The Relation of Plasma Triglyceride, Apolipoprotein B, and High-Density Lipoprotein Cholesterol to Postheparin Lipoprotein Lipase Activity Is Dependent on Apolipoprotein E Polymorphism

J. St-Amand, S. Moorjanit, P.J. Lupien, D. Prud'homme, and J.-P. Després

Postheparin plasma (PH)-lipoprotein lipase (LPL) activity has been reported to be a significant correlate of plasma triglyceride and high-density lipoprotein cholesterol (HDL-C) levels. However, some studies have failed to observe these associations. In this regard, apolipoprotein (apo) E polymorphism may play an important role, since the apo E2 isoform has unfavorable effects on the catabolism of triglyceride-rich lipoprotein particles. We have thus examined the relationships between PH-LPL activity and plasma lipoprotein-lipid levels within groups of men classified on the basis of apo E phenotypes, to verify whether apo E polymorphism could alter these associations. In men carrying the apo E2 isoform (n = 12), PH-LPL activity showed a strong negative correlation with plasma triglyceride ($r = -.72$, $P < .01$), very–low-density lipoprotein (VLDL) triglyceride ([VLDL-TG] $r = -.83$, $P < .001$), and VLDL cholesterol ([VLDL-C] $r = -.57$, $P < .05$) levels and a positive correlation with plasma HDL-C ($r = .87$, $P < .001$) and HDL_2-C ($r = .90$, $P < .001$) concentrations. These correlations were also noted for plasma apo B levels ($r = -.65$, $P < .05$), VLDL-apo B concentrations ($r = -.76$, $P < .01$), and the HDL-C to cholesterol ratio ($r = .85$, $P < .001$). In contrast, none of these associations were found in men carrying the apo E4 isoform (n = 11). In men homozygous for the apo E3 isoform (n = 29), PH-LPL activity was only significantly correlated with plasma HDL_2-C levels ($r = .46$, $P < .01$). Results of the present study indicate that PH-LPL activity is related to plasma triglyceride, VLDL-TG, VLDL-C, VLDL-apo B, apo B, and HDL-C levels and the HDL-C to cholesterol ratio in men carrying the apo E2 isoform, but not in men homozygous for the apo E3 isoform or among apo E4 carriers. Thus, apo E polymorphism appears to modulate the effect of variation in PH-LPL activity on the plasma lipoprotein profile.

LIPOPROTEIN LIPASE (LPL) plays an important role in the catabolism of triglyceride-rich lipoproteins.[1-3] Several studies have reported negative correlations between plasma triglyceride levels and postheparin plasma (PH)-LPL activity.[4-7] High-density lipoprotein cholesterol (HDL-C) and especially HDL_2-C levels are also modulated by LPL[3,8-11] and are positively correlated with PH-LPL activity.[7,12-17] However, the magnitude of these associations between PH-LPL activity and plasma lipoprotein levels has been described as highly variable among studies; some reports showed no significant relationship between PH-LPL activity and plasma triglyceride,[17-22] HDL-C, and HDL_2-C levels.[6,18,22,23]

In this regard, apolipoprotein (apo) E, a protein constituent of chylomicron, very–low-density lipoprotein (VLDL), β-VLDL, intermediate-density lipoprotein (IDL), and HDL,[24] plays an important role in the clearance of triglyceride-rich lipoproteins by acting as a ligand for the lipoprotein remnant receptor and apo B, E receptor.[25] Apo E is polymorphic,[26] and the three common apo E variants are encoded by ε2, ε3, and ε4 alleles at the apo E gene locus.[27] The apo E2 isoform has an amino acid substitution ($Arg_{158} \rightarrow Cys$) in the vicinity of the binding domain, in comparison to apo E3, the most common isoform.[28] Apo E4 also differs from apo E3 by a single amino acid substitution at position 112 ($Cys \rightarrow Arg$). Apo E polymorphism has been shown to have a substantial effect on lipoprotein metabolism.[29] Individuals carrying the ε4 allele usually have higher plasma cholesterol, low-density lipoprotein cholesterol (LDL-C), and apo B levels than individuals who are homozygous for the ε3 allele.[29] Whereas ε2 allele is associated with lower plasma cholesterol, LDL-C, and apo B levels, it also contributes to susceptibility to type III dyslipidemia, which is characterized by accumulation of circulating remnant and IDL particles.[29,30] The low-affinity binding of apo E2 for lipoprotein remnant and LDL receptors is thought to be responsible for its effects on lipoprotein metabolism. Apo E4 shows normal affinity for the remnant or apo B, E receptor, but apo E4–containing triglyceride-rich lipoproteins are cleared more rapidly from the circulation.[31] Thus, it is likely that both apo E polymorphism and LPL activity modulate the clearance of triglyceride-rich lipoproteins, and we have examined this possibility in a sample of 52 healthy men.

SUBJECTS AND METHODS

Subjects

The sample of 52 healthy men aged 30 to 42 years was part of a larger group of 98 men recruited through media advertising.[32] From this previous group, only 52 men had a complete data set, that included plasma lipoprotein levels and PH-LPL activity and apo E phenotype. They provided written consent to participate in this study, which had been approved by the Medical Ethics Committee of Laval University. Each participant underwent a complete medical examination by a physician. Individuals with cardiovascular disease, diabetes, or endocrine disorders or taking medication were excluded. Since physical activity is a known

From the Lipid Research Center, Laval University Medical Research Center, and the Physical Activity Sciences Laboratory, Laval University, Quebec, Canada.

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Address reprint requests to J.-P. Després, PhD, Director, Lipid Research Center, Laval University Medical Research Center, CHUL, 2705 Blvd Laurier, Ste-Foy, Quebec, Canada G1V 4G2.

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correlate of plasma lipoprotein levels and LPL activity, subjects had to be sedentary ($\leq$ one session of 30 minutes continuous endurance exercise per week) to be included in the study. The subjects' body weight was stable for at least 2 months before the study, and smokers were excluded.

Body Fatness

The mean of six hydrostatic weighing measurements was used for estimation of percent body fat from density using Siri's equation.[33] Pulmonary residual volume was determined before immersion in the hydrostatic tank with the closed-circuit helium-dilution method of Meneely and Kaltreider.[34] Waist and hip circumferences were measured following the procedures recommended at the Airlie Conference,[35] and the waist to hip ratio was calculated.

Plasma Lipoprotein-Lipid and Apolipoprotein Analyses

Blood samples were collected in the morning after a 12-hour overnight fast for determination of lipoprotein-lipid and apolipoprotein levels. Venous blood was drawn from an antecubital vein into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) containing EDTA while subjects were in a supine position. Triglyceride and cholesterol concentrations in whole plasma and in lipoprotein fractions were measured enzymatically on an RA-1000 analyzer (Technicon, Tarrytown, NY). Ultracentrifugation was used to isolate plasma VLDL ($d < 1.006$ g/mL),[36] and HDL-C measurements were performed after precipitation of apo B–containing lipoproteins in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$,[37] as previously described.[38] The HDL_3 subfraction was isolated by further precipitation of HDL_2.[39] Apo B concentration was measured in plasma by the rocket immunoelectrophoretic method of Laurell[40] as previously described.[41]

Apo E Phenotyping

Apo E phenotypes were determined as described previously.[42,43] In brief, the VLDL fraction was delipidated with acetone-ethanol 1:1 (vol/vol) followed by diethyl ether at −20°C. The colorless precipitated protein was dried under N_2 at room temperature and stored at −20°C. VLDL-protein was solubilized in 10 mmol/L Tris hydrochloride (pH 8.2) containing 8 mol/L urea and 30 mmol/L dithiothreitol, just before electrophoresis. Apo E isoproteins were separated by isoelectric-focusing electrophoresis in 7.5% polyacrylamide gels containing 8 mol/L urea and a 2.0% mixture of ampholytes pH 4 to 6 (LKB-Produkter, Bromma, Sweden) and pH 5 to 7 (Bio-Rad, Richmond, CA) in a proportion of 4:1. Gels were polymerized in cylindrical tubes and run in a water-cooled column disc electrophoresis apparatus (Hoefer Scientific Instruments, San Francisco, CA) at 4°C for 16 hours at 150V. Samples of known phenotypes, E4/3 and E2/2, were included in each run. Gels were then removed from the tubes and stained with 1% to 2% aqueous Coomassie brilliant blue-R (Sigma Chemical, St Louis, MO) using the method of Malik and Berrie.[44] The various phenotypes were assigned according to the nomenclature as described by Zannis et al.[27] Apo E phenotyping was performed without prior knowledge of the plasma lipoprotein-lipid and apolipoprotein data.

Plasma PH-LPL Activity

Subjects were studied in a 12-hour fasted state for measurement of PH-LPL activity. Blood samples were obtained at 20 minutes after injection of low-dose heparin (10 IU/kg body weight). Plasma was separated by low-speed centrifugation at 4°C, and a 0.5-mL aliquot was frozen and lyophilized overnight. Acetone-ether powders were then prepared from these samples, dried under nitrogen, and stored at −20°C for later assays. LPL activity was measured by a modification of the method of Nilsson-Ehle and Ekman,[45] as previously described.[19] These assays are in agreement with results obtained by selective inhibition of hepatic lipase with antihepatic lipase antibodies.[46] In brief, duplicate aliquots of PH plasma were incubated using glycerol-tri-^{14}C-oleate as substrate in an artificial emulsion in the presence and absence of 1 mol/L NaCl. Free fatty acids released during incubation were selectively extracted and counted for ^{14}C in Aquasol (New England Nuclear, Lachine, Quebec, Canada). LPL activity was calculated as the lipase activity sensitive to 1 mol/L NaCl from the total lipase activity. The coefficient of variation for our PH-LPL assay is 10.1%. LPL activity is expressed as nanomoles of oleic acid released per milliliter plasma per minute. Although the absolute LPL activity measured in plasma after low-dose heparin was less than in other studies that have infused greater amounts of heparin,[4,5,12] we have previously reported that our LPL activity was negatively correlated with plasma VLDL-TG and positively correlated with plasma HDL_2-C levels.[13] Thus, the present LPL assay provides relevant information on subjects' lipoprotein physiology.

Statistical Analyses

Subjects were classified into three groups according to apo E phenotype. Due to the rarity of the homozygous phenotypes E2/2 (n = 2) and E4/4 (n = 3), we have defined the groups as follows: (1) apo E2 group (n = 12), individuals carrying either the E2/2 or E3/2 phenotypes; (2) apo E3 group (n = 29), individuals homozygous for the E3/3 phenotype; and (3) apo E4 group (n = 11), individuals carrying either the E3/4 or E4/4 phenotypes. The two homozygous E2/2 subjects were not outliers in the various distributions. Subjects carrying the E2/4 phenotype were not included in the analyses. The higher frequency of E3/2 and E4/3 in the French-Canadian population is responsible for the enrichment of these phenotypes in the sample.[47] Differences between the three groups were tested for significance by ANOVA. Associations between two variables were quantified using Pearson's product-moment correlation coefficient. The Statistical Analysis System (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Table 1 shows that mean age, body mass index, percent body fat, and waist to hip ratio were comparable among the three apo E phenotype groups. An elevated VLDL-C/TG ratio, which is suggestive of remnant accumulation, was noted in the apo E2 carriers as compared with the other groups. No other significant differences in the plasma lipoprotein-lipid profile were found between the three apo E groups, although apo E4 carriers tended to show the lowest triglyceride and highest LDL-C concentrations.

Relationships between PH-LPL activity and plasma lipoprotein-lipid levels for the overall sample and for each apo E group are shown in Table 2. PH-LPL activity was significantly correlated with plasma VLDL-TG, VLDL-apo B, apo B, HDL-C (Fig 1), and HDL_2-C levels, as well as with the HDL-C to cholesterol ratio, in the whole sample. However, PH-LPL activity was not correlated with plasma triglyceride, cholesterol, VLDL-C, and LDL-C levels in the combined sample. Plasma PH-LPL activity was negatively associated with plasma triglyceride levels in the apo E2 group, whereas no correlation was found in apo E3 homozygotes or apo E4 carriers. Results presented in Fig 2 illustrate that PH-LPL activity was positively associated with plasma HDL-C levels in the apo E2 group, whereas no

Table 1. Characteristics of the Three Groups of Men Defined on the Basis of Apo E Phenotype

Variable	Apo E Phenotype		
	E2	E3	E4
No. of subjects	12	29	11
Age (yr)	37.3 ± 2.8	36.0 ± 3.3	36.4 ± 3.3
Body fat (%)	24.3 ± 7.2	25.0 ± 6.8	24.6 ± 6.9
Body mass index	25.4 ± 3.7	27.0 ± 3.7	26.7 ± 4.7
Waist to hip ratio	0.90 ± 0.08	0.94 ± 0.06	0.92 ± 0.07
TG (mmol/L)	1.70 ± 0.62	1.65 ± 0.92	1.37 ± 0.83
Cholesterol (mmol/L)	4.90 ± 0.78	5.01 ± 0.85	5.08 ± 0.92
Apo B (mg/dL)	84.2 ± 22.0	94.4 ± 21.4	95.2 ± 15.9
VLDL-TG (mmol/L)	1.21 ± 0.65	1.15 ± 0.76	0.90 ± 0.75
VLDL-C (mmol/L)	0.71 ± 0.41	0.58 ± 0.34	0.49 ± 0.44
VLDL-apo B (mg/dL)	9.25 ± 7.32	7.59 ± 3.74	7.18 ± 4.98
LDL-C (mmol/L)	3.17 ± 0.79	3.41 ± 0.77	3.57 ± 0.83
HDL-C (mmol/L)	1.02 ± 0.20	1.02 ± 0.26	1.02 ± 0.20
HDL_2-C (mmol/L)	0.39 ± 0.20	0.35 ± 0.15	0.32 ± 0.11
HDL-C/cholesterol	0.21 ± 0.06	0.21 ± 0.07	0.20 ± 0.04
VLDL-C/TG	0.41 ± 0.12*	0.34 ± 0.05	0.32 ± 0.08
PH-LPL activity (nmol/mL/min)	8.55 ± 5.84	5.72 ± 4.17	6.54 ± 3.15

NOTE. Values are the mean ± SD. E2, subjects homozygous or heterozygous for the E2 isoform; E3, subjects homozygous for the E3 isoform; E4, subjects homozygous or heterozygous for the E4 isoform.
*$P < .05$.

significant relationship was found in apo E3 or E4 groups. Strong and positive correlation coefficients were found between PH-LPL activity and plasma HDL_2-C levels among apo E2 carriers, whereas no associations were noted in the apo E4 group (Table 2). Finally, whereas PH-LPL activity was significantly correlated with plasma VLDL-TG, VLDL-C, VLDL-apo B, and apo B levels and the HDL-C to cholesterol ratio in the apo E2 group, no relationship was found among these variables in apo E3 and E4 groups.

DISCUSSION

Numerous studies have shown that apo E polymorphism affects plasma cholesterol and LDL-C levels, and apo E2 carriers generally have lower levels whereas apo E4 carriers have higher levels of total cholesterol and LDL-C.[29] Apo E2 carriers usually have delayed clearance of apo E2–associated lipoprotein particles from plasma and in contrast apo E4 carriers have enhanced catabolism of chylomicron and VLDL remnants, which can lead to several alterations in lipoprotein metabolism.[48] Despite these observations, there is no evidence for a consistent relationship between apo E phenotype and plasma triglyceride and HDL-C levels,[29] although Dallongeville et al[49] have reported a significant effect of apo E polymorphism on plasma triglyceride and HDL-C concentrations in a meta-analysis. The data presented herein show that plasma LDL-C levels were lower in apo E2 carriers, intermediate in subjects homozygous for apo E3, and elevated in apo E4 carriers. However, the differences were not statistically significant, a phenomenon likely to be attributed to the small sample size. However, even with adequate sample size, the magnitude of the effects of apo E polymorphism varies considerably among populations.[50,51] In the present study, we did not

Table 2. Relationships Between PH-LPL Activity and Plasma Lipoprotein-Lipid Levels in the Whole Sample and in Each Group of Men Defined on the Basis of Apo E Phenotype

Variable	PH-LPL Activity			
	Whole Sample	E2 Carriers	E3 Homozygotes	E4 Carriers
TG	−.14	−.72†	−.03	.12
Cholesterol	.03	−.33	.23	.07
Apo B	−.29*	−.65*	−.11	.11
VLDL-TG	−.29*	−.83‡	−.16	.01
VLDL-C	−.10	−.57*	−.07	.11
VLDL-apo B	−.29*	−.76†	−.04	.20
LDL-C	−.08	−.38	.14	−.07
HDL-C	.43‡	.87‡	.31	.32
HDL_2-C	.60‡	.90‡	.46†	.22
HDL-C/cholesterol	.33†	.85‡	.11	.35

*$P < .05$.
†$P < .01$.
‡$P < .001$.

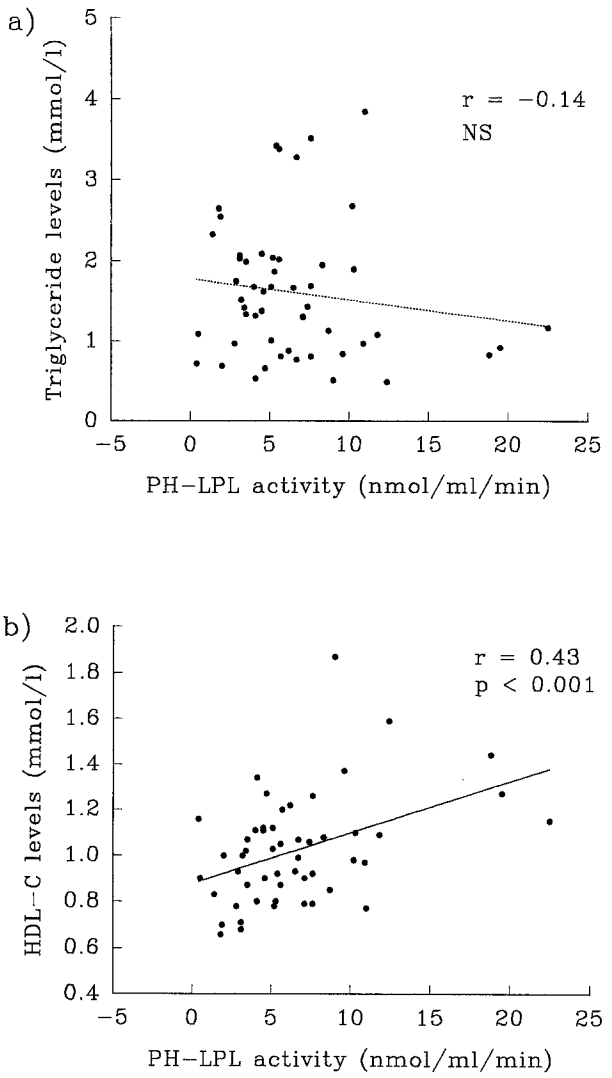

Fig 1. Relationships between PH-LPL activity and plasma triglyceride and HDL-C levels in the whole sample of 52 men.

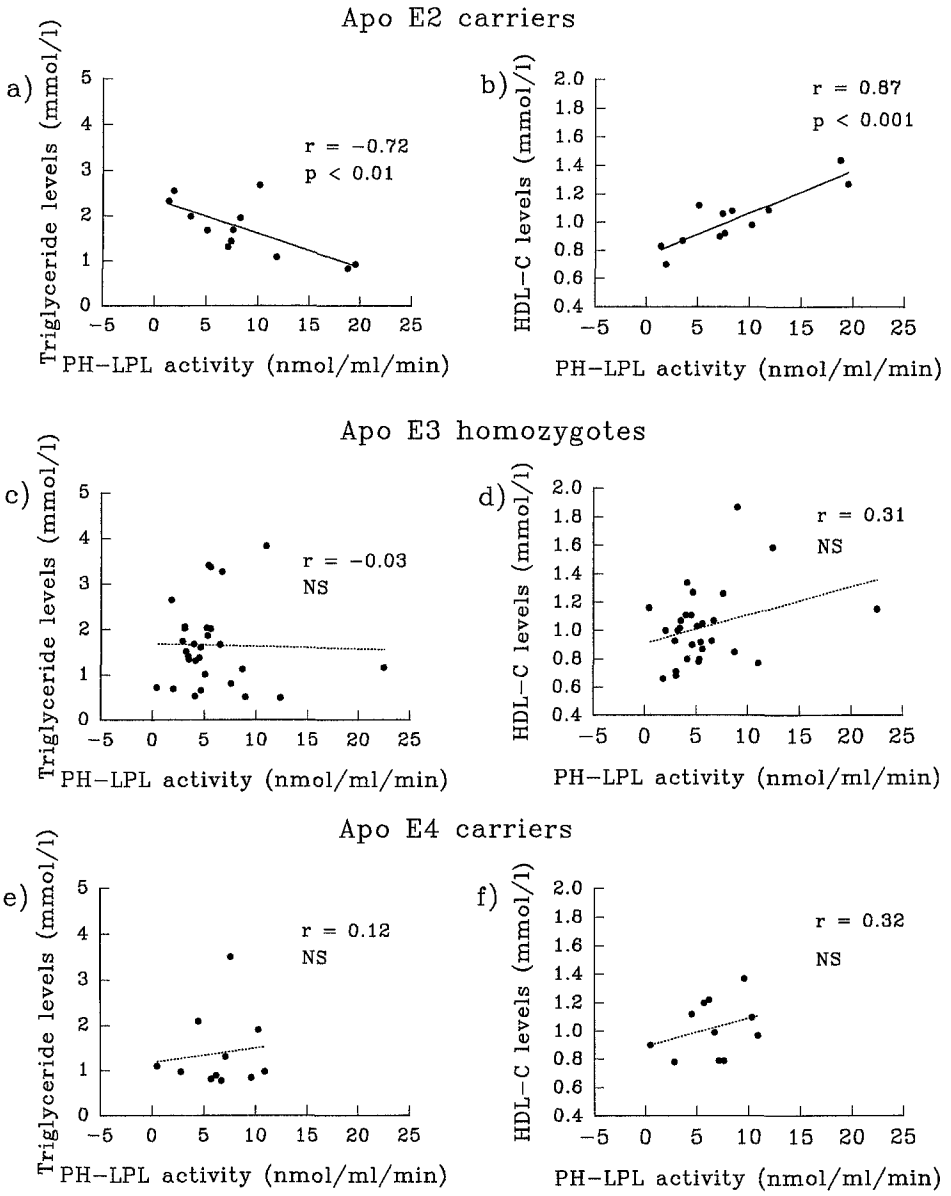

Fig 2. Relationships between PH-LPL activity and plasma triglyceride and HDL-C levels in men defined on the basis of apo E phenotype.

observe any differences in plasma triglyceride and HDL-C levels, which is consistent with several but not all reports.[29,49,52] Interaction between apo E polymorphism and other genetic and environmental factors could be responsible for discrepant results concerning the effect of apo E polymorphism on plasma lipoprotein-lipid levels. Indeed, interactions between apo E polymorphism and apo CII variants,[53] apo B polymorphism,[54] visceral obesity,[55,56] diet,[50,57] and diabetes mellitus[58,59] have been reported to modulate plasma lipoprotein-lipid levels. However, no study has yet investigated the interaction between PH-LPL activity and apo E polymorphism.

We have therefore tested the hypothesis that apo E polymorphism may alter the relationships between PH-LPL activity and plasma lipoprotein-lipid levels. Among apo E2 carriers, PH-LPL activity was strongly associated with plasma triglyceride, VLDL-TG, VLDL-C, VLDL-apo B, apo B, HDL-C, and HDL_2-C levels and the HDL-C to cholesterol ratio. In contrast, no association was found between PH-LPL activity and plasma lipoprotein-lipid levels in apo E4 carriers. In the apo E3 group, only HDL_2-C level was correlated with PH-LPL activity. Without taking apo E polymorphism into account, previous studies have shown a negative correlation between plasma triglyceride levels and PH-LPL activity[4-7] and a positive correlation between plasma HDL-C levels and PH-LPL activity,[7,12,14,15,17] although such associations have not been systematically found.[18,22,23] However, in all studies, including the present one, the correlations were not of high magnitude for either plasma triglyceride or HDL-C levels when apo E polymorphism was not considered. The strong correlation between PH-LPL activity and plasma triglyceride and HDL-C levels found only in apo E2 carriers is indicative that triglyceride and HDL metabolism is particularly modulated by LPL in subjects with the $\epsilon 2$ allele. Thus, the present study may help to account for some of the inconsistent results in the

literature regarding the magnitude of relationships between LPL activity and plasma lipoprotein-lipid levels. These results may also contribute to explain some reported discrepancies regarding the effects of apo E polymorphism on plasma triglyceride and HDL-C levels.

In the present study, PH-LPL activity appeared to be a less critical correlate of plasma triglyceride and HDL levels among subjects with the $\epsilon 4$ allele or with the most common allele, $\epsilon 3$. Indeed, significant associations between PH-LPL activity and plasma triglyceride, VLDL-TG, VLDL-C, VLDL-apo B, apo B, and HDL-C levels and the HDL-C to cholesterol ratio were only found in the E2 group. These results emphasize the importance of apo E in plasma triglyceride and VLDL clearance. Indeed, in the absence of the $\epsilon 2$ allele, LPL activity showed no association with plasma triglyceride and VLDL concentrations. This result is in agreement with the observations of Havel,[60] indicating that as little as 25% of VLDL may normally be converted to LDL, so that direct uptake of VLDL remnants by the liver may be a major pathway of apo B metabolism.

Utermann et al[61] have suggested that type III hyperlipoproteinemia is a polygenic disorder and that other dyslipidemic gene(s) must be present for a homozygous E2/2 to express type III hyperlipoproteinemia. The present results contribute to explaining why the $\epsilon 2$ allele is required to express type III hyperlipoproteinemia ($>90\%$ of type III patients are apo E2/2 homozygotes; the remainder are E2 heterozygotes or homozygotes for rare apo E mutants or compounds), but less than 5% of E2/2 homozygotes are affected by type III hyperlipoproteinemia.[29,48,62] Results of the present study suggest that homozygous E2/2 subjects, who have a lower receptor-mediated clearance of triglyceride-rich lipoprotein remnants, only have elevated plasma triglyceride levels when LPL activity is not sufficiently high to compensate for the reduced clearance of triglyceride-rich lipoproteins. In agreement with the present report, an in vitro study has recently shown that LPL can compensate for the decreased binding of apo E2 to cell receptors.[63] The mechanism responsible for this compensation may be the enhanced lipolysis of triglyceride-rich lipoproteins allowing the VLDL-IDL-LDL cascade and clearance by the LDL receptor to proceed, and/or the structural features of LPL inducing binding to cells.[64-67]

In summary, the present study confirms the role played by LPL as a correlate of plasma triglyceride and HDL-C levels.[1-17] However, the present results indicate that PH-LPL activity is associated with plasma triglyceride, VLDL-TG, VLDL-C, VLDL-apo B, apo B, and HDL-C levels only in individuals with the $\epsilon 2$ allele, and that variation in LPL activity has modest or negligible effects on plasma triglyceride, VLDL-TG, VLDL-C, VLDL-apo B, apo B, and HDL-C levels in men with either apo E3 or apo E4 phenotypes. Thus, the contribution of PH-LPL activity to the regulation of plasma lipoprotein-lipid levels appears to be particularly important among subjects who carry the $\epsilon 2$ allele.

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