

Peroxisome proliferator-activated receptor α polymorphisms and postprandial lipemia in healthy men

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Abstract Peroxisome proliferator-activated receptor α (PPAR α) is a ligand-dependent transcription factor that plays a key role in lipid and glucose homeostasis. This study evaluated whether variants of *PPAR α* are associated with postprandial lipemia. Subjects were given a single fat load composed of 60% calories as fat, 15% as protein, and 25% as carbohydrate. Blood was drawn every hour from baseline to 6 h, then every 2.5 h to 11 h to determine triglyceride (TG) levels. The minor allele of the nonsynonymous p.Leu162Val variant was associated with higher fasting total cholesterol, LDL-cholesterol, and apolipoprotein B. There were no significant associations with all of the postprandial parameters examined. Conversely, the noncoding variant c.140+5435T>C was not associated with fasting lipid concentrations but was significantly associated with decreased postprandial TG and cholesterol in the small TG-rich lipoprotein particle. Although the minor allele carriers displayed lower mean concentrations of TG and cholesterol throughout the postprandial period, the differences were most pronounced in the latter period. **These data suggest that *PPAR α* variants may modulate the risk of cardiovascular disease by influencing both fasting and postprandial lipid concentrations.**—Tanaka, T., J. M. Ordovas, J. Delgado-Lista, F. Perez-Jimenez, C. Marin, P. Perez-Martinez, P. Gomez, and J. Lopez-Miranda. **Peroxisome proliferator-activated receptor α polymorphisms and postprandial lipemia in healthy men.** *J. Lipid Res.* 2007. 48: 1402–1408.

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Heart disease is the leading cause of death in the United States (1). The development of cardiovascular disease (CVD) is multifactorial, with risk factors that are modifiable, such as diet, exercise, and smoking, and others that are not, such as sex, age, and genetics. The behavioral factors influence a number of biochemical markers involved in dyslipidemia, hypertension, inflammation, and

insulin resistance. In terms of the lipid-related risk factors, fasting lipid concentrations are used for CVD risk assessment. However, because people in Westernized societies are mostly in the postprandial state, it has been suggested that nonfasted lipid concentration may be a better reflection of the most common physiological state and, therefore, of dyslipidemia-related CVD risk (2).

After the consumption of fat-containing foods, dietary triglycerides (TGs) as well as other lipids are packaged in the intestine into chylomicrons while the liver secretes endogenous TGs as part of VLDL. The TGs in these lipoprotein particles are hydrolyzed primarily by lipoprotein lipase to form smaller remnant particles. In vitro and in vivo studies have shown that these cholesterol-enriched remnant lipoproteins can penetrate the endothelium, be trapped in the endothelial space, and initiate or exacerbate the atherosclerotic process (3–5). Therefore, efficient metabolism of these proatherogenic remnants may be one mechanism to prevent or delay the development of atherosclerosis.

Peroxisome proliferator-activated receptor α (PPAR α) is a ligand-induced transcription factor that is expressed in tissues that use fatty acids, such as the liver, kidney, skeletal, and cardiac muscle. Natural ligands include fatty acids, namely PUFAs and their metabolites (6). Through the interaction with these ligands, PPAR α alters the expression of genes involved in lipid metabolism, fatty acid oxidation, glucose metabolism, and inflammation (7). In humans, several variants in *PPAR α* have been identified. One variant in exon 6, p.Leu162Val, has been studied extensively and has been associated with body mass index, fasting concentration of total cholesterol (TC), HDL-cholesterol, low density lipoprotein-cholesterol (LDL-C),

Abbreviations: apoB, apolipoprotein B; AUC, area under the curve; CVD, cardiovascular disease; LDL-C, low density lipoprotein-cholesterol; PPAR α , peroxisome proliferator-activated receptor α ; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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apolipoprotein B (apoB), apoA-I, and the progression of atherosclerosis (8–13). Furthermore, this variant has been shown to have an effect on transactivation efficiency in vitro (9, 14). In addition to its effect on fasting lipid parameters, *PPAR* α variants may exert their effect on atherosclerosis progression via the modulation of postprandial lipid metabolism. In this study, we examined the effect of two *PPAR* α polymorphisms in intron 2 and exon 6 on fasting and postprandial lipid metabolism in healthy males.

SUBJECTS AND METHODS

Human subjects

Fifty-nine healthy male students at the University of Cordoba responded to an advertisement and were recruited for the study. The exclusion criteria for the study included the presence of diabetes, liver, renal, or thyroid disease and the administration of vitamin supplements or medications that alter lipid concentrations. The subjects ranged from 18 to 49 years of age. The metabolic study was carried out in the Research Unit of the Reina Sofia University Hospital, and informed consent was obtained from all study participants. The experimental protocol was approved by the Human Investigation Review Committee at the Reina Sofia University Hospital.

Fat-loading test

The subjects were instructed to refrain from alcohol consumption on the day before the test date. All subjects were given a high-fat-enriched meal after a 12 h overnight fast. The meal consisted of two cups of whole milk, eggs, bread, bacon, cream, walnuts, and butter that was consumed in 20 min. The macronutrient composition of this meal was 60% calories as fat, 15% as protein, and 25% as carbohydrates. The meal provided 1 g of fat and 7 mg of cholesterol per kilogram of body weight. The fat composition of the test meal was 63% saturated fatty acid, 33% MUFA, and 4% PUFA. The total calories consumed by the subjects ranged from 825 to 1,583 kcal, depending on their weight. Blood samples were drawn at baseline before the fat load, every hour until 6 h, and every 2.5 h until 11 h after the fat load.

Lipoprotein separation

Blood was collected in tubes containing EDTA to a final concentration of 0.1% EDTA. Plasma was separated from blood cells by centrifugation at 1,500 *g* for 15 min at 4°C. The chylomicron fraction of triglyceride-rich lipoproteins (large-TRLs) was isolated from 4 ml of plasma overlaid with 0.15 M NaCl and 1 mM EDTA (pH 7.4, $d = 1.006$ g/ml) by a single ultracentrifugal spin (28,000 *g*, 30 min, 4°C) in a 50 type rotor (Beckman Instruments, Fullerton, CA). Chylomicrons and VLDLs contained in the top layer were removed by aspiration after cutting the tubes, and the infranatant was centrifuged at a density of 1.019 g/ml for 24 h at 115,000 *g* in the same rotor. The nonchylomicron fraction of TRLs (small-TRLs) was removed from the top of the tube. Large and small TRL fractions were stored at -70°C .

Lipid analysis

Cholesterol and TGs in plasma and lipoprotein fractions were assayed by enzymatic procedures (15, 16). ApoB was determined by turbidimetry (17). HDL-cholesterol was quantified by analyzing supernatant obtained after precipitation of the plasma ali-

quot with dextran sulfate-Mg²⁺, as described by Warnick, Benderson, and Albers (18). LDL-cholesterol was calculated as the difference between the cholesterol at the bottom of the tube after ultracentrifugation at 1.019 g/ml and the HDL-cholesterol.

DNA amplification and genotyping

DNA was extracted from 10 ml of EDTA-containing blood. The single nucleotide polymorphism (SNP) nomenclature used in this study is based on the guidelines suggested by the Human Genome Variation Society (19, 20). Two polymorphisms, p.Leu162Val (rs1800206) and c.140+5435T>C (rs135549; nomenclature based on NM_005036.4), were examined.

The *PPAR* α SNPs were genotyped using the TaqMan 5' nuclease allelic discrimination method (Assay by Design/Demand; Applied Biosystems, Foster City, CA). The primers for p.Leu162Val were 5'-CAGAAACAAATGCCAGTATTGTCGAT-3' and 3'-CCTTACCTACCGTTGTGTGACATC-5', and the primers for c.140+5435T>C were 5'-CCACCGCAGCGAAATTGT-3' and 3'-ACCTGCTCCCTACAGAAGGAAGA-5'. The probes for p.Leu162Val were VIC-ACAAGTGCCTTTCTG-NFQ and FAM-CAAGTGCCTTTCTG-NFQ, and those for c.140+5435T>C were VIC-CAGCTCCCCCACA and FAM-CAGCTTCCCCCACAC. Nucleotides in bold represent the polymorphism. Genotyping was conducted in accordance with the manufacturer's instructions. For c.140+5435T>C, the major allele homozygotes, heterozygotes, and minor allele homozygotes are designated TT ($n = 25$), TC ($n = 25$), and CC ($n = 9$), respectively. The TC and CC were grouped together to test for a dominant genetic model; thus, all genetic comparisons are between TT and TC/CC. Similarly, for p.Leu162Val, there was one minor allele homozygote subject (VV; $n = 1$); therefore, this individual was grouped with the heterozygote subjects (LV; $n = 10$) and compared with the major allele homozygotes (LL; $n = 48$).

Statistical analysis

Each SNP was tested for Hardy-Weinberg equilibrium using the Chi-square test. The strength of pair-wise linkage disequilibrium between the two SNPs was calculated as correlation (r) using Helix Tree software (Golden Helix, Bozeman, MT).

Subsequent analyses were conducted using SAS statistical software (Windows version 9.1; Cary Institute). Distribution of the data was examined, skewed variables were log-transformed to determine significant associations, and means and SEM were estimated using the untransformed variable. Baseline differences by *PPAR* α genotype were assessed with one-way ANOVA. The *PPAR* α genotype effects on postprandial lipid concentrations were examined by two approaches. First, the change in lipid concentrations over time was analyzed using mixed model repeated-measures analysis. Three hypotheses were examined: *a*) the effect of genotype independent of time (P1); *b*) the effect of time independent of genotype (P2); and *c*) the interaction between genotype and time (P3). Four correlation structures (compound symmetry, unstructured, autoregressive, and autoregressive with heterogeneous variance) were examined, and the best fit model was determined using the Akaike information criteria. The effect of genotype at each time point was determined using the SLICE option of LSMEANS. Differences between genotype groups were examined. Second, the area under the curve (AUC) was calculated using the trapezoidal rule. For postprandial TGs, in addition to total AUC, an increase in AUC was calculated by subtracting the area under the baseline value. The AUC values for each lipid class were compared using ANOVA. All postprandial analyses included age, body mass index, smoking, and apoE genotype (E3/E3 vs. E2 or E4 carriers) as covariates. For all analyses, $P < 0.05$ was considered significant.

Some subjects had missing data points. Analyses with list-wise deletion and replacement with the mean were compared. In all cases, the results remained the same. Therefore, results from the list-wise deletion analyses are presented.

RESULTS

Allele frequencies and linkage disequilibrium

The genotype frequencies for both *PPARα* SNPs examined in this study were in Hardy-Weinberg equilibrium (p.Leu162Val, Chi-square = 0.309, $P = 0.579$; c.140+5435T>C, Chi-square = 0.429, $P = 0.513$). The position of the SNPs within the gene is based on the *PPARα* mRNA (NM_005036.4) in the National Center for Biotechnology Information database. There are currently five alternative splice products in the database, and the location of the SNP depends on the mRNA product. In prior studies, p.Leu162Val has been located in exon 5; however, in this mRNA product, the SNP is located in exon 6. The minor allele frequency for this SNP was 10.2%, consistent with prior published reports in a Caucasian population (10, 12). c.140+5435T>C is located in either intron 2 or in the 5' untranscribed region. The minor allele frequency was 36.4%. The two SNPs are in significant linkage disequilibrium ($r = 0.335$, $P = 0.01$); however, the strength of the association is low.

Baseline and postprandial parameters by p.Leu162Val

There were significant baseline differences by genotype in TC, LDL-C, and apoB (Table 1). For each of these measurements, the minor allele carriers displayed greater concentrations compared with the major allele homozygotes.

With the repeated-measures analysis, there were significant genotype effects in postprandial TC ($P_1 = 0.003$), LDL-C ($P_1 = 0.001$), and apoB ($P_1 = 0.008$) (data not shown). A significant time effect was observed for LDL-C ($P_2 < 0.001$) and apoB ($P_2 = 0.02$). For the three measurements, the minor allele carriers maintained greater concentrations compared with the major allele homo-

zygotes throughout the postprandial period. There were no significant genotype and time interactions for all three fractions, suggesting that the pattern of postprandial changes is similar across genotypes. Because there were significant differences in these lipoprotein fractions at baseline, the analysis was repeated with adjustment for these values. After baseline correction, there were no significant differences, suggesting that the genotype effect is not a postprandial phenomenon. Analysis of the AUC was consistent, with the minor allele carriers displaying higher values for TC, LDL-C, and apoB (Table 2).

Baseline and postprandial parameters by c.140+5435T>C

There were no significant differences in the baseline characteristics across c.140+5435T>C genotypes for all parameters examined (Table 1).

The increase and decrease of postprandial TG in the small TRL fraction (sTRL-TG) is reflected by the significant effect of time (Fig. 1A; $P_2 < 0.001$). There was no effect of genotype independent of time ($P_1 = 0.168$); however, there were significant differences in the pattern of postprandial response by genotype, as reflected by the interaction between time and genotype ($P_3 = 0.010$). The major allele homozygotes had greater postprandial response in sTRL-TG compared with the minor allele heterozygotes and homozygotes, with significant differences at times 6 and 8.5 h ($P < 0.05$). Similarly, there was a significant genotype difference in the increase in sTRL-TG AUC (Table 2; $P = 0.008$), in which the major allele homozygotes had higher concentrations compared with the minor allele heterozygotes and homozygotes. The mean TG AUC in total and large TRLs did not differ by genotype group.

There were significant independent genotype and time effects on sTRL cholesterol (sTRL-C) concentrations (Fig. 1B; $P_1 = 0.029$, $P_2 < 0.001$). In addition, there was a significant interaction between genotype and time ($P_3 = 0.033$), in which the major allele homozygotes displayed greater postprandial sTRL-C response compared with the minor allele heterozygotes and homozygotes.

TABLE 1. Baseline characteristics by *PPARα* genotype

Characteristic	c.140+5435T>C			p.Leu162Val		
	TT (n = 25)	TC/CC (n = 34)	<i>P</i>	LL (n = 48)	LV/VV (n = 11)	<i>P</i>
Age, years	24 ± 6	22 ± 4	0.210	23 ± 5	24 ± 6	0.461
Body mass index (kg/m ²)	25 ± 4	25 ± 3	0.972	25 ± 4	25 ± 3	0.891
TC	152 ± 28	152 ± 22	0.896	148 ± 21	171 ± 28	0.003
Small TRL-cholesterol	11 ± 6	9 ± 5	0.119	10 ± 5	10 ± 6	0.894
Large TRL-cholesterol	4 ± 3	4 ± 3	0.856	4 ± 3	4 ± 1	0.423
Total TG ^a	81 ± 31	80 ± 35	0.755	82 ± 34	75 ± 27	0.575
Small TRL-TG ^a	36 ± 21	32 ± 16	0.447	34 ± 19	30 ± 15	0.659
Large TRL-TG ^a	18 ± 12	17 ± 13	0.312	18 ± 14	16 ± 10	0.979
LDL-cholesterol	89 ± 26	93 ± 23	0.531	87 ± 22	112 ± 25	0.001
HDL-cholesterol	47 ± 11	47 ± 11	0.828	47 ± 10	46 ± 13	0.790
ApoB	66 ± 21	64 ± 17	0.661	62 ± 18	80 ± 15	0.004
ApoA-I	99 ± 19	97 ± 15	0.755	98 ± 17	96 ± 17	0.650
Smoking, % (n)	32% (8)	26% (9)	0.221	31% (15)	18% (2)	0.484
ApoE3/E3, % (n)	80% (20)	94% (31)	0.221	85% (40)	100% (11)	0.327

Values shown are means ± SD. All lipid and apolipoprotein values are in mg/dl.

^a*P* values are derived from log-transformed variables.

TABLE 2. Differences in area under the lipid curve by PPAR α genotype

Characteristic	c.140+5435T>C			p.Leu162Val		
	TT (n = 25)	TC/CC (n = 34)	P	LL (n = 48)	LV/VV (n = 11)	P
TC	77.8 \pm 3.1	77.2 \pm 3.4	0.866	76.8 \pm 2.6	87.6 \pm 4.6	0.011
Large TRL-cholesterol	4.4 \pm 0.3	4.2 \pm 0.4	0.664	4.3 \pm 0.3	4.5 \pm 0.5	0.767
Small TRL-cholesterol	7.6 \pm 0.6	6.3 \pm 0.6	0.039	7.0 \pm 0.5	7.2 \pm 1.0	0.823
HDL-cholesterol	23.8 \pm 1.3	23.3 \pm 1.4	0.756	23.7 \pm 1.1	22.7 \pm 2.0	0.615
LDL-cholesterol	42.0 \pm 2.9	43.7 \pm 3.2	0.611	41.9 \pm 2.4	54.0 \pm 4.3	0.003
ApoA-I	51.0 \pm 1.8	48.7 \pm 2.0	0.273	50.1 \pm 1.6	48.5 \pm 2.9	0.532
ApoB	32.4 \pm 2.2	30.6 \pm 2.3	0.462	31.1 \pm 1.8	38.4 \pm 3.2	0.015
Total TG ^a	94.5 \pm 7.7	85.1 \pm 8.3	0.288	90.6 \pm 6.8	88.1 \pm 12.2	0.677
Increase in TG	52.5 \pm 6.2	41.1 \pm 6.6	0.104	47.3 \pm 5.6	50.5 \pm 9.9	0.718
Large TRL-TG ^a	38.7 \pm 4.1	34.2 \pm 4.4	0.342	36.8 \pm 3.6	36.1 \pm 6.5	0.696
Increase in large TRL-TG	30.2 \pm 3.7	25.9 \pm 3.9	0.289	28.3 \pm 3.3	29.5 \pm 5.8	0.815
Small TRL-TG ^a	30.6 \pm 2.3	25.4 \pm 2.5	0.207	28.4 \pm 2.1	27.6 \pm 3.9	0.719
Increase in small TRL-TG	11.0 \pm 1.7	5.8 \pm 1.8	0.008	8.7 \pm 1.6	9.9 \pm 2.9	0.638

Values shown are means \pm SD (mg/dl \times min).

^aP values are derived from log-transformed data.

There were significant genotype effects at times 1, 4, 6, and 8.5 h ($P < 0.05$). Analysis of the AUC of sTRL-C was consistent, with the mean concentrations being higher in major allele homozygotes compared with the minor allele heterozygotes and homozygotes (Table 2; $P = 0.039$). There were no significant differences in TC or large TRL-C.

DISCUSSION

This study demonstrates that variations at the PPAR α gene influence both fasting and postprandial lipid con-

centrations independently of the effects associated with the apoE locus. The minor allele frequencies for both variants studied (p.Leu162Val and c.140+5435T>C) were consistent with prior reports and the frequency reported in the National Center for Biotechnology Information for Caucasian populations (8–13, 21–23); of these, p.Leu162Val is the most widely studied because of its potential functionality (9). We also chose to examine c.140+5435T>C for its high frequency in Caucasian populations and its location in the putative promoter region. In this population, these two variants were in significant linkage disequilibrium. However, the correlation was low.

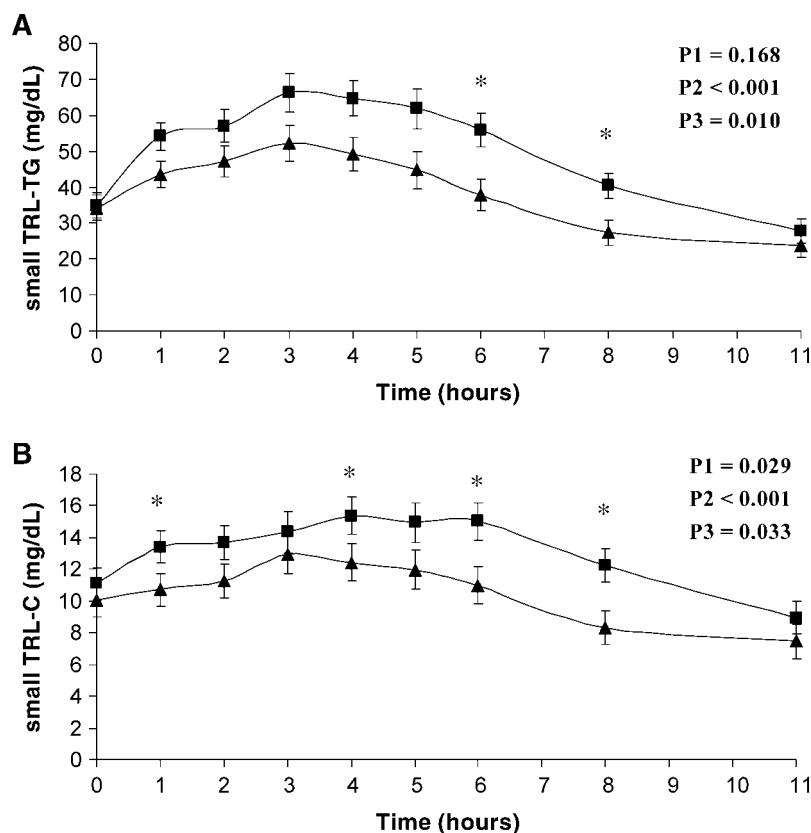


Fig. 1. Changes in postprandial plasma lipids by peroxisome proliferator-activated receptor α (PPAR α) c.140+5435T>C genotype. The mean and SEM of small triglyceride-rich lipoprotein-triglyceride (TRL-TG) (A) and small TRL-cholesterol (TRL-C) (B) during an 11 h postprandial period are shown for TT (n = 25; closed squares) and TC/CC (n = 34; closed triangles). The P values displayed are as follows: P1, genotype effect; P2, time effect; P3, genotype-by-time interaction. * Significant genotype effect at $P < 0.05$.

Therefore, these variants may provide information about different haplotypes of *PPAR α* . Our results show that the p.Leu162Val variant was associated with increased fasting TC, LDL-C, and apoB. This is in agreement with our report from the Framingham Offspring Study in which V-allele male subjects displayed higher TC, LDL-C, apoB, and apoC-III concentrations (12). Significant associations of this polymorphism with these lipid fractions have also been reported in other healthy and diabetic populations (9, 13). Prior studies have suggested that the associations between p.Leu162Val and lipid and lipoprotein concentrations may be modified by dietary fats (11, 24). Our report focuses on the association between variants at this locus and acute response to an oral fat load. Whereas we did not observe significant interaction between plasma postprandial lipid levels and p.Leu162Val, lower postprandial TG and cholesterol response was observed in the small TRL fraction by c.140+5435T>C polymorphism. This can be a reflection of reduced production and/or increased clearance of these particles.

PPAR α modulates the metabolism of TRL both at the stage of production and at clearance of VLDL. Through the regulation of genes involved in the transport and catabolism of fatty acids, *PPAR α* regulates the availability of hepatic TG for VLDL assembly. Fasted *PPAR α* -null mice display hepatic steatosis, hypoglycemia, hyperketonemia, hypothermia, and increased plasma free fatty acids as a result of impaired fatty acid oxidation (25, 26). On a high-fat diet, *PPAR α* /apoE double knockout mice develop higher serum total TG concentrations as a result of increases in VLDL secretion (27). In the absence of *PPAR α* , this change is likely attributable to the greater availability of hepatic TG for VLDL assembly. In humans, the induction of *PPAR α* by fibrates decreases TG levels through decreased production and increased clearance of VLDL (28). The decreased VLDL production may be accounted for by fibrate-induced attenuation of TG synthesis (29). The increased clearance of VLDL as well as chylomicrons is achieved by the increased expression of LPL and a concomitant suppression of apoC-III (30, 31). Increased LPL accelerates clearance through its lipolytic activity and by promoting the interaction of VLDL and chylomicron remnants to LDL receptors. In addition, the accelerated clearance of VLDL may also be attributable to the production of TG-poor particles from the liver that give rise to small dense LDLs that are more quickly metabolized (32).

The p.Leu162Val variant confers an amino acid change in the DNA binding domain of the protein on exon 6. This change results in a protein with altered transactivation efficiency in vitro (9, 14). The protein encoded by the minor allele, or 162V, had greater transactivation efficiency with the addition of a *PPAR α* agonist in a dose-dependent manner (9, 14). In agreement with this observation, a study in the Framingham Heart Study showed that plasma TG and apoC-III concentrations varied with the level of PUFA intake, with carriers of the V-allele having greater differences by dietary fatty acid content (11). The hypotriglyceridemic effect of *PPAR α* was observed in V-allele carriers with high PUFA intake who dis-

played lower TG and higher apoC-III compared with subjects with low PUFA intake. Conversely, in the L162 subjects, TG and apoC-III concentrations remained constant across PUFA intakes. The fat load used in our study is composed primarily of saturated fatty acids that are not ligands for *PPAR α* . This may explain why no interactions were observed between this variant and postprandial lipids. c.140+5435T>C is located ~19 kb upstream of the transcription initiation site for three of the five transcripts and in intron 2 for the other two splice products. Although no functional analysis has been reported to date, it is conceivable that this variant has an effect on the absolute level of *PPAR α* expression or is in linkage disequilibrium with another functional variant within the promoter. If c.140+5435T>C does affect the expression of *PPAR α* , the lower postprandial TG in the minor allele carriers would be consistent with a gain-of-function variant resulting in higher expression levels. Higher *PPAR α* expression may then contribute to lower TG through attenuated hepatic TG production and apoC-III expression and concurrent increased LPL expression. Functional analyses of this variant are necessary to support this hypothesis. In addition, future studies need to examine other SNPs within the gene to determine whether the associations we observed are attributable to linkage disequilibrium with another functional variant.

Epidemiological studies suggest postprandial lipids as an important determinant of CVD risk (2). Various factors predict an unfavorable postprandial lipid response, such as older age, sex, adiposity, and smoking. There is mounting evidence that variants in genes involved in the lipid metabolic pathway, including the apolipoproteins and lipases, affect postprandial lipid response (33, 34). Although the magnitude of the genetic effect on variation in the postprandial lipid response is unknown, each of these genes seems to have a small, yet measurable, effect on postprandial lipid metabolism. Interestingly, polymorphisms in some of the *PPAR α* -mediated genes, such as *LPL*, *APOC3*, *APOA5*, and *SCARB1*, modify the postprandial TG response (35–39). In this study, we report that *PPAR α* is involved in the homeostasis of lipids in the fasted state as well as during the acute postprandial response to dietary fat. Understanding the effect of each of these genes on lipid parameters may provide better assessment methods to predict CVD. In addition, the average diet in Spain, where this study was conducted, is higher in MUFAs compared with other Western countries (15.6–20% in Spain vs. 12.4% in the United States) (40, 41). A MUFA-rich diet has been shown to decrease postprandial TG concentrations compared with diets that are rich in saturated fatty acids (42); therefore, the generalizability of the results of this study will need to be confirmed in future studies. ■

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