patient-oriented and epidemiological research

The effect of *IL6*-174C/G polymorphism on postprandial triglyceride metabolism in the GOLDN study[S]

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Abstract Chronically elevated interleukin-6 (IL-6) affects lipid and lipoprotein metabolism. Individuals genetically predisposed to higher IL-6 secretion may be at risk of dyslipidemia, especially during the postprandial phase. We investigated the effect of genetic variants at the *IL6* locus on postprandial lipemia in US Whites participating in the Genetics of Lipid Lowering Drugs and Diet Network study. Subjects were given a single fat load composed of 3% of calories as protein, 14% as carbohydrate, and 83% as fat. Blood was drawn at 0 h, 3.5 h, and 6 h to determine plasma triglyceride (TG), TG-rich lipoprotein (TRL) and lipoprotein particle size. Homozygotes (GG) and heterozygotes (CG) of the -174C/G variant displayed higher plasma IL-6 concentrations compared with major allele homozygotes (CC) ($P = 0.029$). GG and CG subjects showed higher fasting plasma TG ($P = 0.025$), VLDL ($P = 0.04$), and large VLDL ($P = 0.02$) concentrations than did CC subjects. Moreover, GG and CG subjects experienced greater postprandial response of TG ($P = 0.006$) and TRL, including chylomicrons ($P = 0.005$), total VLDL ($P = 0.029$), and large VLDL ($P = 0.017$) than did CC subjects. These results suggest that the functional polymorphism -174C>G at the *IL6* locus determines the difference in both fasting and postprandial TG metabolism. This phenomenon could be responsible for the observed association of this genetic variant with cardiovascular disease risk.—Shen, J., D. K. Arnett, P. Pérez-Martínez, L. D. Parnell, C-Q. Lai, J. M. Peacock, J. E. Hixson, M. Y. Tsai, R. J. Straka, P. N. Hopkins, and J. M. Ordovás. **The effect of *IL6*-174C/G polymorphism on postprandial triglyceride metabolism in the GOLDN study. *J. Lipid Res.* 2008.** 49: **1839–1845.**

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Current evidence indicates that inflammation plays an instrumental role in atherosclerosis development (1). A number of markers of inflammation, namely fibrinogen, highly sensitive C-reactive protein, interleukin-6 (IL-6), myeloperoxidase, and soluble CD40 ligand, have been explored as means of predicting cardiovascular disease (CVD) (2). Moreover, chronic inflammation has been shown to associate with alterations of lipid and lipoprotein metabolism (3). A more atherogenic lipid profile, characterized by increased triglyceride (TG), small, dense LDLs, and decreased HDL cholesterol (HDL-C), is common among patients with chronic inflammatory diseases such as HIV and rheumatoid arthritis and metabolic abnormalities with a strong inflammatory basis, including obesity, diabetes, and metabolic syndrome (4–8). Moreover, those patients typically experience an elevated postprandial lipemia (PPL) (4, 9–11), which leads to the production of atherogenic triglyceride-rich lipoprotein (TRL) and activation of thrombotic processes (12, 13). It has been well documented that PPL response varies considerably between individuals and is subject to genetic regulation. Thus, many studies have reported that genetic variants of genes involved in the lipid and lipoprotein metabolic pathway are associated with the extent and magni-

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Abbreviations: CVD, cardiovascular disease; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; IL-6, interleukin-6; PPL, postprandial lipemia; SNP, single-nucleotide polymorphism; TG, triglyceride; TRL, TG-rich lipoprotein.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of four tables.

tude of PPL response and consequently modify CVD risk related to dyslipidemia (14).

IL-6 is a major mediator of immune response and inflammatory processes. In addition to immune cells, IL-6 is produced by endothelial and smooth muscle cells, and adipose tissue (15, 16), underpinning its pleiotropic action in the regulation of endothelial and adipose function and metabolism of glucose and lipid (3, 17–19). In humans, increased serum IL-6 levels are correlated with increased fasting serum TG and free fatty acids and low HDL-C levels (20, 21). Animal models support a causal relationship between elevated IL-6 levels and increased hepatic secretion of TG and production of VLDL particles with both high sphingolipid content and longer residence time (22). In addition, increased IL-6 levels perturb HDL metabolism, resulting in both reduced concentrations and a less atheroprotective function of HDL (3). Finally, plasma IL-6 levels are a heritable trait (23), and several potential functional polymorphisms in the 5′ flanking region of the *IL6* locus have been shown to alter *IL6* gene transcription and protein production (24).

Given the interrelation between IL-6 levels and lipoprotein metabolism, it is conceivable that individuals genetically predisposed to higher IL-6 secretion are prone to be in a dyslipidemic state, especially during the postprandial phase. Therefore, the aim of this study was to investigate the effect of putatively functional variants at the *IL6* locus on lipoprotein postprandial response following a fat load challenge among US Whites participating in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.

MATERIALS AND METHODS

Study participants

The study population consisted of 326 male and 394 female with normotriglyceridemia who participated in the GOLDN study. The GOLDN participants were rerecruited from three-generational pedigrees from the ongoing NHLBI Family Heart Study (FHS) (25) in two genetically homogeneous centers (Minneapolis and Salt Lake City) of Caucasian populations. The detailed design and methodology of the study have been described previously (26, 27).

Briefly, participants were asked to fast for >12 h and abstain from using alcohol for >24 h before visiting the clinic. The baseline clinical exam included anthropometric and blood pressure measurements. Questionnaires were administered to solicit demographic and lifestyle information; medical history and medication history. The postprandial fat-load test consisted of a meal formulated according to the protocol of Patsch et al (28). This meal contained 700 calories/m^2 body surface area (2.93 MJ/m^2 body surface area); 3% of calories were derived from protein, 14% from carbohydrate, and 83% from fat sources. Cholesterol content was 240 mg and the ratio of polyunsaturated fat to saturated fat was 0.06. Participants were instructed to consume this high-fat meal within 15 min. Blood samples were drawn immediately before (time 0) and 3.5 and 6 h after the high-fat meal. The protocol for this study was approved by the Human Studies Committee of the Institutional Review Board at University of Minnesota, University of Utah and Tufts University/New England Medical Center. Written informed consent was obtained from all participants.

Biochemical measurements

Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation, Indianapolis, IN) on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). Cholesterol was measured on the Hitachi 911 using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). The same reaction was also used to measure HDL-C after precipitation of nonHDL-C with magnesium/dextran. LDL-C was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, PA) on the Hitachi 911. Lipoprotein particles concentration and size, including TRLs, LDL, and HDL, were measured by NMR. The quantification of the lipoprotein subclass was based on amplitudes of their spectroscopically distinct nuclear magnetic resonance signals of the lipid methyl group (29). Plasma IL-6 was measured using an enzyme-linked immunosorbent assay (R and D Systems Inc.).

DNA isolation and genotyping

Five common *IL6* polymorphisms were selected for genotyping including three promoter single-nucleotide polymorphisms (SNPs) -1363G/T (rs2069827), -599C/T (rs1800797), -174C/G (rs1800795), and 613G/A in intron2 (rs2069832) and 3329A/G in intron 4 (rs2069845). Genomic DNA was extracted from blood samples and purified using commercial Puregene® reagents (Gentra System, Inc..) following the manufacturer's instructions. Genotyping was performed using the 5′nuclease allelic discrimination Taqman assay with ABI 7900HT system (Applies Biosystems, Foster City, CA). The description of primer and probe sequences as well as ABI assay-on-demand IDs are presented in supplementary Table I.

Statistical analyses

Statistical analyses were performed using SAS for Windows version 9.0 (SAS Institute, Cary, NC). A Chi-square test was used to determine if genotype distribution followed Hardy-Weinberg equilibrium. Plasma TG, LDL, chylomicron, nonchylomicron including VLDL and subclasses and particle size were log-transformed to achieve approximate normal distribution. With no significant gender modification observed, men and women were analyzed together. To present these variables on their original scale, geometric means and their 95% confidence intervals (CI) were calculated by taking the exponentiation of adjusted least-squares means and their corresponding confidence intervals. For the cross-sectional analyses between continuous and dichotomous outcomes and genotypes, we used the generalized estimating equation (GEE) linear and logistic regression with exchangeable correlation structure to adjust for the correlated observations due to familial relationship. To compare postprandial lipid responses across genotype groups, we fitted the multilevel model with individual repeated measurements over three time points (level 1) within individuals (level 2) and individuals clustered within pedigrees (level 3) as implemented in Mixed procedure in SAS (30). In this model we tested overall genotype effects independent of time and the interaction between genotype and time. We also calculated postprandial area under the curve (AUC) of lipid parameters using the trapezoidal rule and compared mean values of each lipid class across genotypes using GEE. Covariates included age, gender, BMI, smoking (never, former and current smoker), alcohol use (nondrinker and current drinker), physical activity as expressed as metabolic equivalent task (MET)-hours based on self-reported types and durations of

activities over 24 h. Self-reported use of hormone therapy by women (contraceptives, conjugated estrogens, estradiol or progestin), use of certain medications including aspirin, nonsteroidal anti-inflammatory drugs (NSAID), and drugs for hypertension, diabetes and hypercholesterolemia. A two-tailed P value of <0.05 was considered as statistically significant.

RESULTS

Genotype distributions followed Hardy-Weinberg equilibrium (X^2 test, $P > 0.05$). Minor allele frequencies and SNP pair-wise LD values were evaluated in a subset of 148 unrelated subjects (see supplementary Table II). Four SNPs -599C/T, -174C/G, 613G/A and 3329A/G, were nearly in complete linkage disequilibrium (LD) with $R >$ 0.85 whereas the -1363G/T displayed very weak LD with other four SNPs ($R = 0.13$-0.15) suggesting its involvement in an independent haplotype block. The -174C/G SNP has been the most widely studied at this locus because of its recognized functionality (24). Moreover, this SNP represents a tagSNP for the common haplotype in the LD region. Therefore, we focused our analyses on the -174C/G and -1363G/T. The minor allele frequencies for the -174C/G and the -1363G/T were 43% and 10%, respectively, consistent with the frequency reported by the National Center for Biotechnology Information for American Whites (http://www.ncbi.nlm.nih.gov/). Baseline characteristics and measurements are presented in **Table 1** for all subjects and in **Table 2** according to the -174C/G genotype. We found a statistically significant association between the -174C/G variant and fasting plasma IL-6 concentration with homozygotes (GG) and heterozygotes (CG) for the minor allele having higher concentrations when compared with major allele homozygotes (CC) ($P = 0.029$). GG and CG individuals also showed higher fasting plasma total TG ($P = 0.025$), VLDL ($P = 0.04$) and large VLDL ($P = 0.02$) concentrations than CC subjects. Subjects carrying the G allele also had lower levels of fasting large HDL cholesterol ($P = 0.043$) and smaller fasting HDL size ($P = 0.03$) compared with CC subjects.

We examined the association of the -174C/G genotype with postprandial lipid, lipoprotein and particle size following the fat-load challenge (**Fig. 1A–D**). After multivariate adjustment, there was significant genotype effect on postprandial total plasma TG concentrations ($P = 0.006$), in which GG and CG had higher TG response than CC subjects. We further examined the association with TG in chylomicrons and VLDL. GG and CG subjects had greater postprandial chylomicrons ($P = 0.005$), total VLDL ($P = 0.029$) and large VLDL ($P = 0.017$) than CC subjects. Further adjustment for the respective baseline concentrations did not modify the statistical significance of the associations with postprandial plasma TG and VLDL concentrations ($P = 0.020$ for TG and $P = 0.018$ for VLDL); however, the adjustment attenuated the associations with chylomicrons and large VLDL toward null significance. The genotype associations were maintained over the postprandial period examined in this study and reached highest statistical significance at the 6-h time point ($P < 0.01$ for TG, chylomicrons, VLDL and large VLDL). We also observed the significant genotype-time interaction on the postprandial TG response ($P = 0.039$). Notably, controlling for baseline TG values strengthened this interaction ($P = 0.008$), suggesting that the patterns of TG change over a 6-h postprandial period were significantly different across three genotype groups. There were no significant associations of this SNP with other TRL particles including medium and small VLDL, LDL and HDL particles as well as with the size of lipoprotein particles. To further illustrate a genotype-specific differential response to the fat meal, we compared AUC for postprandial lipid measurements across genotype groups (**Table 3**). GG and CG subjects had higher AUC for TG ($P = 0.013$), chylomicrons ($P = 0.022$), VLDL ($P = 0.024$) and large VLDL ($P = 0.009$) than CC subjects. Carriers of the G minor allele also tended to have lower AUC for large HDL ($P = 0.049$) than

TABLE 1. Baseline characteristics of study participants

Variables	Men (n = 326)	Women (n = 394)
Age, y	48.6(17.3)	47.0(16.5)
BMI, kg/m^2	27.7(4.9)	27.1(6.1)
Cholesterol, mg/dl	178.2(33.3)	180.4(35.1)
Triglycerides, md/dl	92.5(68-116)	83(61-109)
LDL-C, mg/dl	119.7(29.4)	113.0(29.5)
HDL-C, mg/dl	44.2(9.6)	54.6(13.8)
Fasting glucose, mg/dl	101.6(14.4)	95.3(13.8)
Fasting insulin, mU/l	12.6(7.3)	12.0(7.1)
IL6, ng/l	1.25(0.86-1.99)	1.35(0.87-2.12)
Current smoker, n (%)	22(6.75)	27(6.87)
Current drinker, n (%)	150(46)	194(49.2)
Hormones treatment, n (%)		80(20.3)

Values are presented as mean (SD) or median (interquartile range).

TABLE 2. Baseline lipid measurements and IL-6 levels according to *IL6*-174C/G

Variables	CC(n = 230)	CG(n = 358)	GG(n = 128)	P
Total Cholesterol, mg/dl	186.03(2.92)	185.38(2.40)	189.30(3.39)	0.416
Triglycerides, md/dl	93.13(3.08)	97.64(2.63)	99.81(2.65)	0.025
LDL-cholesterol, mg/dl	118.61(2.68)	118.92(2.22)	122.09(3.22)	0.283
HDL-cholesterol, mg/dl	53.47(1.29)	52.07(1.11)	52.86(1.32)	0.454
VLDL size, nM	50.88(1.04)	51.45(0.83)	50.97(0.92)	0.677
LDL size, nM	21.15(0.07)	21.12(0.06)	21.14(0.09)	0.812
HDL size, nM	9.02(0.04)	8.97(0.04)	8.92(0.05)	0.03
Large HDL, mg/dl	27.61(1.18)	25.53(1.16)	24.52(1.36)	0.043
Medium HDL, mg/dl	4.61(0.49)	4.87(0.52)	4.90(0.61)	0.394
Small HDL, mg/dl	20.09(0.50)	20.42(0.51)	21.26(0.63)	0.061
Large LDL, mg/dl	68.96(3.46)	65.65(2.62)	67.78(3.44)	0.975
Small LDL, mg/dl	46.31(2.44)	48.17(2.10)	46.56(2.74)	0.618
Medium small LDL, mg/dl	20.49(1.69)	21.23(1.45)	19.65(1.56)	0.492
Very small LDL, mg/dl	25.64(1.55)	26.98(1.44)	27.05(1.83)	0.338
Total VLDL, mg/dl	60.52(2.76)	63.76(2.38)	66.03(2.39)	0.04
Large VLDL, mg/dl	12.71(1.10)	14.47(0.96)	16.43(1.18)	0.02
Medium VLDL, mg/dl	32.42(2.00)	32.93(1.83)	32.84(1.98)	0.818
Small VLDL, mg/dl	11.19(0.70)	11.78(0.55)	12.07(0.73)	0.308
IL6, ng/l	1.80(0.12)	2.12(0.21)	2.53(0.49)	0.029

Values are means (SEM).

P values for additive model were adjusted for age, gender, BMI, physical activity, smoking status, alcohol intake, drugs for diabetes, hypertension and hypercholesteromia, and hormones use in women.

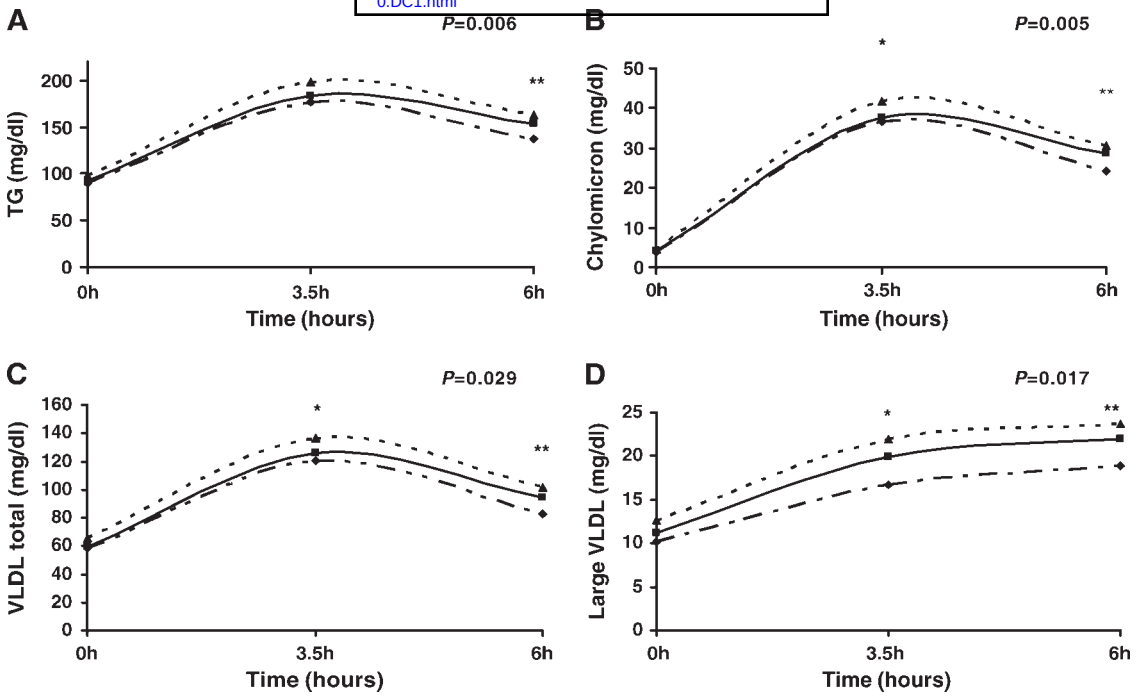

Fig. 1. Postprandial plasma TG (A), chylomicron (B), VLDL total (C) and large VLDL (D) by *IL6*-174C/G genotypes. CC genotype (dotted line, ♦), CG genotype (solid line, ■) and GG genotype (dotted line, ▲). *P* values for overall genotype effect were adjusted for age, gender, BMI, physical activity, smoking status, alcohol intake, drugs for diabetes, hypertension and hypercholesteromia, and hormones use in women. * Significant genotype effect at 3.5 h at $P < 0.05$. ** Significant genotype effect at 6 h at $P < 0.01$.

CC subjects. There were no strong and consistent associations of the -1363G/T with fasting plasma IL-6 concentration and both fasting and postprandial lipid parameters (see supplementary Tables III, IV; Fig. 1.).

DISCUSSION

In this study of a large US White population, we have demonstrated that the presence of the minor G allele at the *IL6*-174C/G SNP, reportedly responsible for higher IL-6 expression, was associated with a more atherogenic fasting lipid profile. Specifically, GG and CG subjects had higher plasma total TG and TRL concentrations, mainly due to increases in total VLDL and large VLDL, and also lower levels of large HDL and smaller HDL particles than CC subjects. More importantly, our study extended this observation to the more dynamic process of PPL following a high-fat meal. Carriers of the G allele experienced greater postprandial responses of TG and TRL including chylomicrons, total VLDL and large VLDL than CC subjects over a 6-h period following a fat-rich meal.

TRL particles including both apoB48- and apoB100-containing lipoproteins reflect the postprandial lipid metabolism. Accumulation of smaller TRL is believed to be atherogenic and thrombogenic as these lipoproteins can penetrate the endothelium, be trapped into the endothelial wall, and initiate or exacerbate the atherosclerotic process (13, 31). In the present study high postprandial responses of chylomicrons and VLDL observed in subjects carrying the minor allele could be due to the consequence of the competition between these two particles for hydrolysis by lipoprotein lipase (LPL) with chylomicron-TG being the preferred substrate (32, 33). G allele carriers, in addition to having higher IL-6 levels, presented higher levels of TRL, mainly chylomicrons and total VLDL and large VLDL than CC subjects, suggesting that those individuals may have increased production and /or low clearance of TRL particles, and this phenomenon could contribute to the increased risk of CVD observed among these subjects (34, 35).

TABLE 3. AUC for lipid parameters by *IL6*-174C/G genotypes

Characteristic	CC (n = 230)	CG (n = 358)	GG (n = 128)	*P*
TG	883(820-952)	931(871-997)	986(920-1,059)	0.013
Chylomicron	156(140-174)	167(151-185)	184(164-207)	0.022
VLDL	549(499-604)	581(533-632)	623(569-681)	0.024
Large VLDL	101(87-117)	117(103-133)	128(111-148)	0.009
Medium VLDL	219(189-254)	215(187-246)	231(199-267)	0.598
Small VLDL	51(44-58)	54(49-60)	56(49-63)	0.173
LDL-cholesterol	818(780-859)	812(779-846)	822(777-869)	0.966
Large LDL	435(389-487)	437(402-475)	450(409-496)	0.613
Medium small LDL	81(66-101)	90(77-107)	87(71-105)	0.421
Small LDL	197(162-240)	222(189-261)	213(177-258)	0.314
HDL-cholesterol	356(339-374)	347(333-362)	347(331-364)	0.259
Large HDL	154(138-173)	146(132-162)	134(119-152)	0.049
Medium HDL	42(36-49)	41(35-48)	44(35-56)	0.682
Small HDL	115(108-123)	118(111-126)	124(115-133)	0.066

Values are geometric means (95% CI) with arbitrary unit.
P values for additive model were adjusted for age, gender, BMI, physical activity, smoking, alcohol intake, drugs for diabetes, hypertension and hypercholesteromia, and hormones use in women.

Our findings for this polymorphism during the fasting state are in accordance with a prior study of healthy Caucasians showing that G allele carriers had higher total and VLDL-TG, higher fasting and postglucose load FFA and lower HDL2-cholesterol than noncarriers (36). Furthermore, our study suggests that carriers of the G allele have greater responses of total TG, TG in chylomicrons and VLDL particles after a fat-load challenge. The strong impact on plasma TG and total VLDL still remained even after controlling for baseline values, suggesting that the genetic effect has an independent postprandial component. Our results reveal the strong influence of this polymorphism on postprandial TG and TRL induced by the fat-load challenge. Interestingly, a previous study suggested that this polymorphism also modulated the exercise-induced change of HDL and its subfraction (37).

Both in vitro and in vivo studies have indicated that the -174C/G polymorphism alters *IL6* gene transcription rate and protein expression (24, 38). In the current study the minor G allele was associated with elevated plasma IL-6 concentrations, supporting previous findings related to the functionality of this polymorphism. However, other studies have shown the common allele of this SNP is responsible for higher IL-6 production (24). The inconsistency may reflect the disease-specific, tissue-specific and/or environment-specific regulation of IL-6 expression. In addition, a recent study has suggested that the promoter haplotypes involving two other SNPs, -1363G/T and -1489CTdel affect the risk of systemic onset juvenile arthritis and these two polymorphisms, primarily -1363G/T, may further differentiate the effect of -174C/G although the functionality of these two SNPs remains to be determined (39). However, our data do not support the functional importance of the -1363G/T regarding the association with plasma IL-6 concentrations and lipid parameters. Nevertheless, current evidence points to the notion that the effect of the -174 C/G may be subject to the control of the regulatory haplotype extending further upstream of the promoter region of *IL6* gene. Our results, together with other reports (38, 40), suggest that the -174 C/G appears to be particularly informative as it represents common haplotypes in the *IL6* locus, spanning eight kbp including all exons, introns, two kbp upstream and one kbp downstream of the transcribed region. Many studies have demonstrated associations of this polymorphism with obesity, insulin resistance, dyslipidemia and CVD (34–36, 41, 42). In line with this notion, the present study demonstrates that the minor G allele was associated with lipid phenotypes from both the fasting and postprandial state that are more atherogenic.

Several mechanisms may underlie the influence of the -174C/G SNP on PPL. The chronic elevation of IL-6 concentrations associated with the minor G allele promotes lipolysis in adipocytes through involvement in the translocation of hormone sensitive lipase (HSL) to the lipid droplet and/or the phosphorylation of proteins, such as perilipin (PLIN), which allows HSL access to the lipid droplets (43). IL-6 increases hepatic de novo FA synthesis and the production of VLDL (44). IL-6 may also increase plasma TG through decreasing VLDL clearance. This effect is mediated via the inhibition of LPL activity in adipose tissue, a key enzyme for hydrolysis of TG in chylomicrons and VLDL (45, 46). In the present study, the minor G allele displayed higher postprandial chylomicrons and total VLDL and large VLDL compared with CC genotypes, suggesting that the minor allele may increase both intestinal and hepatic production of TRL and decrease LPL activity and thus affect the early phase (absorption and synthesis) as well as the late phase (clearance) of PPL. As insulin is an important regulator of LPL activity, IL-6 induced impairment of insulin sensitivity could further suppress LPL activity (47). Moreover, inflammatory cytokines adversely affect enzymes and apolipoproteins associated with HDL, such as lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), paraoxonase 1 (PON1) and APOA1 (3) which results in decreased HDL concentration and altered composition of HDL with less antiatherogenic and antioxidant function. However, our data suggest the -174C/G is unlikely to affect postprandial HDL response in spite of its effect on the fasting large HDL levels and HDL size.

Notably, in our study the genetic effect does not appear to be mediated through fasting plasma IL-6 concentrations as adjustment for plasma IL-6 did not modify the effects of this polymorphism. This could be explained by the fact that a single point measurement of plasma IL-6 concentration may not adequately reflect an individual's long-term exposure of chronic inflammation. Also, circulating IL-6 may be less important than local IL-6 expression as studies have shown that locally produced cytokines possess important auto-/paracrine properties that influence diverse function of other tissue (16, 19). However, due to the lack of measurements of plasma IL-6 response during the postprandial period, we cannot rule out the possibility that the genetic influence on PPL may be mediated through altering postprandial IL-6 responses.

In our study, we performed a number of statistical tests, potentially raising the issue of adjustment of multiple comparisons. However, it is important to underscore that lipid traits are highly correlated phenotypes and genetic markers are also nonindependent. Moreover, statistical tests were hypothesis driven and therefore, with random type I errors, patterns of results, in particular those consistent with previous reports, should be given more weight than isolated results with a single low *P* value (48, 49). Therefore, we did not proceed to adjust for multiple comparisons. Nevertheless, our results still could be a reflection of random association by chance. Replication studies in independent samples will be required to verify the association signals.

In conclusion, our data suggest that the functional polymorphism -174C/G at the *IL6* locus determines differences in both fasting and postprandial TG metabolism. Those individuals carrying the minor G allele are prone to display pro-atherogenic fasting and postprandial lipid phenotypes. Moreover, our data suggest that the hypertriglyceridemic effect of this polymorphism may be due to its impact on both the increase in TG synthesis and the decrease in TG catabolism during the postprandial

phase. These findings provide additional evidence that IL-6 is an important regulator of lipid metabolism and may facilitate the understanding of the causal role of IL-6 in the development of atherosclerosis.

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