PLIN4 polymorphisms could contribute to interindividual differences in the postprandial lipemic response in healthy subjects. Although the results are still hypothetical, the present study proposes a link between adipocyte and plasma TRLs.

The authors’ responsibilities were as follows—JMO, JL-M, DA, and FP-J: the conception and design of the study; MT, RS, EG, MP, JH, JD-L, JR, and IB: the provision of study materials or subjects; NY, PP-M, JH, JR, JD-L, and BG-B: the collection and assembly of data; PP-M, NY, and JMO: the analysis and interpretation of the data; DA, IB, MT, RS, and MP: statistical expertise; JMO and PP-M: the writing of the manuscript draft; FP-J, JL-M, RS, and DA: critical review of the manuscript; and DA, FP-J, JMO, and JL-M: obtained funding. None of the authors had a personal or financial conflict of interest.

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