Linkage and Association Studies of the Lipoprotein Lipase Gene With Postheparin Plasma Lipase Activities, Body Fat, and Plasma Lipid and Lipoprotein Concentrations: The HERITAGE Family Study

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Lipoprotein lipase (LPL) is responsible for the hydrolysis of triglyceride (TG)-rich lipoproteins. The aims of the present study were (1) to test for potential linkages (sib-pair method) between postheparin plasma lipase (lipoprotein and hepatic lipase) activities, body fatness, plasma lipid concentrations, and LPL polymorphisms (Ser447Ter and a tetranucleotide repeat) and microsatellite markers flanking the LPL locus (D8S261 and D8S258); and (2) to investigate associations between the LPL Ser447Ter (S447X) polymorphism and these phenotypes. Data on 190 parents and 312 adult offspring from 99 Caucasian families participating in the HERITAGE Family Study were available for this study. Data were adjusted for the effects of age within sex, and lipases, lipid variables, and abdominal visceral fat were further adjusted for fat mass. A suggestive linkage was observed only between the S447X polymorphism and very-low-density (VLDL)-apolipoprotein B (apo B) (332 sib-pairs, $P = .013$). The S447X polymorphism was not associated with body fat phenotypes or postheparin plasma LPL (PH-LPL) activity (men, $P = .19$; women, $P = .47$). In contrast, the X447 allele carriers had lower plasma TG (men and women, $P = .01$), VLDL-TG (men and women, $P = .01$), and VLDL-apo B (men and women, $P = .009$). The relationships between the X447 allele and plasma TG, VLDL-TG, and VLDL-apo B in both genders were observed in obese (body mass index [BMI] ≥ 30 kg/m^2) but not in normal-weight (BMI < 25 kg/m^2) subjects. Thus, the S447X polymorphism of the LPL gene is not associated with body fatness and postheparin plasma lipase activities. However, the obese carriers of the X447 allele have plasma TG, VLDL-TG, and plasma cholesterol/high-density lipoprotein cholesterol (HDL-C) levels equivalent to those of normal-weight sedentary adults.

LIPOPROTEIN LIPASE (LPL) is responsible for the extrahepatic hydrolysis of triglyceride (TG) in circulating chylomicrons and very–low-density lipoprotein (VLDL). The enzymatic action of LPL results in the formation of monoglycerides and diglycerides, as well as free fatty acids, which can be oxidized to meet energy demands in skeletal and heart muscle or reesterified for storage in adipose tissue. LPL is also responsible for apolipoprotein and phospholipid exchange between VLDL and high-density lipoprotein (HDL),[1] thereby affecting HDL_3 conversion to HDL_2 and low-density lipoprotein (LDL) generation derived from VLDL hydrolysis.[2] HDL_2-C is correlated positively and HDL_3-C negatively with skeletal muscle LPL activity.[3] Hepatic lipase (HL) is negatively correlated with HDL_2-C but not with HDL_3-C.[4] Thus, the balance between LPL and HL activities in postheparin plasma is an important correlate of plasma HDL-C levels.[5]

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Animal models indicate that LPL activity in adipose tissue could be under genetic control.[6] These observations suggest that DNA sequence variation in the LPL gene could explain some of the heterogeneity commonly found in the adiposity and plasma lipid profile. The LPL gene is located on chromosome 8p22.[7] Several LPL polymorphisms have been investigated in different populations for an association with disorders such as diabetes mellitus, obesity, or dyslipoproteinemia, and recent data show a high degree of sequence variation in the LPL gene.[8] Linkage disequilibrium is observed between the LPL Ser447Ter (S447X) and intron 8 *Hin*dIII polymorphisms.[9] The *Hin*dIII polymorphism is associated with the body mass index (BMI) in overweight subjects,[10] and the H+/H+ genotype is associated with high plasma TG levels in subjects with elevated levels of abdominal visceral adipose tissue.[11] The S447X polymorphism is a C to G transversion that produces a mature protein truncated by 2 amino acids (Ser-Gly) at the C-terminal end.[12] Recent in vitro studies have proposed that the truncation of the 2 C-terminal residues results in a protein with greater[13] or normal[14] LPL activity compared with the native protein. Among coronary artery disease (CAD) patients, carriers of the X447 allele have higher postheparin plasma lipoprotein lipase (PH-LPL) but normal postheparin HL (PH-HL) activities.[15] Furthermore, the X447 allele is associated with higher HDL-C levels in CAD patients[15,16] and with elevated levels in healthy German subjects,[17] but this relationship was not found among subjects with premature CAD.[18] The same allele has also been associated with low TG levels in CAD patients,[15,16] in premature CAD patients,[9,18] and in the ECTIM (Étude Cas-Témoins de l'Infarctus du Myocarde) Study.[19] Recently, in a study of 54 male monozygotic twin pairs, the X447 allele was associated with significantly smaller within-pair differences in total cholesterol and HDL-C levels.[20]

The aim of the present study was to test the hypothesis that carriers of the X447 allele of the S447X polymorphism have higher PH-LPL activity, lower plasma TG, and higher HDL-C

levels than noncarriers. We also studied genetic linkages between body fatness, PH lipase activities, and plasma lipoprotein profile traits and the S447X and a tetranucleotide repeat in intron 6 of the LPL gene and 2 microsatellite markers (D8S261 and D8S258) at 1.6 cM telomeric and 1.7 cM centromeric, respectively, to the LPL gene in white subjects who participated in the HERITAGE Family Study. Finally, we investigated potential associations between the S447X polymorphism and a variety of body fatness and metabolic variables.

SUBJECTS AND METHODS

Subjects

The subjects of the present study were participants in the HERITAGE Family Study, a multicenter study designed to investigate the role of genetic factors in the cardiovascular and metabolic adaptations to 20 weeks of endurance training in white and black families. The HERITAGE Family Study design and methods have been described elsewhere.[21] All subjects were required to be sedentary and in good health to participate in the HERITAGE Family Study protocol and to meet a set of inclusion criteria.[21] For the present study, only baseline (before training) data measured in Caucasians were analyzed. A total of 190 parents (97 men and 93 women) and 312 adult offspring (148 sons and 164 daughters) were recruited and tested. The study protocol was approved by each Institutional Review Board of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

Phenotype Measurements

Anthropometric and body density measurements. These measurements have been described in detail previously.[22] The BMI was calculated as weight in kilograms divided by height in meters squared. The sum of 8 skinfolds was used to assess the level of subcutaneous fat.[22] Hydrostatic weighing was used to assess body density, and the percent body fat was estimated from body density using conventional equations as described elsewhere.[22]

Abdominal visceral, subcutaneous, and total fat areas. Abdominal fat was assessed by computed tomography as previously described.[23] Scans were obtained between the fourth and fifth lumbar (L4-L5) vertebrae with subjects in the supine position with arms stretched above the head. The total fat area was calculated using an attenuation range of −190 to −30 Hounsfield units. The abdominal visceral fat area was defined by drawing a line within the inner portion of the muscle wall surrounding the abdominal cavity.

LPL activity. Blood samples were collected after a 12-hour overnight fast, 10 minutes after an intravenous injection of heparin (60 IU/kg body weight). The PH-LPL and PH-HL activities were measured using a modification of the method of Nilsson-Ehle and Ekman[24] as previously described.[25] The activity was expressed as nanomoles of oleic acid released per milliliter of plasma per minute.

Plasma lipids and lipoproteins. Fasting TG and cholesterol levels were determined at baseline on 2 separate days at least 24 hours apart. Blood was drawn and prepared according to a standard protocol and then sent to the Lipid Core Laboratory (Lipid Research Center, Laval University Medical Center). Measurements were performed on plasma and lipoprotein fractions by enzymatic methods using a Technicon RA-500 analyzer (Technicon Instruments, Tarrytown, NY).[26] Plasma VLDL ($d < 1.006$ g/mL) was isolated by ultracentrifugation, whereas the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$.[27] Cholesterol and TG levels in the infranatant fraction were measured before and after the precipitation step. The cholesterol content of HDL_2 and HDL_3 subfractions was determined after further precipitation of HDL_2 with dextran sulfate.[28] TG levels were measured in plasma, VLDL, LDL, and HDL fractions, and cholesterol was assessed in plasma, VLDL, and LDL fractions and HDL_2 and HDL_3 subfractions. Apolipoprotein B (apo B) concentrations were measured by the rocket immunoelectrophoretic method of Laurel,[29] as previously described,[26] in the total plasma and LDL fraction ($d > 1.006$ g/L). Apo A1 concentrations were measured in the infranatant using the same method.

Genotype Determination

Polymerase chain reaction amplification of the S447X polymorphism. DNA was extracted from lymphoblastoid cell lines after digestion by proteinase K and purification with phenol-chloroform.[30] Polymerase chain reaction (PCR) amplification was performed in a vol of 20 μL containing 500 ng DNA, 200 μmol/L each of dATP, dCTP, dGTP, and dTTP, 5% dimethylsulfoxide, 1.5 mmol/L $MgCl_2$, 1× buffer (50 mmol/L NaCl, 10 mmol/L Trishydrochloride, 10 mmol/L $MgCl_2$, and 1 mm dithiothreitol, pH 7.5, at 25°C), 300 nmol/L of each primer, and 0.5 U Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT). The forward primer was 5′-CATCCATTTTCTTCCACAGG-3′ and the reverse primer was 5′-CCCAGAATGCTCACCAGACT-3′. These primers generated a product of 137 bp, which was cut into fragments of 117 + 20 bp in the presence of the *Hin*fI cutting site (X447 allele).[31] The amplification protocol was (1) 1 cycle of denaturation at 96°C for 3 minutes, (2) 40 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 1 minute, and (3) 1 final elongation cycle at 72°C for 10 minutes. A negative control without DNA was performed in every amplification.

After each amplification, the PCR product was digested overnight at 37°C after adding 10 U restriction enzyme *Hin*fI to the PCR mixture. The resulting fragments were separated by electrophoresis in 10% acrylamide gels. Each gel was allowed to run for 2 hours at 150 V, stained with ethidium bromide, and photographed under UV transmitted light. The X174 DNA digested with *Hae*III was used as a length marker to estimate the size of the digested DNA fragments. The allele without the *Hin*fI restriction site is designated here as the S447 allele (137 bp), whereas the allele with the *Hin*fI restriction site is the X447 allele (117 bp + 20 bp).

PCR amplification of the D8S261, LPL tetranucleotide repeat, and D8S258 microsatellites. PCR amplification of the 3 microsatellites was performed in a vol of 10 μL containing 250 ng DNA, 125 μmol/L each of dATP, dCTP, dGTP, and dTTP, 10X buffer (100 mmol/L Tris hydrochloride, 500 mmol/L KCl, 15 mmol/L $MgCl_2$, and 0.01% gelatin, pH 8.3, at 25°C), 0.1 pmol forward primer, 0.4 pmol reverse primer, and 0.3 U Taq polymerase (Perkin Elmer-Cetus). For the D8S261 microsatellite, the forward primer was 5′-TGCCACTGTCTTGAAATTCC-3′ and the reverse primer was 5′-TATGGCCCAGCAATGTGTAT3′. For the LPL tetranucleotide repeat microsatellite,[32] the forward primer was 5′-ATCTGACCAAGGATAGTGG-3′ and the reverse primer was 5′-TCCTGGGTAACTGAGCGAGAC3′. For the D8S258 microsatellite, the forward primer was 5′-CTGCCAGGAATCAACTGAG-3′ and the reverse primer was 5′-TTGACAGGGACCCACG-3′. The amplification protocol was (1) 1 cycle of denaturation at 93°C for 5 minutes, (2) 1 cycle of annealing at 57°C (D8S261 and LPL tetranucleotide repeat) or 55°C (D8S258) for 60 seconds, (3) 10 cycles of denaturation at 94°C for 20 seconds and annealing at 57°C for 60 seconds, and (4) 24 cycles of denaturation at 94°C for 20 seconds and annealing at 52°C for 60 seconds. All 3 forward primers were labeled with an infrared tag (IRD41 and IRD800; Li-Cor, Lincoln, NE).

After amplification, 1 μL of each PCR product was loaded on a 6% acrylamide urea gel on a DNA automatic sequencer (model 4000; Li-Cor). Genotyping was assessed with computer software (ONE-Dscan; Scanalytics, Billirica, MA). Mendelian inheritance was confirmed within families for all markers typed on all subjects involved in this study.

Statistical Analysis

All statistical analyses were performed using the SAS[33] and SAGE[34] software. Plasma TG, VLDL-TG, VLDL-cholesterol, and VLDL-apo B and the PH-HL/PH-LPL and HDL_2-C/HDL_3-C ratios were logarithmically transformed. A chi-square test was performed to determine if the genotype frequencies of the S447X polymorphism were in Hardy-Weinberg equilibrium. Alleles of genotypes with fewer than 5 subjects were pooled for the chi-square tests. We also calculated the heterozygosity and the polymorphic information content.[35]

Linkage analysis. Phenotypes were adjusted for the age effects (age, age^2, and age^3) in each of the 6 sex × age (<25, ≥25 to <45, and ≥45 years) groups using a stepwise regression, keeping only terms that were significant at the 5% level. Lipases, lipid variables, and abdominal visceral fat were further adjusted for fat mass. For adjustment purposes, the outliers defined as those beyond ±3 SD of the mean for each sex and age group–specific distribution were temporarily set aside. The regression parameters were then used to calculate the residuals for all subjects including the outliers. Residuals were then standardized to a mean of 0 and a SD of 1. The sib-pair linkage method implemented in the SIBPAL version 3.1 software from SAGE[34] was used to test for linkage between phenotypes and LPL polymorphisms. In this nonparametric analysis, the relationship between the number of alleles identical by descent (IBD) shared by sibs and the within-pair variability for the trait is analyzed by regression. If a locus related to the phenotype is located at or near the tested marker, the phenotypic variance will be smaller among sib-pairs sharing more alleles IBD than among sibs who share fewer alleles IBD.

Association. Two genotype groups, carriers (genotypes S447X and X447X) and noncarriers (genotype S447S) of the X447 allele, were considered. Associations between the S447X polymorphism and each phenotype were investigated separately in men and women using an ANCOVA (General Linear Model) procedure that included age as a covariate (age, age^2, and age^3) in each of the 3 age (<25, ≥25 to <45, and ≥45 years) groups. Lipases, lipid variables, and abdominal visceral fat were further analyzed with age and fat mass as covariates. To test for potential interactions between obesity and the S447X polymorphism, associations were investigated separately in normal-weight (BMI < 25 kg/m^2) and obese (BMI ≥ 30 kg/m^2) subjects.

RESULTS

Body fatness and metabolic variables are summarized in Table 1. Men less than 25 years of age and women less than age 45 years were not overweight (mean BMI < 25 kg/m^2). However, men older than 25 years and women older than 45 years were, on average, overweight (25 ≤ BMI < 30 kg/m^2). All body fatness variables increased significantly across the 3 age groups in both men and women. In both genders, all plasma and lipoprotein phenotypes increased significantly across age groups, except apo A1 (men only), HDL-C (men only), and HDL_3-C and the HDL_2-C/HDL_3-C ratio (women only). Overall, differences among age groups were generally less important in men than in women. Furthermore, HDL-C levels were significantly higher in young women (<25 years) than in men of the same age. For all age groups, men had a higher PH-HL/PH-LPL ratio than women.

Allelic Frequencies

Allele and genotype frequencies of the S447X polymorphism calculated with parental data alone are presented in Table 2. The genotype frequency distribution is in Hardy-Weinberg equilib-

Table 1. Body Fatness and Lipid Traits for Each Sex and Age Group

Trait	Men (age, yr)			Women (age, yr)		
	<25	≥25 to <45	≥45	<25	≥25 to <45	≥45
No. of subjects	77-72	72-68	96-83	95-92	72-68	90-78
Age (yr)	20.5 ± 0.3	30.8 ± 0.5	53.7 ± 0.5	20.9 ± 0.2	32.1 ± 0.6	52.4 ± 0.5
BMI (kg/m^2)	23.9 ± 0.5	27.4 ± 0.6	28.4 ± 0.4¶	23.2 ± 0.5	24.4 ± 0.5	27.5 ± 0.5¶
Fat mass (kg)	13.4 ± 1.2	21.5 ± 1.2	24.6 ± 0.9¶	16.7 ± 1.0*	20.4 ± 1.1	26.8 ± 1.2¶
Fat (%)	16.2 ± 1.1	23.8 ± 0.9	27.6 ± 0.7¶	25.2 ± 0.9‡	29.2 ± 1.0‡	36.6 ± 0.9‡¶
Sum of 8 skinfolds (mm)	100 ± 6	145 ± 6	146 ± 5¶	146 ± 5‡	154 ± 6	196 ± 6‡¶
Abdominal visceral fat area (cm^2)	57 ± 3	103 ± 5	158 ± 6¶	45 ± 2†	65 ± 4‡	119 ± 6‡¶
Abdominal total fat area (cm^2)	208 ± 17	367 ± 22	428 ± 15¶	276 ± 17*	349 ± 19	481 ± 18*¶
Apo B (mg/dL)	71.9 ± 2.0	91.6 ± 2.7	104.8 ± 2.2¶	75.0 ± 2.3	79.4 ± 2.6†	90.6 ± 2.1‡¶
Plasma TG (mmol/L)	1.13 ± 0.06	1.53 ± 0.11	1.93 ± 0.10¶	1.05 ± 0.05	1.13 ± 0.07†	1.39 ± 0.07‡¶
Plasma cholesterol (mmol/L)	3.82 ± 0.08	4.62 ± 0.11	5.12 ± 0.09¶	4.07 ± 0.09*	4.34 ± 0.10	4.89 ± 0.08¶
VLDL-apo B (mg/dL)	6.7 ± 0.3	9.0 ± 0.6	11.5 ± 0.6¶	6.4 ± 0.3	7.4 ± 0.6	8.3 ± 0.4‡‖
VLDL-TG (mmol/L)	0.75 ± 0.05	1.07 ± 0.10	1.36 ± 0.09¶	0.60 ± 0.04*	0.64 ± 0.05‡	0.83 ± 0.05‡‖
VLDL-C (mmol/L)	0.36 ± 0.02	0.54 ± 0.05	0.70 ± 0.05¶	0.31 ± 0.02	0.34 ± 0.03†	0.43 ± 0.02‡‖
LDL-apo B (mg/dL)	65.2 ± 1.9	82.6 ± 2.4	93.3 ± 1.9¶	68.6 ± 2.1	71.9 ± 2.3†	82.3 ± 1.9‡¶
LDL-C (mmol/L)	2.49 ± 0.07	3.14 ± 0.09	3.49 ± 0.08¶	2.67 ± 0.08	2.83 ± 0.09*	3.27 ± 0.07*¶
Apo A1 (mmol/L)	1.12 ± 0.02	1.14 ± 0.02	1.16 ± 0.02	1.16 ± 0.02	1.21 ± 0.02†	1.27 ± 0.02‡¶
HDL-C (mmol/L)	0.96 ± 0.02	0.92 ± 0.02	0.91 ± 0.02	1.09 ± 0.02‡	1.16 ± 0.03‡	1.20 ± 0.03‡§
HDL_3-C (mmol/L)	0.67 ± 0.01	0.68 ± 0.01	0.65 ± 0.01	0.70 ± 0.01	0.71 ± 0.01	0.74 ± 0.02‡
HDL_2-C (mmol/L)	0.29 ± 0.01	0.24 ± 0.01	0.26 ± 0.01§	0.39 ± 0.02‡	0.45 ± 0.02‡	0.46 ± 0.02‡§
HDL_2-C/HDL_3-C	0.44 ± 0.02	0.36 ± 0.02	0.40 ± 0.02§	0.57 ± 0.02‡	0.63 ± 0.03‡	0.64 ± 0.03‡
Plasma cholesterol/HDL-C	4.08 ± 0.12	5.27 ± 0.19	5.89 ± 0.16¶	3.87 ± 0.10	3.92 ± 0.14‡	4.31 ± 0.12‡§
PH-HL activity (nmol/min/mL)	263 ± 7	251 ± 6	221 ± 6¶	179 ± 6*	165 ± 7*	177 ± 7*
PH-LPL activity (nmol/min/mL)	49 ± 3	46 ± 3	52 ± 3	56 ± 2	64 ± 4†	72 ± 4‡‖
PH-HL/PH-LPL	9.0 ± 1.8	9.5 ± 1.5	7.4 ± 1.2	5.5 ± 1.0	3.7 ± 0.4‡	3.8 ± 0.4*

NOTE. Results are the mean ± SEM.

Sex differences by ANOVA: *$P < .05$, †$P < .005$, ‡$P < .0005$.

Age group differences within each sex by ANOVA: §$P < .05$, ‖$P < .005$, ¶$P < .0005$.

Table 2. Allele and Genotype Frequency of the LPL S447X Polymorphism and Three Microsatellites

	No. of Subjects	PIC	Hz	Allele Frequency S447		Allele Frequency X447		Genotype Frequency S447S		Genotype Frequency S447X		Genotype Frequency X447X		
LPL S447X	190	0.16	0.18	0.90		0.10		0.82		0.16		0.02		
Microsatellites														
D8S261														
Alleles in bp				124	126	128	130	132	134	136	138	140	142	144
		0.75	0.78	0.003	0.013	0.045	0.25	0.024	0.042	0.356	0.128	0.099	0.034	0.006
LPL tetranucleotide repeat														
Alleles in bp				112	116	120	124	128	132	136	140			
		0.65	0.70	0.003	0	0.037	0.394	0.307	0.214	0.042	0.003			
D8S258														
Alleles in bp				144	146	148	150	152	154	156	158			
		0.65	0.70	0.089	0.005	0.354	0.157	0.376	0.016	0	0.003			

Abbreviations: PIC, polymorphism information content; Hz, heterozygosity.

rium ($X^2 = 1.39$, $P > .05$). No sex differences were observed in the allele frequency of lean subjects ($X^2 = 2.29$, $P > .05$) and obese subjects ($X^2 = .01$, $P > .05$). The frequency of the X447 allele reported herein is comparable to[17,19,31,36] or greater than[9,18,37] reported in other studies.

Allele frequencies of the D8S261, LPL tetranucleotide repeat, and D8S258 microsatellites are presented in Table 2. Additional alleles besides those previously reported (Genome Data Base) were detected for the D8S261 (alleles 124 bp and 126 bp), LPL tetranucleotide repeat (alleles 112 bp and 140 bp), and D8S258 (allele 158 bp).

Linkage Analyses

A minimum of 301 and a maximum of 364 sib-pairs were available. Suggestive evidence of linkage was observed only between the LPL S447X polymorphism and VLDL-apo B (332 sib-pairs, $P = .013$; results not shown).

Association Analyses

No significant association of the LPL S447X polymorphism was observed in either gender with body fatness phenotypes including abdominal visceral fat (results not shown). The LPL S447X polymorphism was not associated with PH-LPL activity in men (carriers *v* noncarriers, 52.9 ± 4.2 *v* 47.0 ± 2.1 nmol/min/mL, $P = .19$) and women (carriers *v* noncarriers, 67.3 ± 5.1 *v* 63.3 ± 2.4 nmol/min/mL, $P = .47$; Table 3). However, when PH-LPL was not adjusted for fat mass, we observed a significant association in men (carriers *v* noncarriers, 56.1 ± 4.0 *v* 47.1 ± 2.1 nmol/min/mL, $P = .04$).

In men, carriers of the X447 allele exhibited significantly lower VLDL-apo B and VLDL-C concentrations (for raw and log-transformed data; Table 3) and lower plasma TG (1.29 ± 0.13 *v* 1.65 ± 0.06 mmol/L, $P = .01$) and VLDL-TG (0.85 ± 0.11 *v* 1.16 ± 0.06 mmol/L, $P = .01$) than noncarriers (Fig 1). In women, the associations between the LPL S447X polymorphism and VLDL-apo B and VLDL-C were equivalent to those observed in men (Table 3). In contrast, women carriers exhibited a lower plasma cholesterol to HDL-C ratio (3.63 ± 0.17 *v* 4.05 ± 0.07 mmol/L, $P = .02$) than noncarriers, which may result from the trend observed for HDL-C (carriers *v* noncarriers, 1.222 ± 0.039 *v* 1.142 ± 0.018 mmol/L, $P = .06$). The latter can be attributed to an increase in the cholesterol content of the HDL_3 subfraction (carriers *v* noncarriers, 0.756 ± 0.021 *v* 0.713 ± 0.009 mmol/L, $P = .06$) (Table 3).

Table 3. Association of the LPL S447X Polymorphism With Metabolic Traits Measured in Men and Women

	Men					Women				
Trait	No.	Noncarriers of the X447 Allele	No.	Carriers of the X447 Allele	*P*	No.	Noncarriers of the X447 Allele	No.	Carriers of the X447 Allele	*P*
Apo B (mg/dL)	188	91.2 ± 1.5	40	90.8 ± 3.1	.90	204	81.8 ± 1.5	43	75.5 ± 3.2	.08
Plasma cholesterol	188	4.60 ± 0.06	40	4.55 ± 0.13	.69	204	4.43 ± 0.06	43	4.28 ± 0.12	.28
VLDL-apo B (mg/dL)	188	9.79 ± 0.38	40	7.55 ± 0.78	.009	204	7.57 ± 0.27	43	5.83 ± 0.60	.009
VLDL-C (mmol/L)	188	0.600 ± 0.031	40	0.422 ± 0.063	.009	204	0.372 ± 0.015	43	0.278 ± 0.033	.01
LDL-apo B (mg/dL)	188	81.4 ± 1.4	40	83.2 ± 2.9	.55	204	74.2 ± 1.3	43	69.6 ± 2.9	.15
LDL-C (mmol/L)	188	3.06 ± 0.06	40	3.15 ± 0.11	.47	204	2.92 ± 0.05	43	2.78 ± 0.11	.27
Apo A1 (mmol/L)	188	1.138 ± 0.012	40	1.168 ± 0.025	.28	204	1.210 ± 0.012	43	1.218 ± 0.026	.78
HDL-C (mmol/L)	188	0.925 ± 0.015	40	0.966 ± 0.030	.21	204	1.142 ± 0.018	43	1.222 ± 0.039	.06
HDL_2-C (mmol/L)	188	0.263 ± 0.008	40	0.276 ± 0.017	.50	204	0.423 ± 0.013	43	0.467 ± 0.029	.25
HDL_3-C (mmol/L)	188	0.661 ± 0.009	40	0.690 ± 0.019	.17	204	0.713 ± 0.009	43	0.756 ± 0.021	.06
HDL_2-C/HDL_3-C	188	0.40 ± 0.01	40	0.40 ± 0.02	.87	204	0.62 ± 0.02	43	0.62 ± 0.04	.97
Plasma cholesterol/HDL-C	188	5.22 ± 0.10	40	4.85 ± 0.21	.11	204	4.05 ± 0.07	43	3.63 ± 0.17	.02
PH-HL activity (nmol/min/mL)	186	243 ± 5	40	257 ± 9	.18	192	173 ± 4	41	178 ± 10	.64
PH-LPL activity (nmol/min/mL)	186	47.0 ± 2.1	40	52.9 ± 4.2	.19	192	63.3 ± 2.4	41	67.3 ± 5.1	.47
PH-HL/PH-LPL	186	8.8 ± 1.1	40	9.8 ± 2.2	.68	192	4.4 ± 0.5	41	4.6 ± 1.1	.91

NOTE. Data are the least-square mean ± SEM. TG values are shown in Fig 1.

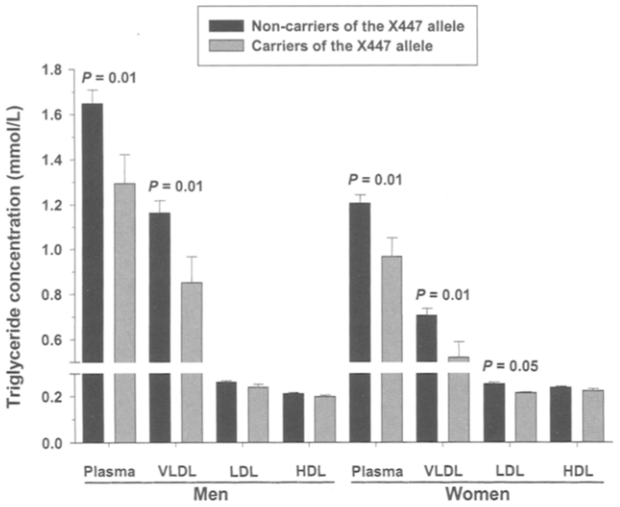

Fig 1. TG concentrations in plasma, VLDL, LDL, and HDL particles by X447 allele carrier status for the LPL S447X polymorphism. Data are the least-square mean ± SEM for men (n = 188) and women (n = 204) noncarriers and men (n = 40) and women (n = 43) carriers of the X447 allele. *P* values were obtained using ANCOVA with age and fat mass as covariates.

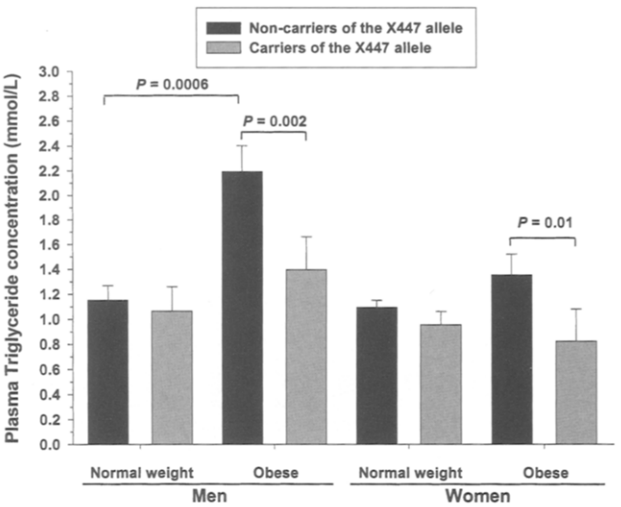

Fig 2. Associations between the LPL S447X polymorphism and plasma TG concentrations measured in normal-weight men (BMI = 22.4 ± 1.8 kg/m²; noncarriers, n = 79; carriers, n = 17) and women (BMI = 21.8 ± 1.9 kg/m²; noncarriers, n = 121; carriers, n = 26) and obese men (BMI = 33.9 ± 3.3 kg/m²; noncarriers, n = 39; carriers, n = 11) and women (BMI = 34.8 ± 3.8 kg/m²; noncarriers, n = 26; carriers, n = 8). Data are the least-square mean ± SEM. *P* values were obtained using ANCOVA with age and fat mass as covariates.

No difference between carriers and noncarriers was observed for the HDL_2-C/HDL_3-C ratio in either sex.

No significant differences in plasma TG, VLDL-TG, and VLDL-apo B concentrations, the plasma cholesterol to HDL-C ratio, and PH-LPL activity were observed between carriers and noncarriers in normal-weight men (BMI = 22.4 ± 1.8 kg/m²) or women (BMI = 21.8 ± 1.9 kg/m²). However, obese men (BMI = 33.9 ± 3.3 kg/m²) who were carriers of the X447 allele showed 36% lower plasma TG (1.39 ± 0.27 *v* 2.19 ± 0.21 mmol/L, $P = .002$; Fig 2), 46% lower VLDL-TG (0.88 ± 0.24 *v* 1.63 ± 0.18 mmol/L, $P = .001$), and 42% lower VLDL-apo B (7.34 ± 1.71 *v* 12.67 ± 1.30 mmol/L, $P = .001$) concentrations, a 14% lower plasma cholesterol to HDL-C ratio (4.88 ± 0.42 *v* 5.70 ± 0.32, $P = .05$; Fig 3), and a 66% higher PH-LPL activity (70.8 ± 8.9 *v* 42.7 ± 6.9 nmol/min/mL, $P = .001$; Fig 4) than noncarriers. Plasma TG ($P = .36$), VLDL-TG ($P = .48$), VLDL-apo B ($P = .77$), plasma cholesterol to HDL-C ($P = .37$), and PH-LPL activity ($P = .11$) for obese carriers of the X447 allele were not significantly different from those observed in normal-weight subjects.

Obese women (BMI = 34.8 ± 3.8 kg/m²) exhibited the same association pattern as men for plasma TG, VLDL-TG, and VLDL-apo B. Obese women carriers of the X447 allele had 39% lower plasma TG (0.82 ± 0.26 *v* 1.35 ± 0.217 mmol/L, $P = .01$; Fig 2), 51% lower VLDL-TG (0.43 ± 0.20 *v* 0.87 ± 0.13 mmol/L, $P = .01$), and 45% lower VLDL-apo B (4.58 ± 1.63 *v* 8.40 ± 1.07 mmol/L, $P = .006$) concentrations than noncarriers. No significant association was observed between the LPL S447X polymorphism and the plasma cholesterol to HDL-C ratio (3.67 ± 0.47 *v* 4.31 ± 0.31, $P = .11$; Fig 3) and PH-LPL activity (84.97 ± 15.13 *v* 72.3 ± 10.04 nmol/min/mL, $P = .33$; Fig 4).

DISCUSSION

The functional significance of the C-terminal dipeptide Ser-Gly of the LPL gene remains uncertain, but it is highly conserved across all known mammalian LPLs[13] and may be involved in binding the enzyme to the endothelial cell surface. It

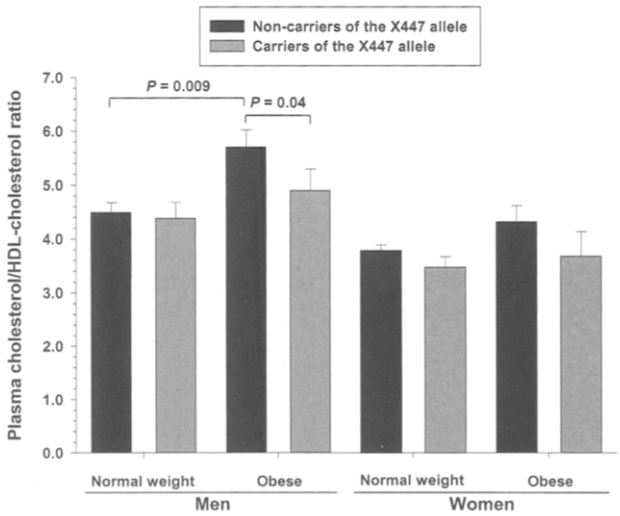

Fig 3. Associations between the LPL S447X polymorphism and the plasma cholesterol to HDL-C ratio measured in normal-weight men (BMI = 22.4 ± 1.8 kg/m²; noncarriers, n = 79; carriers, n = 17) and women (BMI = 21.8 ± 1.9 kg/m²; noncarriers, n = 121; carriers, n = 26) and obese men (BMI = 33.9 ± 3.3 kg/m²; noncarriers, n = 39; carriers, n = 11) and women (BMI = 34.8 ± 3.8 kg/m²; noncarriers, n = 26; carriers, n = 8). Data are the least-square mean ± SEM. *P* values were obtained using ANCOVA with age and fat mass as covariates.

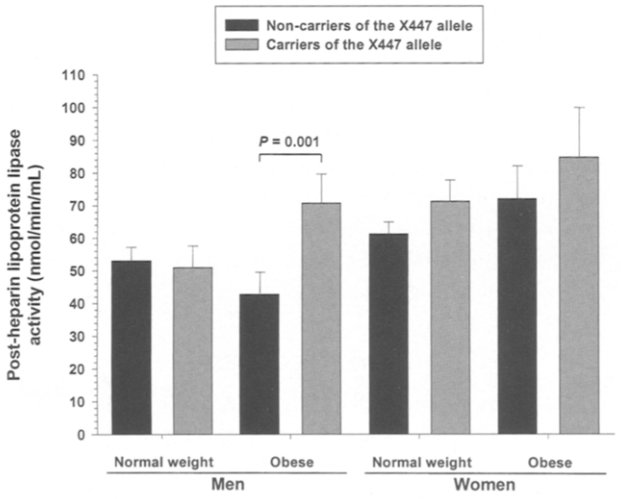

Fig 4. Associations between the LPL S447X polymorphism and PH-LPL activity measured in normal-weight men (BMI = 22.4 ± 1.8 kg/m²; noncarriers, n = 77; carriers, n = 17) and women (BMI = 21.8 ± 1.9 kg/m²; noncarriers, n = 114; carriers, n = 25) and obese (BMI ≥ 30 kg/m²) men (BMI = 33.9 ± 3.3 kg/m²; noncarriers, n = 38; carriers, n = 11) and women (BMI = 34.8 ± 3.8 kg/m²; noncarriers, n = 24; carriers, n = 7). Data are the least-square mean ± SEM. *P* values were obtained using ANCOVA with age and fat mass as covariates.

has been postulated that the X447 allele may be responsible for a defect in lipid substrate interaction.[38] In vitro studies proposed that LPL truncation by 2 residues has either normal[14] or higher[13] LPL activity. On the other hand, the previously reported association of the X447 allele with higher PH-LPL activity[15] was not confirmed in the present study when we adjusted PH-LPL activity for age and fat mass, although it should be noted that the former observations were obtained in CAD patients using β-blockers. Indeed, in the study by Groenemeijer et al,[15] when the effect of β-blockers, which have an unfavorable effect on PH-LPL activity, was considered, the association disappeared. Thus, the LPL S447X polymorphism or the other markers tested in the present study cannot account for the genetic variance component of PH-LPL activity previously observed in the HERITAGE Family Study (females, $h^2 = 76\%$; males, $h^2 = 30\%$).[39] It should be noted that all of the above-mentioned studies have been performed on Caucasian subjects and little is known about race differences in the LPL S447X polymorphism and its impact on lipids and lipoproteins.

LPL activity is involved in the clearance of TG-rich lipoproteins from the circulation and contributes to the formation of HDL_2 particles, since hydrolysis of TG-rich lipoproteins leads to the transfer of phospholipids, TG, and apolipoproteins to nascent HDL (HDL_3) particles. Kuivenhoven et al[17] have recently demonstrated that the X447 allele is more frequent in the highest tertile of the HDL-C distribution in a sample of 241 healthy Dutch males. In the present study, the HDL-C subfraction affected (and only in women) by the X447 allele was HDL_3, which represents about two thirds of plasma HDL-C levels. CAD subjects with the X447 allele have been shown to exhibit a lower plasma cholesterol to HDL-C ratio reflecting the beneficial effect of this allele on the lipoprotein profile in adult males.[16] Results reported herein reproduced this association of the X447 allele with the plasma cholesterol to HDL-C ratio in women, but failed to link the variation in PH-LPL activities to this association. The elevated level of HDL_3-C in carriers of the X447 allele can perhaps be explained by the fact that a lower HDL-TG concentration in these subjects may have led to a decrease in HDL catabolism. If PH-LPL activity was increased by the truncation of the last two residues, we would expect to observe a higher HDL_2-C concentration, which is not the case. The increase in HDL_3-C is related to a decrease in HDL_3 to HDL_2 transformation and a decrease in catabolism compared with normal HDL particles with normal HDL-TG levels. Also, the HDL_2-C/HDL_3-C ratio, which is increased by high PH-LPL activity, is not affected by the LPL S447X polymorphism. Thus, we speculate that a decrease of HDL catabolism caused by a decrease in TG content rather than an increase in the HDL_3 to HDL_2 transformation accounts for the effect of the polymorphism on HDL-C.

In humans, the X447 allele has been reported to be more frequent in healthy controls than in hypertriglyceridemic and low-HDL-C subjects.[12] Stocks et al[31] showed that the X447 allele was less frequent among patients with primary hypertriglyceridemia. In addition, the X447 allele was associated with lower TG levels in subjects of the ECTIM Study.[19] Furthermore, the X447 allele was associated with lower fasting and postprandial TG levels in subjects with a paternal history of myocardial infarction.[9] Humphries et al[9] also proposed that the X447 allele may affect a nonenzymatic function of LPL such as its ligand role. This is in concordance with our findings of a significant association between the X447 allele and lower TG and VLDL levels in both genders. The significant association with a lower LDL-TG level in women may be the result of lower TG levels (VLDL) observed in carriers. A proposed mechanism is that VLDL receptors may interact with VLDL particles in a stronger manner when subjects are carriers of the X447 allele. LPL is known to enhance binding to VLDL receptors via both the formation of bridges between lipoproteins and heparan sulfate proteoglycans and its lipolytic effect.[40] One could speculate that the lower plasma concentrations of VLDL-TG, VLDL-C, and VLDL-apo B observed in carriers may result from a higher relative amount of internalization of VLDL particles via the LDL receptor protein[41] for the truncated LPL protein. This molecular interaction is thought to be dependent on the carboxy-terminal domain of the LPL protein.[42] Because LPL activity is associated with the dimeric form and ligand activity with the monomeric form, we may speculate that the truncated protein is present in the plasma in the monomeric form in a greater amount than the mature protein, thus resulting in higher ligand activity. Mutational analyses in COS-1 cells support the fact that the truncated protein exhibits higher mass and, more specifically, higher specific activity than the nontruncated protein.[13] Thus, it would be of interest to investigate the impact of the LPL S447X polymorphism on the protein form (dimeric or monomeric) and mass.

Three different haplotypes were found for the S447X and the intron 8 *Hin*dIII polymorphisms: H+S447, H−S447, and H−X447.[9] In our results, obese carriers of the X447 allele had the same plasma TG, VLDL-TG, and cholesterol to HDL-C

ratio as those observed in normal-weight subjects. Thus, the effect of the *Hin*dIII polymorphism on TG levels in previous reports[9-11] may likely be attributed to the S447X polymorphism, as the 2 markers are in linkage disequilibrium.[9] When we tested for potential interactions between obesity and the S447X polymorphism, the effect of this polymorphism on plasma TG, VLDL-TG, VLDL-apo B, cholesterol to HDL-C ratio, and PH-LPL activity was observed mainly in obese subjects. This effect was stronger in women than in men, especially for the association with PH-LPL activity. The effect of the X447 allele on the TG concentration is concordant with its effect on PH-LPL activity, which is higher in obese carriers of the X447 allele compared with noncarriers.

A weak but significant linkage was observed between the S447X polymorphism and VLDL-apo B. The fact that none of the microsatellites around the LPL gene exhibited significant linkage with VLDL-apo B suggests that the linkage is likely attributable to the LPL gene itself and not another gene. In addition, the significant association between the same marker and the same trait provides further support for the notion that the S447X marker has a significant metabolic impact on plasma VLDL-apo B concentrations.

In conclusion, the LPL S447X polymorphism is not associated with the body fatness, visceral fat level, and postheparin plasma lipase activities. Although this polymorphism is only marginally associated with the plasma cholesterol to HDL-C ratio, it is strongly associated with plasma TG, VLDL-TG, and VLDL-apo B levels, and to a greater extent in sedentary obese men versus women. Obese carriers of the X447 allele exhibit cardiovascular risk factors, such as plasma TG, VLDL-TG, and the cholesterol to HDL-C ratio, equivalent to those of normal-weight sedentary individuals.

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REFERENCES

1. Patsh JR, Prasad S, Gotto AM, et al: HDL2 relationships of the plasma levels of this lipoprotein species to its composition, to the magnitude of post prandial lipaemia and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 80:341-347, 1987

2. Garfinkel AS, Schotz MC: Lipoprotein lipase, in Gotto AM Jr (ed): Plasma Lipoproteins. New York, NY, Elsevier Science, 1987, pp 335-357

3. Taskinen M-R, Nikkilä EA: High density lipoprotein subfractions in relation to lipoprotein lipase activity of tissues in man—Evidence for reciprocal regulation of HDL_2 and HDL_3 levels by lipoprotein lipase. Clin Chim Acta 112:325-332, 1981

4. Kuusi T, Saarinen P, Nikkilä EA: Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein2 in man. Atherosclerosis 36:589-593, 1980

5. Tikkanen MJ, Nikkilä EA: Regulation of hepatic lipase and serum lipoproteins by sex steroids. Am Heart J 113:562-567, 1987

6. Ben-Zeev O, Lusis AJ, Leboeuf RC, et al: Evidence for independent and genetic regulation of heart and adipose lipoprotein lipase activity. J Biol Chem 258:13632-13636, 1983

7. Sparkes RS, Zollman S, Klisak I, et al: Human genes involved in lipolysis of plasma lipoproteins: Mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21. Genomics 1:138-144, 1987

8. Nickerson DA, Taylor SL, Weiss KM, et al: DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene. Nat Genet 19:233-240, 1998

9. Humphries SE, Nicaud V, Margalef J, et al: Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglyceride. The European Atherosclerosis Research Study (EARS). Arterioscler Thromb Vasc Biol 18:526-534, 1998

10. Jemaa R, Tuzet S, Portos C, et al: Lipoprotein lipase gene polymorphisms: Associations with hypertriglyceridemia and body mass index in obese people. Int J Obes Relat Metab Disord 19:270-274, 1995

11. Vohl M-C, Lamarche B, Moorjani S, et al: The lipoprotein lipase *Hin*dIII polymorphism modulates plasma triglyceride levels in visceral obesity. Arterioscler Thromb Vasc Biol 15:714-720, 1995

12. Hata A, Robertson M, Emi M, et al: 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, 1990

13. Kozaki K, Gotoda T, Kawamura M, et al: Mutational analysis of human lipoprotein lipase by carboxy-terminal truncation. J Lipid Res 34:1765-1772, 1993

14. Faustinella F, Chang A, Van Biervliet JP, et al: Catalytic triad residue mutation ($Asp^{156} \rightarrow Gly$) causing familial lipoprotein lipase deficiency. Co-inheritance with a nonsense mutation ($Ser^{447} \rightarrow Ter$) in a Turkish family. J Biol Chem 266:14418-14424, 1991

15. Groenemeijer BE, Hallman MD, Reymer PWA, et al: Genetic variant showing a positive interaction with β-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser^{447}-Stop substitution in the lipoprotein lipase gene. Circulation 95:2628-2635, 1997

16. Galton DJ, Mattu R, Needham EW, et al: Identification of putative beneficial mutation for lipid transport. Z Gastroenterol 34:56-58, 1996 (suppl 3)

17. Kuivenhoven JA, Groenemeyer BE, Boer JMA, et al: Ser447Stop mutation in lipoprotein lipase is associated with elevated HDL cholesterol levels in normolipidemic males. Arterioscler Thromb Vasc Biol 17:595-599, 1997

18. Zhang Q, Cavanna J, Winkelman BR, et al: Common genetic polymorphisms of lipoprotein lipase that relate to lipid transport in patients with premature coronary artery disease. Clin Genet 48:293-298, 1995

19. Jemaa R, Fumeron F, Poirier O, et al: Lipoprotein lipase gene polymorphisms: Associations with myocardial infarction and lipoprotein levels. The ECTIM Study. J Lipid Res 36:2141-2146, 1995

20. Thorn JA, Needham EWA, Mattu R, et al: The Ser^{447}-Ter mutation of the lipoprotein lipase gene relates to variability of serum lipid and lipoprotein levels in monozygotic twins. J Lipid Res 39:437-441, 1998

21. Bouchard C, Leon AS, Rao DC, et al: The HERITAGE Family Study: Aims, design, and measurement protocol. Med Sci Sports Exerc 27:721-729, 1995

22. Wilmore JH, Stanforth PR, Domenick MA, et al: Reproducibility of anthropometric and body composition measurements: The HERITAGE Family Study. Int J Obes 21:297-303, 1997

23. Ferland M, Després J-P, Tremblay A, et al: Assessment of adipose tissue distribution by computed axial tomography in obese women: Association with body density and anthropometric measurements. Br J Nutr 61:139-148, 1989

24. Nilsson-Ehle P, Ekman R: Specific assays for lipoprotein lipase and hepatic lipase activities of post-heparin plasma, in Peeters H (ed): Protides of Biological Fluids, vol 25. Oxford, UK, Pergamon, 1978, pp 243-246

25. St-Amand J, Després J-P, Lemieux S, et al: Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women. Metabolism 44:491-498, 1995

26. Moorjani S, Dupont A, Labrie F, et al: Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism 36:244-250, 1987

27. Burstein M, Samaille J: Sur un dosage rapide du cholesterol lié aux β-lipoprotéines du sérum. Clin Chim Acta 5:609-610, 1960

28. Gidez LI, Miller GJ, Burstein M, et al: Separation and quantification of subclass of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 23:1206-1223, 1982

29. Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 5:609-610, 1960

30. Dionne FT, Turcotte L, Thibault M-C, et al: Mitochondrial DNA sequence polymorphism, VO_2max, and response to endurance training. Med Sci Sports Exerc 23:177-185, 1991

31. Stocks J, Thorn JA, Galton DJ: Lipoprotein lipase genotype for a common premature termination codon mutation detected by PCR-mediated site-directed mutagenesis and restriction digestion. J Lipid Res 33:853-857, 1992

32. Zuliani G, Hobbs HH: Tetranucleotide repeat polymorphism in the LPL gene. Nucleic Acids Res 18:4958, 1990

33. SAS Institute: Statistical Analysis System, release 6.12. SAS Institute, Cary, NC

34. SAGE: Statistical Analysis for Genetic Epidemiology, release 3.1. Computer program package available from the Department of Biometry and Genetics, LSU Medical Center, New Orleans, LA, 1997

35. Ott J: Analysis of Human Genetic Linkage. Baltimore, MD, The Johns Hopkins University Press, 1991

36. Mattu RK, Needham EWA, Morgan R, et al: DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population. Arterioscler Thromb 14:1090-1097, 1994

37. Peacock RE, Hamsten A, Nilsson-Ehle P, et al: Association between lipoprotein lipase gene polymorphism and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. Atherosclerosis 97:171-185, 1992

38. Kobayashi J, Nishida T, Ameis D, et al: A heterozygous mutation (the codon for Ser447 → a stop codon) in lipoprotein lipase contributes to a defect in lipid interface recognition in a case with type I hyperlipidaemia. Biochem Biophys Res Commun 182:70-77, 1992

39. Pérusse L, Rice T, Després JP, et al: Familial resemblance of plasma lipids, lipoproteins and post-heparin lipoprotein and hepatic lipases in the HERITAGE Family Study. Arterioscler Thromb Vasc Biol 17:3263-3269, 1997

40. Takahashi S, Suzuki J, Kohno M, et al: Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. J Biol Chem 270:15747-15754, 1995

41. Beisiegel U, Weber W, Bengtsson-Olivecrona G: Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor–related protein. Proc Natl Acad Sci USA 88:8342-8346, 1991

42. Nykjaer A, Bengtsson-Olivecrona G, Lookene A, et al: The α2-macroglobulin receptor/low density lipoprotein receptor–released protein binds lipoprotein lipase and migrating very low density lipoprotein lipase associated with the lipase. J Biol Chem 268:15048-15055, 1993