Lipoprotein lipase activity is decreased in a large cohort of patients with coronary artery disease and is associated with changes in lipids and lipoproteins

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Abstract **Lipoprotein lipase (LPL) is crucial in the hydrolysis of triglycerides (TG) in TG-rich lipoproteins in the formation of HDL particles. As both these lipoproteins play an important role in the pathogenesis of atherosclerotic vascular disease, we sought to assess the relationship between post-heparin LPL (PH-LPL) activity and lipids and lipoproteins in a large, well-defined cohort of Dutch males with coronary artery disease (CAD). These subjects were drawn from the REGRESS study, totaled 730 in number and were evaluated against 75 healthy, normolipidemic male controls. Fasting mean PH-LPL activity in the CAD subjects was 108 (46) mU/ml, compared to 138 (44) mU/ml in controls ($P < 0.0001$). When these patients were divided into activity quartiles, those in the lowest versus the highest quartile had higher levels of TG ($P < 0.001$), VLDLc and VLDL-TG ($P = 0.001$). Conversely, levels of TC, LDL, and HDLc were lower in these patients ($P = 0.001$, $P = 0.02$, and $P = 0.001$, respectively). Also, in this cohort PH-LPL relationships with lipids and lipoproteins were not altered by apoE genotypes. The frequency of common mutations in the LPL gene associated with partial LPL deficiency (N291S and D9N carriers) in the lowest quartile for LPL activity was more than double the frequency in the highest quartile (12.0% vs. 5.0%; $P = 0.006$). By contrast, the frequency of the S447X LPL variant rose from 11.5% in the lowest to 18.3% ($P = 0.006$) in the highest quartile. This study, in a large cohort of CAD patients, has shown that PH-LPL activity is decreased (22%; $P = 0.001$) when compared to controls; that the D9N and N291S, and S447X LPL variants are genetic determinants, respectively, in CAD patients of low and high LPL PH-LPL activities; and that PH-LPL activity is strongly associated with changes in lipids and lipoproteins.**—Henderson, H. E., J. J. P. Kastelein, A. H. Zwinderman, E. Gagne, J. W. Jukema, P. W. A. Reymer, B. E. Groenemeyer, K. I. Lie, A. V. G. Bruschke, M. R. Hayden, and H. Jansen. **Lipoprotein lipase activity is decreased in a large cohort of patients with coronary artery disease and is associated with changes in lipids and lipoproteins.** ***J. Lipid Res.*** **1999.** 40: **735–743.**

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The role of lipoprotein lipase (LPL) in the modulation and determination of plasma lipid and lipoprotein levels is now well recognized. This is particularly evident in individuals who have a genetic deficiency of LPL (Type 1 hyperlipoproteinemia) and present with markedly elevated chylomicron levels and decreased VLDL, LDL, and HDLc concentrations. Individuals heterozygous for these null genetic defects, with approximately 50% activity, often also show abnormal lipid and lipoprotein levels in fasting plasma (1–5). Variations of LPL activity in normal subjects are likewise known to influence plasma lipids. This is particularly evident for HDLc, where studies in healthy adults (6–9), twins (10), and dyslipidemic patients (11–13) have shown a statistically significant positive correlation with fasting post-heparin plasma LPL (PH-LPL) levels. This finding is in keeping with the hydrolytic action of LPL on triglyceride in the triglyceride-rich lipoproteins and with the entry of the excess surface phospholipid, cholesterol, and apolipoproteins into the HDL pool. The relationship of PH-LPL to fasting plasma TG levels is less clear, as the findings of a negative correlation in healthy adults (6, 14–16), dyslipidemic, obese and diabetic subjects (16–19), has not been substantiated in other studies (20–24).

Abbreviations: LPL, lipoprotein lipase; TG, triglycerides; PH, post-heparin; CAD, coronary artery disease; HDL, high density lipoproteins; LDL, low density lipoproteins; DGGE, density gradient gel electrophoresis; PCR, polymerase chain reaction.

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Significant difficulties in the assessment of some of these studies have been the small numbers of subjects and the resulting low discriminatory power, the presence of mixed genders, and the measurement of LPL activity in different laboratories, with differing assay methods. Here we have re-examined this question in a large single-gender cohort, comprising 730 Dutch males with angiographically defined coronary artery disease. Further design features of significance and relevance were the assay of PH-LPL levels in one laboratory using a single assay procedure and a rigorously controlled protocol of heparin injection and blood withdrawal for LPL determination.

Here we demonstrate that PH-LPL levels are lower in CAD subjects when compared to normolipidemic controls with a 22% decrease in the mean activity level. We further demonstrate unequivocally that PH-LPL activity in male CAD patients correlates inversely with fasting plasma TG and directly with HDLc levels. These correlations are in keeping with the role of LPL in the lipolysis of lipoprotein triglyceride and the generation of HDL particles. Also, the apoE genotype in these subjects was found to be of minor significance in determining the relationships between PH-LPL levels, with lipids and lipoproteins.

We, and others, have previously shown that the D9N and N291S variants in the LPL gene are associated both in vitro and in vivo, with lowered catalytic activity (16, 25–28). We, therefore, determined the frequency of these variants in our CAD cohort and demonstrate that carrier status for these variants is a genetic determinant of low PH-LPL in CAD patients. The joint frequency of these variants in patients in the lowest quartile of PH-LPL levels was more than double that of the frequency in subjects in the highest quartile. Conversely, we demonstrate that the S447X gene variant is a likely contributory factor to the determination of PH-LPL levels in those subjects in the upper quartile of LPL activities.

Collectively these data from the REGRESS study suggest that depressed LPL catalytic function is a significant risk factor for CAD.

MATERIALS AND METHODS

Patients

Post-heparin plasma LPL activity measurements were available from 730 of the 885 subjects in the REGRESS study (29) which was designed as a double-blind, placebo-controlled, multi-center study to assess the effect of pravastatin treatment on the progression and regression of coronary atherosclerosis. No selection bias was evident for the 730 individuals examined. All patients were males of Caucasian descent, below 70 years of age, and had angiographically documented coronary artery disease (>50% stenosis of a major vessel). Patients were specifically excluded who had unstable angina or who suffered a myocardial infarction within the preceding 6 months. All patients had to have total cholesterol levels between 4 and 8 mmol/L and triglyceride levels below 4 mmol/L.

Controls (n = 75) were drawn from the general population, and were healthy, normolipidemic males, off lipid-lowering medication, and between 25 and 55 years of age with a mean age of 46 (5.1) years. All patients participating in the REGRESS trial signed written informed consent and the study protocol was approved by all institutional review boards of all respective hospitals.

Lipid and lipoprotein analysis

All laboratory tests were carried out at the Lipid Reference Laboratory, Academic Center, Amsterdam. Serum cholesterol, HDL cholesterol, and triglycerides were measured on fasting blood samples by standard methodologies (29). LDL cholesterol was calculated according to the Friedewald formula (30). The Lipid Reference Laboratory is an international member of the USA National Cholesterol Reference Method Laboratory Network chaired by the Centers for Disease Control and Prevention (Atlanta, GA).

DNA analysis

In a search for LPL mutations associated with lowered catalytic activity, DNA from all patients in the lowest quartile of LPL activity was subjected to mutation analysis by PCR and density gradient gel electrophoresis (DGGE), with sequencing, where indicated. This search covered the promoter region (−450 to −36) and the translated sequence of exons 1–10 of the LPL gene. The PCR primers used for the amplification of individual exons are given in **Table 1**. In addition, the entire subject cohort was screened for the Asp9Asn (D9N), Asn291Ser (N291S), and Ser447Stop (S447X) mutations in the LPL gene. This was achieved by PCR and restriction endonuclease digestion of amplified product, as described previously (16, 31, 32).

ApoE genotypes were determined by PCR and digestion with the restriction endonuclease Hha1 as described (33).

Post-heparin plasma lipase activity

Lipase activities were obtained on enrollment and were determined in fasting plasma, separated from blood collected after IV heparin (50 IU/kg). Patients were kept sedentary for 20 min after which heparin was injected as a bolus in normal saline. Twenty min later blood was drawn from the alternate arm and immediately placed on wet ice for transport to the central laboratory. All samples were received within 6 h of collection. LPL was determined after inhibition of hepatic lipase (HL) by co-incubation with a goat antibody to human HL (14). All activity assays utilized gum acacia-stabilized [^{3}H]trioleoylglycerol as substrate. Released fatty acids were extracted as calcium salts and counted in a liquid scintillation counter.

The number of subjects in this cohort was large enough to allow for subdivision into groupings of varying LPL levels, which increased the comparative value of the study. Subjects were therefore partitioned into quartiles of PH-LPL activities. Following current convention, quartiles 2 and 3 were combined to give categories of subjects with low, intermediate, and high levels of PH-LPL.

Statistical analysis

The distribution of PH-LPL activity was checked for its shape and was transformed by the Box-Cox method for stabilization and normalization. The association between PH-LPL activity and baseline patient characteristics was assessed using ANOVA, univariate and multiple regression analysis. The frequency of mutations in different quartiles of LPL activity was tested using a two-sided Fishers Exact test.

RESULTS

Patient characteristics

PH-LPL levels were determined in 730 patients. The mean age of this cohort was 56 ± 8.1 years; whereas 73%

TABLE 1. Sets of flanking intronic primers for PCR and DDGE analysis

Region	DGGE Primers	Fragment Size bp
Promoter-1	5′-$(CG)_{20}$ATGTGTGTCCCTCTATCCCTACATT-3′ 5′-GAAAGGGCAGACGGAAAAATTTGCT-3	341 (−450 to −200)
Promoter-2	5′-$(CG)_{20}$AGCAAATTTTTCCGTCTGCCCTTTC-3′ 5′-CTTATGTGACTGGAAATATGCAAA-3′	228 (−176 to −36)
Exon 1	5′-$(CG)_{20}$ATATTTCCAGTCACATAAGCAGCCT-3′ 5′-AGGGGAGTTTGCGCGCAAA-3′	382
Exon 2	5′-$(CG)_{20}$CTCATATCCAATTTTTCC-3′ 5′-CTCTTCCCCAAAGAGCCT-3′	251
Exon 3	5′-$(CG)_{20}$AAGCTTGTGTCATCAT-3′ 5′-CTGGCTCCAGTCAAAAACACTGT-3′	299
Exon 4	5′-CCTATATTTGGAAAACAATATTTATATTCA-3′ 5′-$(CG)_{20}$CCACACATGTGGGTATTTAACAAAATT-3′	347
Exon 5	5′-$(CG)_{20}$TGCCAGTGCATTCAAATGATGAGCAGTGAC-3′ 5′-AAGGGTTAAGGATAAGAGTGACATTTAATT-3′	415
Exon 6	5′-$(CG)_{20}$CAAATGAACACTCTTTGTCAATTTCT-3′ 5′-AGGACTCCTTGGTTTCCTTATTATTTA-3′	391
Exon 7	5′-GATACTTCTGTGGTTCTGAATTGCCTG-3′ 5′-$(CG)_{20}$CAAGGGTTATGGCAGGAGAGGGACT-3′	303
Exon 8	5′-TATTTGGAGAGGAGAAAAAAAAGTGGG-3′ 5′-$(CG)_{20}$GAATTGTGAAGGCCCCTGAAATACAG-3′	391
Exon 9	5′-$(CG)_{20}$CCTGACAGAACTGTACCTTTGTGAACA-3′ 5′-GAATGCATGAAGCTGCCTCCCTTAG-3′	293

were current users of β-blockers and 87% reported a past history of smoking. In the group as a whole (**Table 2**), no correlation was evident between PH-LPL activity, age (r = 0.05; P = 0.11), BMI (r = 0.01; P = 0.22), and systolic or diastolic blood pressure (r = 0.04; P = 0.16 and r = 0.03; P = 0.97, respectively). Other characteristics, notably CAD risk factors, CAD-related parameters, or pharmacological treatment, did not differ among patients in the lowest, intermediate, or highest quartiles of LPL activity (Table 2).

LPL-activity and lipid levels

Mean PH-LPL activity for the whole group was 108 mU/mL (SD = 46) with values ranging from 13 to 293 mU/mL and having a median of 103 mU/mL. The distribution of LPL activity was skewed to the right (Kolmogorov-Smirnov test: P = 0.002). The maximum likelihood estimate of the power parameter of the Box-Cox transformation was 0.4 with a 95% confidence interval (CI) of 0.32–0.56. Consequently, we used the square-root transformation (power = 0.5) to normalize and standardize the LPL distribution. In

TABLE 2. Characteristics of CAD patients in LPL activity quartiles

	Quartile				
Variables	1[a] (n = 191)	2[b] (n = 179)	3[c] (n = 181)	4[d] (n = 179)	*P* Value
Risk factors					
Age (yr)	55 (8)	57 (8)	57 (8)	56 (8)	0.11
Body mass index (kg/m^2)	25.9 (2.8)	26.0 (2.8)	26.5 (2.9)	25.6 (2.3)	0.22
Systolic BP (mm Hg)	133 (19)	137 (20)	137 (20)	135 (17)	0.16
Diastolic BP (mm Hg)	81 (11)	82 (11)	81 (10)	82 (9)	0.97
Current smoking: n (%)	53 (28%)	54 (30%)	48 (27%)	42 (23%)	0.47
Insulin (U/L)	11.88 (9.51)	11.26 (7.29)	11.1 (7.73)	10.13 (6.55)	0.24
Glucose (mmol/L)	5.28 (1.35)	5.41 (1.33)	5.39 (1.47)	5.17 (1.17)	0.28
Fibrinogen (g/L)[e]	1.12 (0.41)	1.17 (0.39)	1.14 (0.38)	1.12 (0.39)	0.80
Medical treatment					
Beta-blockers	144 (75%)	133 (74%)	139 (77%)	127 (71%)	0.62
Calcium channel blockers	124 (65%)	109 (61%)	109 (60%)	107 (59%)	0.72
ACE inhibitors	13 (7%)	15 (8%)	20 (11%)	19 (11%)	0.45
Pravastatin	86 (45%)	96 (53%)	100 (55%)	82 (48%)	0.19

Results are given as mean (SD) or number (SD) unless otherwise stated; BP, blood pressure; n, number of patients.
[a] Quartile 1: PH-LPL activity, ⩽77 mU/ml.
[b] Quartile 2: PH-LPL activity, 78–103 mU/ml.
[c] Quartile 3: PH-LPL activity, 104–132 mU/ml.
[d] Quartile 4: PH-LPL activity, ⩾133 mU/ml.
[e] Result reported on the logarithmic scale.

TABLE 3. LPL activity in controls and CAD patients

	Controls (n = 75)	CAD Patients (n = 730)	*P* Value
	mU/ml		
LPL activity	138 ± 44	108 ± 46	<0.0001

Values given as mean ± SD; LPL, lipoprotein lipase; CAD, coronary artery disease.

addition, mean and median of the square-root distribution of LPL activity were almost equal (10.16 vs. 10.15) and the Kolmorov-Smirnov test showed a *P* value of 0.58, indicating Gaussian distribution. The square root of LPL activity was subsequently used in all statistical inferences. The percentage variance of TG and HDLc attributable to PH-LPL is equal to the squares of their respective correlations (reported in Table 4), and were found to be $(-0.02)^2 = 4\%$ and $(0.025)^2 = 6.3\%$, respectively.

Last, we divided the 730 patients into quartiles of LPL activity. The first quartile (PH-LPL, 13–77 mU/mL) contained 191 patients. The second (78–103 mU/ml) and third quartiles (104–132 mU/mL) contained 179 and 181 patients, respectively, and the fourth quartile (133–293 mU/mL) contained 179 patients (Table 2). The sample size in the first quartile is not equal to that of the others as the boundary between the first and second quartiles bisected a large number of patients who yielded PH-LPL activities of exactly 77 mU/ml. All these patients were therefore included in the first quartile.

Comparison of PH-LPL levels in REGRESS subjects and controls

PH-LPL activities were available for 75 normolipidemic controls, with a mean level of 138 (44) mU/mL (**Table 3**). CAD patients and controls had significantly different LPL activities, 108 (46) versus 138 (44) mU/mL; $P < 0.0001$, which indicates a decrease of 22% in CAD patients. Significance was still obtained ($P = 0.001$) if the study subjects were divided into those on or off beta-blockers, 106 (42) (n = 543) and 114 (53) mU/ml (n = 188), respectively.

Lipids and lipoproteins in different quartiles of LPL activity

Patients in the lowest versus the highest quartile of LPL activity had higher levels of triglycerides {log transformed; 0.58 (0.43) vs. 0.35 (0.42) mmol/l, $P < 0.0001$} and higher levels of both VLDL-cholesterol and VLDL-triglycerides, and IDL-triglycerides {log transformed; −0.69 (0.84) vs. −1.26 (1.08) mmol/l, $P = 0.002$; −0.09 (0.83) vs. −0.62 (0.93), $P = 0.002$, respectively; −2.18 (0.83) vs. −2.49 (0.60), $P = 0.02$} (**Table 4**). Conversely, levels of total cholesterol {5.96 (0.85) vs. 6.15 (0.84) mmol/l, $P = 0.01$}, LDL-cholesterol {4.21 (0.80) vs. 4.43 (0.77) mmol/l, $P = 0.026$} and HDL-cholesterol {0.86 (0.26) vs. 1.02 (0.23) mmol/l, $P < 0.0001$} were significantly lower in patients in the lowest versus the highest quartile of LPL activity. IDL-cholesterol {log transformed; −1.92 (0.84) vs. −2.08 (0.86) mmol/l, $P = 0.42$} levels, were not statistically different (Table 4).

PH-LPL activity correlated positively with LDL-cholesterol ($r = 0.14$; $P = 0.011$) and HDL-cholesterol ($r = 0.25$; $P < 0.0001$), but just missed significance ($r = 0.13$; $P = 0.059$) for total cholesterol. Conversely, a significant but negative association was evident for PH-LPL activity and triglycerides ($r = -0.20$; $P < 0.0001$). Values for triglycerides, VLDL, and IDL-triglycerides, were based on log-transformation, with means ± SD being given in the tables. Plots of PH-LPL activity versus plasma triglyceride and HDL-cholesterol are depicted in **Fig. 1A** and **1B**, respectively.

ApoE genotypes

ApoE genotypes were available for 606 of the 730 subjects in this study cohort; no bias was discernable in the ascertainment of these genotypes. Statistically significant associations between PH-LPL activities, and lipid and lipoprotein levels were evident in each genotype grouping, with significance in the E3/3 cohort (n = 356) only being very similar to that of the associations in the total subject cohort. Here, significance was shown for PH-LPL and plasma TG, TC, LDLc, and HDLc. Weaker associations were found in the other genotype divisions with the combined E2/2 + E2/3 subjects failing to show significance between PH-LPL activities and either TG or HDLc levels, but reach-

TABLE 4. Lipids and lipoproteins in CAD patients according to PH-LPL activity

	Quartile					
	1[a] (n = 191)	2[b] (n = 179)	3[c] (n = 181)	4[d] (n = 179)	*r* Value	*P* Value (All)
TG[e]	0.58 (0.43)	0.53 (0.45)	0.48 (0.42)	0.35 (0.42)	−0.20	<0.0001
IDL-TG[e]	−2.18 (0.83)	−2.32 (0.66)	−2.26 (0.67)	−2.49 (0.60)	−0.14	0.0467
VLDL-TG[e]	−0.09 (0.83)	−0.05 (0.94)	−0.45 (1.01)	−0.62 (0.93)	−0.23	0.0003
TC	5.96 (0.85)	5.97 (0.84)	6.12 (0.87)	6.15 (0.84)	0.13	0.059
VLDL-C[e]	−0.69 (0.84)	−0.78 (1.02)	−1.03 (1.02)	−1.26 (1.08)	−0.22	0.0033
IDL-C[e]	−1.92 (0.84)	−1.99 (0.77)	−1.94 (0.76)	−2.08 (0.86)	−0.05	0.65
HDL-C	0.86 (0.26)	0.89 (0.21)	0.96 (0.23)	1.02 (0.23)	0.25	<0.0001
LDL-C	4.21 (0.80)	4.23 (0.74)	4.38 (0.76)	4.43 (0.77)	0.14	0.0112

Results in mmol/l; n, number of patients.
[a] Quartile 1: lipoprotein lipase activity, ≤77 mU/ml plasma.
[b] Quartile 2: lipoprotein lipase activity, 78–103 mU/ml plasma.
[c] Quartile 3: lipoprotein lipase activity 104–132 mU/ml plasma.
[d] Quartile 4: lipoprotein lipase activity ≥133 mU/ml plasma.
[e] Log transformed values.

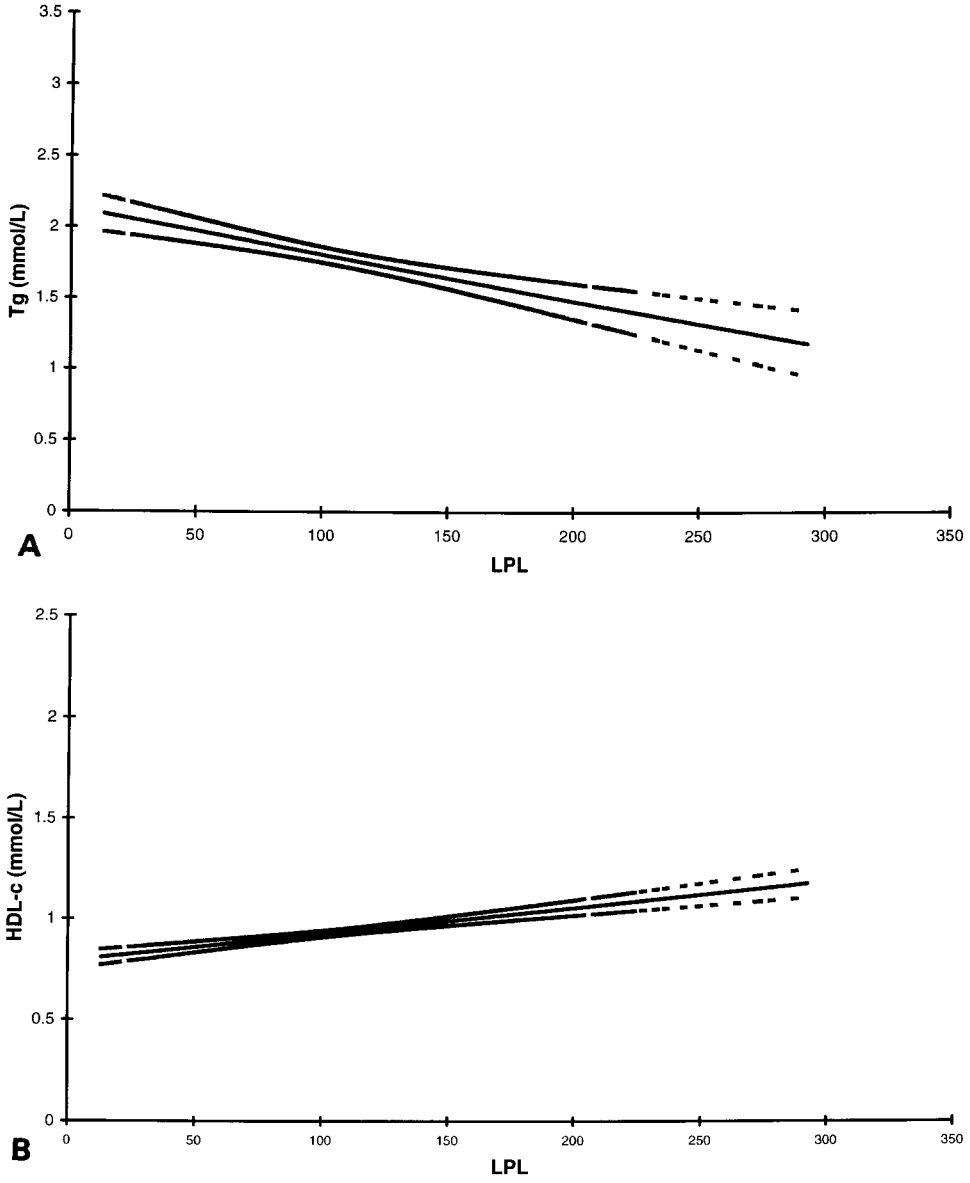

Fig. 1. (A) A plot of post-heparin plasma lipoprotein lipase (PH-LPL) activity versus plasma TGs and (B) versus high density lipoprotein cholesterol (HDLc) for the 730 subjects from the REGRESS study. Solid lines represent the estimated regression lines between PH-LPL activity and TG and HDLc, respectively. Dotted lines indicate the 95% confidence interval around these regression lines.

ing significance for total and LDL cholesterol. The combined E4/3 + E4/4 subjects only gave significant associations between PH-LPL, and TG and HDLc.

Contribution of LPL gene mutations to lowered PH-LPL activity in REGRESS subjects

Functional variants of LPL have been shown to occur relatively frequently in the general population (34). Therefore, to determine the contribution of mutations in the LPL gene to decreased PH-LPL activity, DNA samples of the 191 CAD patients in the lowest quartile of activity ($\leqslant$77 mU/mL) were subjected to comprehensive mutation analysis of the promoter and exonic regions of the gene.

As anticipated, sequence changes were detected in exons 2, 6, and 7, which, respectively, represent the Asp9Asn (D9N), Asn291Ser (N291S), and Ser447$\rightarrow$Stop (S447X) amino acid substitutions, previously described (27, 35, 36). The $-$93T/G promoter mutation (37) was found in this group, and in each case was linked with the D9N mutation. No additional base substitutions were identified in these subjects, other than the three known (35, 38) silent base substitutions in exons 3 ($G_{405}\rightarrow$A), 4 ($G_{435}\rightarrow$A), and 8 ($C_{1164}\rightarrow$A), which occurred at carrier frequencies of 7.5, 3.3, and 1.5%, respectively. Individuals in the other quartiles were not subjected to detailed mutation analysis but were screened for the D9N, N291S, and S447X mutations. The distribution and frequency of carriers of these LPL variants is given in Table 2. In the entire cohort we found 27 D9N and 37 N291S carriers. One of these patients carried both amino acid substitutions. Carriers of the S447X

TABLE 5. Frequencies of the D9N, N291S and S447X alleles according to PH-LPL activity

Allele	Quartile 1[a] (n = 191)	Quartile 2[b] (n = 179)	Quartile 3[c] (n = 181)	Quartile 4[d] (n = 179)	*P* Value (All)
N291S: n (%)	14 (7.3%)	8 (4.5%)	5 (2.8%)	5 (2.8%)	0.022
D9N: n (%)	9 (4.7%)	6 (3.4%)	5 (2.8%)	4 (2.2%)	0.17
N291S+D9N: n (%)	23 (12.0%)	14 (7.8%)	10 (5.0%)	9 (5.0%)	0.006
S447X: n (%)	22 (11.5%)	25 (14%)	31 (16.5%)	33 (18.3%)	0.047

[a] Quartile 1: LPL activity ⩽77 mU / ml.
[b] Quartile 2: LPL activity 78–103 mU / ml.
[c] Quartile 3: LPL activity 104–132 mU / ml.
[d] Quartile 4: LPL activity ⩾133 mU / ml.

allele totaled 118; 4 were homozygous, while 6 also carried either a D9N or a N291S substitution. The total number of compound heterozygotes detected was 7, and these individuals were excluded from the analysis. No carriers of the predominant Caucasian mutation, Gly188Glu (G188E) were detected in this cohort.

Frequencies for D9N, N291S, and S447X carriers differed significantly between the lowest and highest quartiles of LPL activity. The combined frequency of D9N and N291S carriers in the lowest quartile was more than double the frequency in the highest quartile (12.0% vs. 5.0%: $P = 0.006$), while conversely in the high quartile the S447X frequency rose to 18.3% (11.5% vs. 18.3%: $P = 0.006$) as depicted in **Table 5**. These 447stop frequency differentials are similar to those reported previously by us for a smaller subgroup of the REGRESS cohort (32).

DISCUSSION

The determination of PH-LPL levels in subjects with CAD, from the REGRESS study, provided a unique opportunity for a rigorous appraisal of PH-LPL levels, and the relationships of these levels with circulating lipids and lipoproteins, particularly as a large number of single gender subjects were available together with a highly controlled laboratory assay for PH-LPL activity.

To our knowledge, our study is the first to demonstrate directly through lipase activity measurements, that males with CAD have lower mean levels of PH-LPL than normal controls. Earlier studies (39–41) have examined this aspect but have not been able to record differing mean LPL activities; however, the numbers of subjects in the cohorts examined, in each case, were low. A large number of our study subjects were on beta-blockers, which have been suggested to inhibit LPL activity (42, 43). However, even when we excluded these individuals, a significant reduction in mean PH-LPL activity was obtained for the CAD patients. Another potential factor of influence to be considered here would be that of advancing age where a decrease in PH-LPL activity has been reported (44). However, the difference in the mean ages between the control and CAD subjects was not a factor in our study as neither of these cohorts yielded a significant correlation of PH-LPL activity with age.

The finding of lower PH-LPL activities in our study cohort would suggest that this group is enriched in individuals who have reduced PH-LPL levels and would thereby implicate a reduction in PH-LPL activity as an etiological factor in CAD. This would most likely be on the basis of the atherogenicity of the small dense LDL, low HDLc, elevated plasma TGs, and prolonged post-prandial lipemia accompanying depressed PH-LPL activities (1, 45, 46). This conclusion is supported by several studies that showed that men with hypoalphalipoproteinemia and mild hypertriglyceridemia have significantly enhanced postprandial lipemia (46–48). Also, a preponderance of small, LDL particles has been linked to increased risk of myocardial infarction, and a dense and protein-rich LDL has proved to be characteristic of patients with CAD (49–51).

Plasma total triglyceride was found to correlate negatively with PH-LPL activity in our study cohort (Fig. 1A). This finding is similar to that of previous studies (6, 14, 15, 17) and confirms this relationship in the largest cohort of CAD patients examined to date. This relationship was manifest by both the VLDL and IDL fractions. HDLc was positively correlated with PH-LPL activity, again confirming and extending previous findings (6–8, 10, 11) to a large cohort of CAD patients. These relationships in our subjects are in accordance with the role of LPL in the metabolism of the triglyceride-rich lipoproteins, and the maintenance of HDL levels. LPL is located on the capillary endothelium and is active in the lipolysis and removal of the triglycerides in chylomicrons, VLDL, and IDL. A reduction in LPL activity would therefore result in an increase in plasma TG, through slower TG hydrolysis, and a decrease in HDL due to decline in the rate of surface component influx into the HDL pool.

Apolipoprotein E is an important component of the clearance pathways for triglyceride-rich lipoproteins, through its participation in their uptake through a receptor-mediated process. Three isoforms are common in humans (E2,3,4) and are known to independently influence lipid and lipoprotein levels (51). They are therefore an important factor to consider in the evaluation of the impact of other components of the same clearance pathway on lipid and lipoprotein levels. In this context, a recent study in 52 healthy males (13) provides evidence that apoE may modulate the effect of variations of LPL activity on lipid and lipoprotein levels. However, unexpectedly, this effect was

TABLE 6. Relationships (r) of PH-LPL activity with lipid/lipoprotein levels in subjects categorized according to apoE genotypes

	Entire Cohort (n = 730)	E2/E2 + E2/E3 (n = 69)	E3/E3 (n = 356)	E4/E3 + E4/E4 (n = 167)	*P* value
Total cholesterol	0.13[a]	0.33[b]	0.16[b]	0.07	0.15
HDLc	0.25[a]	0.23	0.30[a]	0.23[b]	0.83
LDLc	0.14[a]	0.34[b]	0.19[a]	0.08	0.18
Total triglyceride	−0.2[a]	−0.08	−0.23[a]	−0.17[c]	0.46
			mU/ml		
LPL activity	108 (46)	121(49)	109 (45)	107 (41)	0.075

ApoE genotypes were assessed for 606 of the 730 study subjects; 14 E2/4 individuals were excluded from the analysis.

[a] $P \leq 0.001$.

[b] $P \leq 0.01$.

[c] $P \leq 0.05$.

limited to carriers of the E2 isoform (E2/2 and E2/3; n = 12), which showed statistically significant correlations between PH-LPL, and VLDL and HDL levels. No correlations reached significance in the other two divisions, E3/3 (n = 29) and E3/4, E4/4 (n = 11). We have therefore also studied the impact of apoE isoforms on PH-LPL relationships in our cohort of males and found no specific effect of the E2 isoform (**Table 6**). In fact, the association between PH-LPL and TGs was weakest in the E2/2 + E2/3 grouping; this is in direct contrast to the previous study (13). All three genotype categories showed statistically significant relationships, with the E3/3 division in particular, paralleling the associations of the total subject cohort, by yielding highly significant ($P = <0.001$) relationships with plasma–TG, LDLc, and HDLc. These data contradict prior findings and, originating from a much larger cohort, suggest that the apoE polymorphism is of minor importance in assessing the relationships between PH-LPL activity and plasma lipid and lipoprotein levels.

We have shown here that CAD subjects in the REGRESS study have a lower PH-LPL level than normal subjects. In an attempt to characterize the factors determining the lower level in these subjects, we determined their carrier frequency for LPL variants known to be associated with lower catalytic activities. Only two previously reported functional variants, D9N and N291S, were detected in this cohort, clearly indicating that no other additional mutations in the LPL gene are likely to contribute to lowered LPL activity levels in the Dutch population. The contribution of these variants to the depression of PH-LPL levels was evident in comparisons of their carrier frequencies in the lowest versus the highest quartile divisions of the REGRESS cohort. Both variants were detected at an increased prevalence in the former division, where a joint carrier frequency of 12.0% was found. This is more than double that of the 5.0% found in the upper quartile division. Clearly, while these variants contribute significantly to the etiology of the lower PH-LPL levels in this division, other factors must be involved. The identity of these factors is undetermined as likely candidates such as age, BMI, and beta-blockers did not differ significantly among the quartile divisions.

Several studies examining the relationships between LPL gene variants and lipid and lipoprotein levels in humans (16, 32, 52–57) have provided direct and indirect evidence that the S447X variant is associated with an increase in LPL catalytic activity which manifests with lower plasma TGs and higher HDLc levels in carriers of this variant. Based on these data, the frequency of carrier status for the S447X variant was predicted to be elevated in subjects in the upper quartile of PH-LPL activities. This prediction was validated as the carrier frequency of 18.3% (n = 179) was significantly elevated ($P = 0.006$) above the 11.5% recorded for individuals (n = 191) in the lowest quartile of PH-LPL activities. These data thus significantly strengthen the in vivo evidence that the S447X variant manifests with a higher catalytic activity when compared to wild type LPL. In vitro data supporting this hypothesis have been sought in several mutagenesis and transfection systems (26, 58–60) but thus far, little difference has been discovered between the functional activities of this variant and wild type LPL which may point to a selective mechanism for the in vivo effect.

In conclusion, this study demonstrates that post-heparin plasma LPL activity is an important determinant of lipid and lipoprotein levels in humans, relatively independent of secondary factors such as age and BMI. In addition, the REGRESS study provides evidence that low PH-LPL activity is a risk factor for CAD and that common mutations in the LPL gene can contribute to this risk through their impact on the variation of PH-LPL levels in these patients.

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REFERENCES

1. Miesenbock, G., B. Holzl, B. Foger, E. Brandstatter, B. Paulweber, F. Sandhofer, and J. R. Patsch. 1993. Heterozygous lipoprotein lipase deficiency due to a missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities. *J. Clin. Invest.* **91:** 448–455.
2. Wilson, D. E., M. Emi, P. H. Iverius, A. Hata, L. L. Wu, E. Hillas, R. R. Williams, and J. M. Lalouel. 1990. Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. *J. Clin. Invest.* **86:** 735–750.
3. Bijvoet, S., S. E. Gagne, S. Moorjani, C. Gagne, H. E. Henderson, J. C. Fruchart, J. Dallongeville, P. Alaupovic, M. Prins, J. J. P. Kastelein, and M. R. Hayden. 1996. Alterations in plasma lipoproteins and apolipoproteins before the age of 40 in heterozygotes for lipoprotein lipase deficiency. *J. Lipid Res.* **37:** 640–650.
4. Sprecher, D. L., B. V. Harris, E. A. Stein, P. S. Bellet, L. M. Keilson, and L. A. Simbartl. 1996. Higher triglycerides, lower high-density cholesterol, and higher systolic blood pressure in lipoprotein lipase-deficient heterozygotes. A preliminary report. *Circulation.* **94:** 3239–3245.
5. Nordestgaard, B. G., S. Abildgaard, H. H. Wittrup, R. Steffensen, G. Jensen, and A. Tybjaerg-Hansen. 1997. Heterozygous lipoprotein lipase deficiency: frequency in the general population, effect

on plasma lipid levels and risk of ischemic heart disease. *Circulation*. **96:** 1737–1744.
6. Jackson, R. L., M. T. Yates, C. A. McNerney, and M. L. Kashyap. 1990. Relationship between post-heparin plasma lipases, triglycerides and high density lipoproteins in normal subjects. *Horm. Metab. Res.* **22:** 289–294.
7. Kekki, M. 1980. Lipoprotein-lipase action determining plasma high density lipoprotein cholesterol level in adult normolipaemics. *Atherosclerosis*. **37:** 143–150.
8. Glaser, D. S., T. J. Yost, and R. H. Eckel. 1992. Preheparin lipoprotein lipolytic activities: relationship to plasma lipoproteins and postheparin lipolytic activities. *J. Lipid Res.* **33:** 209–214.
9. Lamarche, B., J. P. Despres, M. C. Pouliot, S. Moorjani, P. J. Lupien, G. Theriault, A. Tremblay, A. Nadeau, and C. Bouchard. 1992. Is body fat loss a determinant factor in the improvement of carbohydrate and lipid metabolism following aerobic exercise training in obese women?. *Metab. Clin. Exp.* **41:** 1249–1256.
10. Kuusi, T., Y. A. Kesaniemi, M. Vuoristo, T. A. Miettinen, and M. Koskenvuo. 1987. Inheritance of high density lipoprotein and lipoprotein lipase and hepatic lipase activity. *Arteriosclerosis.* **7:** 421–425.
11. Taskinen, M. R., and E. A. Nikkila. 1980. Effect of acute vigorous exercise on lipoprotein lipase activity of adipose tissue and skeletal muscle in physically active men. *Artery.* **6:** 471–483.
12. Kuusi, T., C. Ehnholm, J. Viikari, R. Harkonen, E. Vartiainen, P. Puska, and M. R. Taskinen. 1989. Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia. *J. Lipid Res.* **30:** 1117–1126.
13. St. Amand, J., S. Moorjani, P. J. Lupien, D. Prud'Homme, and J. P. Despres. 1996. The relation of plasma triglyceride, apolipoprotein B, and high-density lipoprotein cholesterol to postheparin lipoprotein lipase activity is dependent on apolipoprotien E polymorphism. *Metab. Clin. Exp.* **45:** 261–267.
14. Huttunen, J. K., C. Ehnholm, M. Kekki, and E. A. Nikkila. 1976. Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridaemia: correlations to sex, age and various parameters of triglyceride metabolism. *Clin. Sci. Mol. Med.* **50:** 249–260.
15. Pollare, T., B. Vessby, and H., Lithel. 1991. Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler. Thromb.* **11:** 1192–1203.
16. Mailly, F., Y. Tugrul, P. W. Reymer, T. Bruin, M. Seed, B. E. Groenemeyer, A. Asplund-Carlson, D. Vallance, A. F. Winder, G. J. Miller, J. J. P. Kastelein, A. Hampsten, G. Olivecrona, S. E. Humphries, and P. Talmud. 1995. A common variant in the gene for lipoprotein lipase (Asp9→Asn). Functional implications and prevalence in normal and hyperlipidemic subjects. *Arterioscler. Thromb. Vasc. Biol.* **15:** 468–478.
17. Boberg, J., 1972. Heparin-released blood plasma lipoprotein lipase activity in patients with hyperlipoproteinemia. *Acta. Med. Scand.* **191:** 97–102.
18. Alvarez, J. J., A. Montelongo, A. Iglesias, M. A. Lasuncion, and E. Herrera. 1996. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J. Lipid Res.* **37:** 299–308.
19. Couillard, C., N. Bergeron, D. Prud'homme, J. Bergeron, A. Tremblay, C. Bouchard, P. Mauriege, and J. P. Despre. 1998. Postprandial triglyceride response in visceral obesity in men. *Diabetes.* **47:** 953–960.
20. Taskinen, M. R., C. J. Glueck, M. L. Kashyap, L. S. Srivastava, B. A. Hynd, G. Perisutti, K. Robinson, P. J. Kinnunen, and T. Kuusi. 1980. Post-heparin plasma lipoprotein and hepatic lipases. Relationships to high density lipoprotein cholesterol and to apolipoprotein CII in familial hyperalphalipoproteinemic and in normal subjects. *Atherosclerosis.* **37:** 247–256.
21. Applebaum-Bowden, D., S. M. Haffner, P. W. Wahl, J. J. Hoover, G. R. Warnick, J. J. Albers, and W. R. Hazzard. 1985. Postheparin plasma triglyceride lipases. Relationships with very low density lipoprotein triglyceride and high density lipoprotein2 cholesterol. *Arteriosclerosis.* **5:** 273–282.
22. St. Amand, J., J. P. Despres, S. Lemieux, B. Lamarche, S. Moorjani, A. Prud'homme, C. Bouchard, and P. J. Lupien. 1995. Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women?. *Metab. Clin. Exp.* **44:** 491–498.
23. Kuusi, T., E. A. Nikkila, P. Saarinen, P. Varjo, and L. A. Laitinen. 1982. Plasma high density lipoproteins HDL2, HDL3 and postheparin plasma lipases in relation to parameters of physical fitness. *Atherosclerosis.* **41:** 209–219.
24. Peltonen, P., J. Marniemi, E, E. Hietanen, I. Vuori, and C. Ehnholm. 1981. Changes in serum lipids, lipoproteins, and heparin releasable lipolytic enzymes during moderate physical training in man: a longitudinal study. *Metab. Clin. Exp.* **30:** 518–526.
25. Knudsen, P., S. Murtomaki, M. Antikainen, S. Ehnholm, C. Ehnholm, and M. R. Taskinen. 1997. The Asn291→Ser and Ser447→ stop mutations of the lipoprotein lipase gene and their significance for lipid metabolism in patients with hypertriglyceridemia. *Eur. J. Clin. Invest.* **27:** 928–935.
26. Zhang, H., H. E. Henderson, E. Gagne, S. M. Clee, L. Miao, G. Liu, and M. R. Hayden. 1996. Common sequence variants of lipoprotein lipase: standardized studies of in vitro expression and catalytic function. *Biochim. Biophys. Acta.* **1302:** 159–166.
27. Ma, Y., T. C. Ooi, M. S. Liu, H. Zhang, R. McPherson, A. L. Edwards, I. J. Forsythe, J. Frohlich, J. D. Brunzell, and M. R. Hayden. 1994. High frequency of mutations in the human lipoprotein lipase gene in pregnancy-induced chylomicronemia: possible association with apolipoprotein E2 isoform. *J. Lipid Res.* **35:** 1066–1075.
28. Busca, R., J. Peinado, E. Vilella. J. Auwerx, S. S. Deeb, S. Vilaro, and M. Reina. 1995. The mutant Asn291→Ser human lipoprotein lipase is associated with reduced catalytic activity and does not influence binding to heparin. *FEBS Lett.* **367:** 257–262.
29. Jukema, J. W., A. V. Bruschke, A. J. van Boven, J. H. Reiber, E. T. Bal, A. H. Zwinderman, H. Jansen, G. J. Boerma, F. M. van Rappard, and K. I. Lie. 1995. Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The Regression Growth Evaluation Statin Study (REGRESS). *Circulation*. **91:** 2528–2540.
30. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
31. Ma, Y., M. S. Liu, H. Zhang, I. J. Forsythe, J. D. Brunzell, and M. R. Hayden. 1993. A 4 basepair deletion in exon 4 of the human lipoprotein lipase gene results in type I hyperlipoproteinemia. *Hum. Mol. Genet.* **2:** 1049–1050.
32. Groenemeijer, B. E., M. D. Hallman, P. W. Reymer, E. Gagne, J. A. Kuivenhoven. T. Bruin, H. Jansen, K. I. Lie, A. V. Bruschke, E. Boerwinkle, M. R. Hayden, and J. J. P. Kastelein. 1997. Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS Study Group. *Circulation.* **95:** 2628–2635.
33. Hixson, J. E., and D. T. Vernier. 1990. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J. Lipid Res.* **31:** 545–548.
34. Hokanson, J. E. 1997. Lipoprotein lipase gene variants and the risk of coronary disease: a quantitative analysis of population-based studies. *Int. J. Clin. Lab. Res.* **27:** 24–34.
35. Elbein, S. C., C. Yeager, L. K. Kwong, A. Lingam, I. Inoue, J. M. Lalouel, and D. E. Wilson. 1994. Molecular screening of the lipoprotein lipase gene in hypertriglyceridemic members of familial noninsulin-dependent diabetes mellitus families. *J. Clin. Endocrin. Metab.* **79:** 1450–1456.
36. Hata, A., M. Robertson, M. Emi, and J. M. Lalouel. 1990. Direct detection and automated sequencing of individual alleles after electrophoretic strand separation: identification of a common nonsense mutation in exon 9 of the human lipoprotein lipase gene. *Nucleic Acids Res.* **18:** 5407–5411.
37. Yang, W-S., D. N. Nevin, R. Peng, J. D. Brunzell, and S. S. Deeb. 1992. A mutation in the promoter of the lipoprotein lipase (LPL) gene in a patient with a familial combined hyperlipoproteinemia and low LPL activity. *Proc. Natl. Acad. Sci.* USA. **92:** 4462–4466.
38. Gagne, E., J. J. Genest, H. Zhang, L. A. Clarke, and M. R. Hayden. 1994. Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. *Arterioscler. Thromb.* **14:** 1250–1257.
39. Karpe, F., P. Tornvall, T. Olivecrona, G. Steiner, L. A. Carlson, and A. Hamsten. 1993. Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis.* **98:** 33–49.
40. Zambon, A., M. A. Austin, B. G. Brown, J. E. Hokanson, and J. D. Brunzell. 1993. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arterioscler. Thromb.* **13:** 147–153.

41. Katzel, L. I., P. J. Coon, M. J. Busby, S. O. Gottlieb, R. M. Krauss, and A. P. Goldberg. 1992. Reduced HDL2 cholesterol subspecies and elevated postheparin hepatic lipase activity in older men with abdominal obesity and asymptomatic myocardial ischemia. *Arterioscler. Thromb.* **12:** 814–823.
42. Kihara, S., M. Kubo, N. Ikeda, S. Yokoyama, Y. Matsusawa, S. Tauri, A. Yamamoto, and K. Y. Hostetler. 1989. Inhibition of post-heparin lipoprotein lipase by beta-adrenergic blockers in vitro. *Biochim. Pharmacol.* **38:** 407–411.
43. Day, J. L., J. Metcalfe, N. Simpson, and L. Lowenthal. 1984. Adrenergic mechanisms in the control of plasma lipids in man. *Am. J. Med.* **76:** 94–96.
44. Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. 1988. Postprandial plasma lipoprotein changes in human subjects of different ages. *J. Lipid Res.* **29:** 469–479.
45. Zambon, A., and J. E. Hokansen. 1998. Lipoprotein classes and coronary disease regression. *Curr. Opin. Lipidol.* **9:** 329–336.
46. Ooi, T.C., E. Simo, and J. A. Yakichuk. 1992. Delayed clearance of postprandial chylomicrons and their remnants in the hypoalphalipoproteinemia and mild hypertriglyceridemia syndrome. *Arterioscler. Thromb.* **12:** 1184–1190.
47. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and J. R. Patsch. 1987. High density lipoprotein2. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *J. Clin. Invest.* **80:** 341–347.
48. Patsch, J. R., J. B. Karlin, L. W. Scott, L. C. Smith, and A. M. Gotto, Jr. 1983. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc. Natl. Acad. Sci. USA.* **80:** 1449–1453.
49. Slyper, A. H. 1994. Low-density lipoprotein density and atherosclerosis. *J. Am. Med. Assoc.* **272:** 305–308.
50. Austin, M. A., J. E. Hokanson, and J. D. Brunzell. 1994. Characterization of low-density lipoprotein subclasses: methodologic approaches and clinical relevance. [Review]. *Curr. Opin. Lipidol.* **5:** 395–403.
51. Austin, M. A., and K. L. Edwards. 1996. Small, dense low density lipoproteins, the insulin resistance syndrome and noninsulin-dependent diabetes. [Review]. *Curr. Opin. Lipidol.* **7:** 167–171.
52. Gerdes, C., R. M. Fisher, V. Nicaud, J. Boer, S. E. Humphries, P. J. Talmud, and O. Faergeman. 1997. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations: studies in the fasting and postprandial states: the European Atherosclerosis Research Studies. *Circulation*. **96:** 733–740.
53. Dallongeville, J., S. Lussier-Cacan, and J. Davignon. 1992. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J. Lipid Res.* **33:** 447–454.
54. Kuivenhoven, J. A., B. E. Groenemeyer, J. M. Boer, P. W. Reymer, R. Berghuis, T. Bruin, H. Jansen, J. C. Seidell, and J. J. P. Kastelein. 1997. Ser447stop mutation in lipoprotein lipase is associated with elevated HDL cholesterol levels in normolipidemic males. *Arterioscler. Thromb. Vasc. Biol.* **17:** 595–599.
55. Fisher, R. M., S. E. Humphries, and P. J. Talmud. 1997. Common variation in the lipoprotein lipase gene: effects on plasma lipids and risk of atherosclerosis. *Atherosclerosis.* **135:** 145–159.
56. Peacock, R. E., A. Temple, V. Gudnason, M. Rosseneu, and S. E. Humphries. 1997. Variation at the lipoprotein lipase and apolipoprotein AI-CIII gene loci are associated with fasting lipid and lipoprotein traits in a population sample from Iceland: interaction between genotype, gender, and smoking status. *Genet. Epidemiol.* **14:** 265–282.
57. Hegele, R. A., S. Gahndi, J. H. Brunt, and P. W. Connelly. 1996. Restriction isotyping of the premature termination variant of lipoprotein lipase in Alberta Hutterites. *Clin. Biochem.* **29:** 63–66.
58. Faustinella, F., A. Chang, J. P. Van Biervliet, M. Rosseneu, N. Vinaimont, L. C. Smith, S. H. Chen, and L. Chan. 1991. Catalytic triad residue mutation (Asp156—Gly) causing familial lipoprotein lipase deficiency. Co-inheritance with a nonsense mutation (Ser447—Ter) in a Turkish family [published erratum appears in *J. Biol. Chem.* 1992; **267:** 7194]. *J. Biol. Chem.* **266:** 14418–14424.
59. Kobayashi, J., T. Nishida, D. Ameis, G. Stahnke, M. C. Schotz, H. Hashimoto, I. Fukamachi, K. Shirai, Y. Saito, and S. Yoshida. 1992. A heterozygous mutation (the codon for Ser^{447}→a stop codon) in lipoprotein lipase contributes to a defect in lipid interface recognition in a case with type I hyperlipidemia. *Biochem. Biophys. Res. Commun.* **182:** 70–77.
60. Previato, L., O. Guardamagna, K. A. Dugi, R. Ronan, G. D. Talley, S. Santamarina-Fojo, and H. B. Brewer, Jr. 1994. A novel missense mutation in the C-terminal domain of lipoprotein lipase (Glu^{410}→ Val) leads to enzyme inactivation and familial chylomicronemia. *J. Lipid Res.* **35:** 1552–1560.