Heterozygosity for Asn^{291} → Ser mutation in the lipoprotein lipase gene in two Finnish pedigrees: effect of hyperinsulinemia on the expression of hypertriglyceridemia

M. Syvänne,[1,*] M. Antikainen,† S. Ehnholm,§ H. Tenkanen,§ S. Lahdenperä,* C. Ehnholm,§ and M-R. Taskinen*

Department of Medicine,* Helsinki University, Central Hospital, Haartmaninkatu 4, FIN-00290 Helsinki, Finland; Children's Hospital,† University of Helsinki, Finland; and Department of Biochemistry,§ National Public Health Institute, Helsinki, Finland

Abstract: We describe two Finnish kindreds with the Asn^{291} → Ser mutation (A291S) of the lipoprotein lipase (LPL) gene. Sixteen subjects (9 male, 7 female) heterozygous for this mutation were studied and compared with 17 unaffected family members or spouses (family controls) and 19 unrelated healthy subjects (population controls). In the group of subjects heterozygous for the A291S mutation, postheparin plasma LPL activity was on average 23% lower than in the family controls and 29% lower than in the population controls. In agreement, in vitro expression studies with COS-7 cells showed that the mutant protein exhibits approximately 50% of the lipolytic activity of the wild-type protein. Median serum triglyceride concentration was 2.90 mmol/l in the group of heterozygotes, compared with 1.14 mmol/l in the family controls ($P < 0.01$) and 0.99 mmol/l in the population controls ($P < 0.001$). The heterozygotes also had a marked preponderance of small dense low density lipoproteins (LDL) as assessed by gradient gel electrophoresis. Nine of the heterozygous subjects were hypertriglyceridemic (serum triglyceride concentration > 2.0 mmol/l). Age or body mass index were not related to the presence of hypertriglyceridemia. By contrast, all hypertriglyceridemic subjects were either hyperinsulinemic (serum insulin concentration > 10 mU/l, n = 7) or had diabetes (n = 2). In a multivariate regression analysis, very low density lipoprotein (VLDL) triglyceride level was significantly and independently related to serum apolipoprotein B concentration, the presence of the A291S mutation, serum insulin concentration, and postheparin plasma LPL activity. The Asn^{291} → Ser mutation of the LPL gene results in reduced lipolytic activity. However, dyslipidemia appears to manifest only if VLDL production is also increased. Hyperinsulinemia was the major determinant of excessive VLDL synthesis and dyslipidemia among the subjects heterozygous for the A291S mutation in this study.—**Syvänne, M., M. Antikainen, S. Ehnholm, H. Tenkanen, S. Lahdenperä, C. Ehnholm, and M-R. Taskinen.** Heterozygosity for Asn^{291} → Ser mutation in the lipoprotein lipase gene in two Finnish pedigrees: effect of hyperinsulinemia on the expression of hypertriglyceridemia. *J. Lipid Res.* 1996. **37:** 727–738.

Supplementary key words LPL deficiency • dyslipidemia • insulin resistance

Lipoprotein lipase (LPL: EC 3.1.1.34) plays a central role in lipoprotein metabolism, hydrolyzing both dietary and endogenous triglycerides (TG) transported in chylomicrons and in VLDL particles (1, 2). LPL is synthesized mainly in adipose tissue, muscle, and the lactating mammary gland (3). The mature protein is secreted from parenchymal cells and transported to capillaries where LPL is bound to glycosaminoglycan components of the capillary endothelium (1, 4). Hydrolysis of TG by LPL requires the presence of apolipoprotein (apo)C-II (5, 6). Another small apolipoprotein, apoC-III, inhibits LPL in vitro (7, 8). LPL can be released into the circulation by an intravenous injection of heparin; thus its activity can be measured in postheparin plasma (9).

The human LPL gene has been assigned to chromosome 8p22 (10). It extends over 30 kb and consists of 10 exons (11, 12). The enzyme contains 448 amino acids, which constitute several functional domains, including a signal peptide, a catalytic domain, and binding sites for the substrate, for heparin, and for apoC-II (6, 11, 12). Mutations leading to amino acid substitutions have been

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Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; apo, apolipoprotein; PCR, polymerase chain reaction; A291S, Asn^{291} → Ser mutation of the LPL gene; NIDDM, non-insulin-dependent diabetes mellitus; HL, hepatic lipase; SSCP, single-strand conformation polymorphism; BMI, body mass index; HTG, hypertriglyceridemic; NTG, normotriglyceridemic.

[1]To whom correspondence should be addressed.

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found in most of the exons in the LPL gene (13). The majority of the missense mutations causing LPL deficiency have, however, been localized to exon 5 which codes for the amino acids 154 to 231 of the mature protein (13, 14).

A missense mutation in the human LPL gene, $Asn^{291} \rightarrow Ser$ (A291S), was first described by Ma and coworkers (15) who suggested that this mutation might be one of the genetic factors involved in the development of type III hyperlipoproteinemia in apoE 2/2 homozygotes. Similar findings and conclusions were obtained later by Zhang et al. (16). Minnich et al. (17) found this mutation in 6 out of 95 unrelated French Canadian type IV hyperlipoproteinemic subjects but in none of 72 normolipidemic control subjects. They concluded that the A291S mutation is associated with type IV hyperlipoproteinemia. The A291S mutation has also been found to be associated with reduced HDL levels and premature atherosclerosis (18). The same authors further reported that a relatively high frequency of this mutation was found among patients with familial combined hyperlipidemia (19). They observed that the carriers of the A291S mutation developed a phenotype of hypertriglyceridemia and low HDL cholesterol in the presence of obesity.

In this paper we report a missense mutation $Asn^{291} \rightarrow Ser$ (A291S) in the human LPL gene in two Finnish pedigrees. We also explored the underlying characteristics that determine whether an individual carrying the mutant allele expresses hypertriglyceridemia.

SUBJECTS AND METHODS

Subjects

Two propositi heterozygous for the A291S mutation of the LPL gene were identified in screening programs. Propositus #1 (subject 1.1.1) was a 60-year-old man with previously diagnosed coronary artery disease, a participant in a secondary prevention trial among post-coronary-bypass men. He had two brothers and three sisters (subjects 1.1.2 through 1.1.6), all of whom participated in the study (**Fig. 1A**). Thus, the first generation of family # 1 consisted of 6 subjects. The second generation of family # 1 consisted of 11 subjects (6 men and 5 women), who also all participated. The subjects of the second generation had altogether 14 children (third generation), 9 of whom were younger than 10 years of age and were either not studied (n = 5) or were excluded from the present analysis. The remaining 5 adolescent subjects were all included in the study.

In addition to the propositus of family # 1, subjects 1.1.2 (a 66-year-old male), 1.1.4 (a 64-year-old female), and 1.1.5 (67 years, female) had symptoms compatible with coronary artery disease. Subject 1.1.4 also had a previous diagnosis of non-insulin-dependent diabetes mellitus (NIDDM) treated with diet only. In addition, subject 1.1.2 had been on lipid-lowering drugs for two decades, but the medication was interrupted for 1 month before participating in this study. The two remaining subjects in the oldest generation of family # 1 were clinically healthy.

All subjects in the second generation of family # 1 were healthy except subject 1.2.7, a 37-year-old man, who had suffered three attacks of acute pancreatitis. At the time of this study, he had had symptoms of hyperglycemia for several months. Diabetes (fasting blood glucose 14.5 mmol/l and glycosylated hemoglobin A_{1c} 13.0% [upper limit of normal, 6.0%]) was diagnosed and insulin therapy was instituted. Based on family history, on the age of onset, and on absence of ketosis, his disease was classified as NIDDM, although the previous episodes of pancreatitis most likely contributed to the development of β-cell failure and frank hyperglycemia.

All the children and adolescents in the third generation of family # 1 were clinically healthy and none of them had a previously diagnosed dyslipidemia.

The propositus (subject 2.1.1) of family # 2 (Fig. 1B) was a healthy 54-year-old man. He had 1 brother (subject 2.1.2) and 1 sister (2.1.3) who participated in the study. The propositus had 3 children one of whom was 1 year old and was excluded from this study. The remaining son and daughter participated. Subject 2.1.2 had 2 children who were not studied. The son and daughter of subject 2.1.3 participated. Thus, 2 men and 2 women of the second generation in this family were studied. They were all clinically healthy and had no children.

Among the 2 kindreds (Fig. 1) there were 16 subjects (9 men and 7 women) heterozygous for the A291S mutation. They constituted group 1 in this study. In addition, 4 spouses (those of subjects 1.1.1, 1.1.2, 1.1.4, and 1.2.7) were studied and found to be negative for the A291S mutation. Together with the non-affected family members, they constituted the family control group (group 2, n = 17, 7 men and 10 women). Another control group (the population controls) consisted of 19 unrelated individuals (8 men and 11 women). They were volunteers recruited among hospital staff and their relatives with no known diseases and no regular medications (including hormones), selected to have age and sex distributions similar to those in the two other groups. All were assessed for the A291S mutation and were found to be negative.

Most study subjects were not taking any medications at the time of the study. No one was on β-blockers or diuretics. One female carrier and 1 family control subject were on a postmenopausal hormone-replacement regimen (estrogen and a combination of estrogen and

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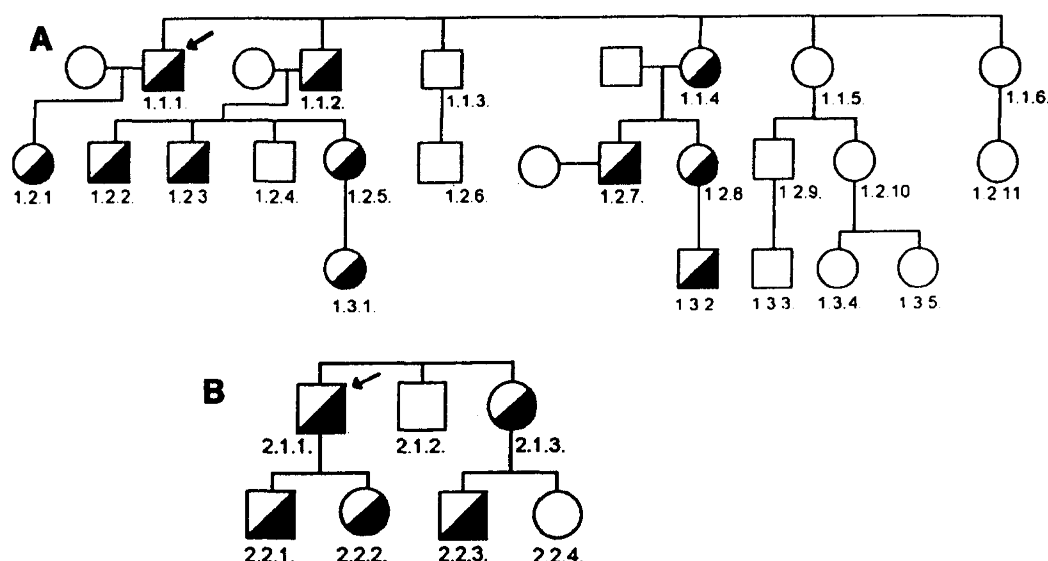

Fig. 1. Pedigrees showing the study participants in family # 1 (panel A) and in family # 2 (panel B). Panel A also shows the spouses that were included in the family control group. The arrows indicate the propositi. Squares indicate male subjects and circles indicate female subjects. Subjects heterozygous for the $Asn^{291} \rightarrow Ser$ mutation of the LPL gene are shown by half-shaded symbols.

progestin, respectively). Of the younger women, 1 carrier and 2 family control subjects were taking low-estrogen oral contraceptives. One diabetic male family control subject (the spouse of subject 1.1.4) was treated with insulin.

All subjects gave their informed consent to participate in the study, which was approved by the appropriate Ethics Committee.

Measurements

Lipid, lipoprotein, and apolipoprotein analyses. Venous blood was collected after an overnight fast in Vacutainer tubes containing EDTA. Plasma was centrifuged (2000 rpm for 15 min at 4°C) and the cells for DNA extraction were separated. Lipoproteins were separated from fresh fasting serum by sequential ultracentrifugation (Beckman L 8-70, Beckman Inc., Palo Alto, CA). If serum was turbid, chylomicrons were first isolated by centrifugation for 30 min at 13,000 rpm using a Beckman Ti 50.4 rotor. VLDL, IDL, LDL, HDL_2, and HDL_3 were isolated at 2°C at densities 1.006 g/ml (35,000 rpm, 18 h), 1.019 g/ml (35,000 rpm, 24 h), 1.063 g/ml (35,000 rpm, 24 h), 1.120 g/ml (35,000 rpm, 48 h), and 1.210 g/ml (35,000 rpm, 48 h), respectively. Cholesterol and TG were measured in serum and in the lipoprotein fractions by enzymatic methods (Cobas Mira autoanalyzer, Hoff-

TABLE 1. Characteristics of the study groups

	Group 1	Group 2	Group 3	P (ANOVA)	P (Adjusted ANOVA)[a]
n (male/female)	16 (9/7)	17 (7/10)	19 (8/11)	–	–
Age, yr	39 ± 16	41 ± 18	46 ± 8	0.338	–
Body mass index, kg/m²[b]	27.6 ± 4.2	26.1 ± 4.6	23.9 ± 2.4	0.016	0.007
Waist-to-hip ratio	0.90 ± 0.08	0.87 ± 0.10	NA	0.382	0.591
Systolic blood pressure, mm Hg	137 ± 25	134 ± 23	NA	0.725	0.337
Diastolic blood pressure, mm Hg	83 ± 15	78 ± 9	NA	0.246	0.103
Serum insulin mU/l[c]	9.4 ± 5.3	9.2 ± 5.7	5.0 ± 1.4	0.003	0.007

The data are mean ± SD (except number of subjects). Group 1, subjects heterozygous for the $Asn^{291} \rightarrow Ser$ mutation of the LPL gene; group 2, nonaffected family members and spouses of the subjects in group 1 (family controls); group 3, unrelated healthy control subjects (population controls). NA indicates data not available.

[a]Adjusted for sex and age.

[b]Between-group comparisons adjusted for sex and age and for multiple comparisons (Tukey-Kramer adjustment): group 1 vs. group 2, P = 0.519; group 1 vs. group 3, P = 0.006; group 2 vs. group 3, P = 0.081.

[c]Between-group comparisons of logarithmically transformed data adjusted for sex and age and for multiple comparisons (Tukey-Kramer adjustment): group 1 vs. group 2, P = 0.997; group 1 vs. group 3, P = 0.016; group 2 vs. group 3, P = 0.017.

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TABLE 2. Serum and lipoprotein triglyceride and cholesterol concentrations and apolipoprotein levels in the study groups

Variable	Group 1	Group 2	Group 3	P (ANOVA)	P (Adjusted ANOVA)[a]	P (Group 1 vs. group 2)[b]	P Group 1 vs. group 3)[b]	P Group 2 vs. group 3)[b]
Serum TG[c]	2.90 (0.78 to 145.00)	1.14 (0.61 to 3.00)	0.99 (0.48 to 1.87)	0.0002	–	0.009	0.0003	0.954
VLDL TG[d]	2.28 (0.43 to 60.25)	0.56 (0.15 to 2.26)	0.47 (0.23 to 1.19)	< 0.0001	< 0.0001	0.0003	0.0001	0.368
IDL TG[d]	0.17 ± 0.10	0.11 ± 0.04	0.11 ± 0.04	0.093	0.053	0.082	0.086	0.999
LDL TG[d]	0.26 ± 0.09	0.24 ± 0.09	0.25 ± 0.09	0.702	0.291	0.483	0.284	0.926
HDL TG[d]	0.26 ± 0.14	0.19 ± 0.04	0.21 ± 0.06	0.362	0.115	0.114	0.256	0.886
HDL_2 TG[d]	0.14 ± 0.09	0.10 ± 0.03	0.12 ± 0.06	0.598	0.317	0.286	0.653	0.778
HDL_3 TG[d]	0.13 ± 0.08	0.10 ± 0.02	0.09 ± 0.03	0.022	0.020	0.204	0.015	0.465
Serum cholesterol[c]	5.34 (2.75 to 67.00)	5.26 (3.69 to 9.51)	5.84 (3.61 to 9.43)	0.488	–	1.000	1.000	0.802
VLDL-C[d]	0.90 (0.20 to 16.75)	0.33 (0.04 to 1.43)	0.20 (0.06 to 0.64)	< 0.0001	0.0001	0.002	0.0002	0.483
IDL-C[d]	0.23 ± 0.13	0.22 ± 0.15	0.22 ± 0.16	0.935	0.709	0.891	0.686	0.925
LDL-C	2.33 ± 1.13	3.54 ± 1.14	3.70 ± 1.27	0.003	0.008	0.029	0.010	0.925
HDL-C	1.00 ± 0.42	1.22 ± 0.42	1.60 ± 0.32	0.0001	0.0001	0.518	0.0002	0.002
HDL_2-C	0.48 ± 0.27	0.59 ± 0.31	0.94 ± 0.28	< 0.0001	< 0.0001	0.849	0.0002	0.0003
HDL_3-C	0.53 ± 0.19	0.63 ± 0.12	0.67 ± 0.15	0.029	0.058	0.285	0.047	0.635
ApoA-I	132 ± 37	137 ± 34	160 ± 23	0.023	0.014	0.988	0.042	0.024
ApoA-II	38 ± 11	37 ± 7	36 ± 5	0.896	0.754	0.794	0.781	1.000
ApoB	128 ± 48	100 ± 34	98 ± 36	0.066	0.035	0.119	0.035	0.844
ApoC-II[d]	6.4 (1.8 to 66.0)	3.2 (1.6 to 7.3)	2.9 (1.5 to 7.6)	0.001	0.0002	0.004	0.0004	0.574
ApoC-III[d]	13.8 (4.7 to 258.0)	9.3 (4.3 to 15.7)	7.9 (4.7 to 13.8)	0.001	0.0004	0.003	0.0008	0.788

TG indicates triglyceride; C, cholesterol; and apo, apolipoprotein. Group 1, subjects heterozygous for the $Asn^{291} \rightarrow$ Ser mutation of the LPL gene; group 2, nonaffected family members and spouses of the subjects in group 1 (family controls); group 3, unrelated healthy control subjects (population controls). Data are mean ± SD or median (range). TG and cholesterol values are in mmol/l, apolipoprotein concentrations are in mg/dl.

[a]Adjusted for sex and age.

[b]Between-group comparisons were adjusted for sex and age and for multiple comparisons (Tukey-Kramer adjustment) except serum TG and cholesterol (see footnote[c]).

[c]Statistical comparisons were performed by use of the Kruskal-Wallis ANOVA and the pairwise between-group comparisons were done by the Mann-Whitney U test, Bonferroni-adjusted for multiple comparisons.

[d]Statistical comparisons were performed using logarithmically transformed data.

man-La Roche, Basel, Switzerland). ApoA-I and apoA-II (Boehringer Mannheim, Germany), and apoB (Orion Diagnostica, Espoo, Finland) were measured by immunoturbidimetry. ApoC-II and apoC-III concentrations were determined by the single radial immunodiffusion technique (Daiichi Pure Chemicals, Tokyo, Japan). ApoE phenotyping was performed by the method of Havekes et al. (20). The mean particle diameter of the major LDL peak was determined by nondenaturing polyacrylamide gradient gel electrophoresis (21) as described (22). Serum insulin concentrations were measured by radioimmunoassay using the Phadeseph Insulin RIA kit (Pharmacia, Uppsala, Sweden).

LPL activity and protein concentration. Postheparin plasma samples were collected after an overnight fast into chilled tubes containing lithium heparin. Blood was sampled 15 min after an intravenous bolus injection of heparin (100 IU/kg, Leiras, Turku, Finland). The activities of LPL and hepatic lipase (HL) were determined using an antiserum inhibition method (9). LPL protein concentration was measured by ELISA. A polyclonal, affinity-purified, chicken anti-human LPL was used as a capture and monoclonal anti-human LPL (Monoclonal Antibody, LPL (Ab-1), Cat LP01, Oncogene Science, New York, N.Y.) as a detection antibody (23).

Amplification of the exons of the lipoprotein lipase gene by PCR. Exon 6 of the human LPL gene was amplified by PCR technique in all subjects (24). All the exons of subject 1.2.7 were amplified. PCR primers, homologous to intron sequences flanking the exons of the LPL gene, were designed based on the published LPL gene structure (12). The primers were synthesized on an Applied Biosystems Model 381A DNA synthesizer (25). The PCR was carried out as described (26) in a total volume of 50 µl, except that 1 µCi of [α-^{32}P]dCTP (Amersham International plc, Amersham, U.K.) was included in each reaction for SSCPs. The cycles of denaturation (1 min at 95°C), annealing (1 min at 57°C), and elongation (2.5 min at 72°C) were repeated 30 times.

Analysis of single-strand conformation polymorphism (SSCP). The PCR products were diluted 1:5 in 0.1% SDS, 10 mM EDTA, and mixed with an equal volume of 95%

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formamide, 20 mM EDTA, containing 0.05% bromophenol blue, and 0.05% xylene cyanol. The samples were denatured at 90°C for 2 min and cooled on ice. Aliquots of 2 to 4 μl were analyzed for SSCPs (27) on nondenaturing 5% polyacrylamide gel containing 10% glycerol at 360 V for 18 h at room temperature. After electrophoresis, the gel was transferred to Whatman 3 filter paper (Whatman International Ltd, Maidstone, U.K.) and dried in a vacuum slab dryer. Autoradiographs were developed for 1 to 3 days at -70°C using Kodak XAR film (Rochester, N.Y.).

DNA sequencing. Exon 6 which showed an aberrant migration in SSCP was amplified for sequencing using the same primers as for SSCP. The PCR products were sequenced by the dideoxy chain termination method (28) with modifications (29).

Solid-phase minisequencing. The principle of the method has been described by Syvänen et al. (30). The upstream PCR primer was 5′-(ATC TTG GTG TCT CTT TTT TAC CC)-3′ and the downstream primer 5′-(CAA TCT GGG CTA TGA GAT CA)-3′ was biotinylated as described (31). Five nanograms of DNA was amplified under conditions given for the amplification of exons of the LPL gene except that the biotinylated primer was used at 0.2 μM and the annealing temperature was lowered to 55°C.

For each minisequencing reaction, a 10-μl aliquot of the PCR mixture and 40 μl 20 mM sodium phosphate buffer, pH 7.5, containing 0.1% Tween 20 were added to microtitration wells (Labsystems, Helsinki, Finland) that had been coated with streptavidin. The samples were incubated with gentle shaking at 37°C for 1.5 h and washed three times with 200 μl 40 mM Tris-HCl, pH 8.8, 1 mM EDTA, 50 mM NaCl, 0.1% Tween 20, at room temperature. The wells were treated twice with 100 μl 50 mM NaOH for 5 min at room temperature and washed as above. A reaction mixture consisting of 0.2 μM detection primer 5′-(CAA TCT GGG CTA TGA GAT CA)-3′ and 0.4 μM [^{3}H]dATP (TRK 625, 67 Ci/mmol) to detect A at the second position of codon 291 or 0.4 μM [3H]dGTP (TRK 627, 37 Ci mmol) to detect G at this position was added to each well. The samples were incubated at 50°C for 10 min and washed as above. The microtitration plates were treated with 60 μl of 50 mM NaOH for 5 min at room temperature and the eluted radioactivity was measured in a liquid scintillation counter.

Site-directed mutagenesis of lipoprotein lipase cDNA. Human LPL cDNA was kindly provided by Dr. Michael C. Schotz (32). The LPL cDNA was amplified by the PCR (24). Sac I and BamHI restriction sites were added to the primers (Primer I: 5′-ACG TGA GCT CCC CCT CTT CCT CCT CCT CAA G-3′; Primer II: 5′-ACG TGG ATC CCA TTC TTC ACA GAA TTC ACA TGC C-3′). The PCR product was subcloned to the Sac I–BamH I site of the replicative form of M13mp18. Oligonucleotide directed site-specific mutagenesis of LPL-cDNA in M13mp18 single-stranded DNA was carried out by the phosphorothioate DNA selection method (33) using a commercial kit (RPN 1523, Amersham International plc, Amersham, U.K.). The oligonucleotide used for mutagenesis was 5′-(TGT GAC TTT ACT GAT CTC ATA CCC)-3′. Mutants were verified by dideoxynucleotide sequencing (28).

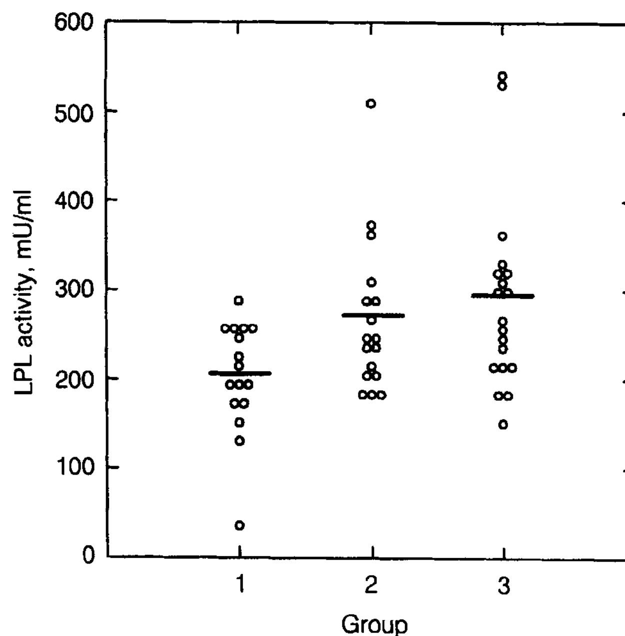

Fig. 2. Dot plot showing postheparin plasma lipoprotein lipase (LPL) activities in three groups of subjects. Horizontal bars denote group mean levels. Group 1, subjects heterozygous for the Asn^{291} → Ser mutation of the LPL gene; group 2, nonaffected family members and spouses of the subjects in group 1 (family controls); group 3, unrelated healthy control subjects (population controls).

Expression of the wild-type and mutant LPL cDNAs in COS-7 cells. Wild-type and mutant LPL cDNAs were inserted into the Sac I and BamH I sites of expression vector pSVL (Pharmacia, Uppsala, Sweden). COS-7 cells (ATCC, CRL 1651), maintained in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% fetal calf serum, were seeded 1 day prior the transfection at a confluence of 10^6 cells per a 10 cm petri dish. The cells were transfected with 5 μg of the pSVL plasmid DNA constructs by the liposome transfection method (34) using lipofectin (Lipofectin reagent, BRL, Life Technologies Inc., Gaithersburg, MD) as described (26). For LPL activity and protein concentration measurements, culture medium containing heparin (20 U/ml) was collected at 48 and 72 h after transfection. Cells were collected, washed in PBS, solubilized in 1 ml of 50 mM NH_3/NH_4Cl (pH 8.1) containing heparin, and sonicated.

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Media and cell extracts were stored at -70°C until assayed for LPL (9, 23).

Lifestyle questionnaire. The carriers of the A291S mutation, their relatives, and the spouses studied were asked to fill out a questionnaire about their physical activity at work and in spare time, smoking habits, and drinking habits. They were classified as sedentary, moderately active, or active; as current smokers or nonsmokers (including ex-smokers); and as using alcohol once weekly at the most or more frequently than once a week. Similar data on smoking and drinking were available in 15 subjects of group 3, but physical activity data were not recorded for this group.

Statistical methods. Data are presented as mean ± SD unless otherwise indicated. Variables with skew distributions or heteroscedasticity among the study groups were transformed to their natural logarithms before statistical analyses. Differences among the groups were first evaluated by an unadjusted ANOVA, followed by ANOVA adjusted for sex and age. The adjusted ANOVA model was further used for pairwise comparisons (group 1 vs. 2, group 1 vs. 3, and group 2 vs. 3). In these analyses, multiple comparisons were accounted for by the Tukey-Kramer adjustment. If a reasonably normal distribution and homoscedasticity could not be reached by transformations, the nonparametric Kruskal-Wallis ANOVA was used, followed by pairwise comparisons using Bonferroni-adjusted Mann-Whitney U tests. Proportions were compared by use of Fisher's exact test.

We used multivariate linear stepwise regression analysis to evaluate which variables predicted VLDL TG, VLDL cholesterol, and HDL cholesterol concentrations in our study population. The following candidate predictors were entered into the preliminary model: the presence or absence of the A291S mutation, sex, age, body mass index (BMI), serum insulin concentration, postheparin plasma LPL and HL activities, apoB concentration (only when VLDL TG or cholesterol was the dependent variable), apoA-I and apoA-II concentrations (only when HDL cholesterol was the dependent variable), and the apoE phenotype (coded as the presence or absence of apoE2 and apoE4, respectively). Forward stepping was performed with an alpha-to-enter = 0.15 and alpha-to-remove = 0.15 until addition or deletion of variables no longer improved the fit of the model (35). The tolerance option was used to prevent multicollinearity among the independent variables (35).

RESULTS

Figure 1 illustrates the pedigrees of the two Finnish families studied. Eleven subjects were heterozygotes for A291S mutation in family # 1. Five affected individuals for this mutation were found in family # 2. **Table 1** lists selected characteristics of the study groups. The age and sex distribution did not differ significantly among the groups. The A291S positive subjects (group 1) and the family controls (group 2) were more obese than the population controls (group 3). Groups 1 and 2 had similar waist-to-hip ratios and blood pressures. They also had similar serum insulin concentrations that were significantly higher than those in the population control group. We found that 3 subjects in group 1 and 1 subject in group 2 had evidence of coronary artery disease. We also found that 4 and 2 subjects among the A291S heterozygotes and family controls, respectively, had hypertension, and 2 and 1, respectively, had diabetes.

Regarding physical activity, 2, 8, and 6 subjects in group 1 and 4, 8, and 5 subjects in group 2 were sedentary, moderately active, and active, respectively (P = 0.456, Mann-Whitney U test). There were 4 and 6 current smokers in groups 1 and 2, respectively, whereas no one in group 3 smoked (data not available in 4 subjects). Alcohol use more frequently than once weekly was reported by 3, 3, and 4 subjects in groups 1 through 3, respectively (between-group differences not significant). Drinking data were missing in 4 subjects in group 3.

Serum and lipoprotein TG and cholesterol. As a group, the A291S heterozygotes had markedly higher serum TG levels compared with groups 2 and 3 (**Table 2**). Four

TABLE 3. Multivariate linear regression model selected to predict log VLDL triglyceride concentrations

Variable	Coefficient	SE	Std Coeff[a]	P
ApoB	0.015	0.002	0.600	< 0.001
A291S[b]	0.698	0.143	0.324	< 0.001
Serum insulin[c]	0.393	0.121	0.208	0.002
LPL activity[c]	-0.498	0.208	-0.151	0.021

The model was selected by automated stepwise regression analyses (see Methods for details). Apo indicates apolipoprotein; and LPL, lipoprotein lipase.
[a]Standardized regression coefficient.
[b]Variable indicating the presence or absence of the mutant $Asn^{291} \rightarrow$ Ser allele of the LPL gene.
[c]Transformed to natural logarithms.

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TABLE 4. Multivariate linear regression model selected to predict log VLDL triglyceride concentrations when apolipoprotein B concentration was not among the predictor variables

Variable	Coefficient	SE	Std Coeff[a]	P
A291S[b]	0.946	0.246	0.383	< 0.001
LPL activity[c]	-1.082	0.267	-0.378	< 0.001
Body mass index	0.064	0.032	0.226	0.050
ApoE4[d]	0.494	0.213	0.216	0.025
Serum insulin[c]	0.402	0.218	0.183	0.072
Age	0.011	0.007	0.136	0.142

The model was selected by automated stepwise regression analyses (see Methods for details). LPL indicates lipoprotein lipase; and apo, apolipoprotein.
[a]Standardized regression coefficient.
[b]Variable indicating the presence or absence of the mutant $Asn^{291} \rightarrow Ser$ allele of the LPL gene.
[c]Transformed to natural logarithms.
[d]Variable indicating the presence or absence of the ε4 allele of the apoE gene.

heterozygotes had chylomicronemia in the fasting serum, whereas no one in either control group did ($P < 0.05$ vs. both control groups). We found that VLDL TG concentrations were significantly higher in group 1 than in the control groups. We also found the highest HDL_3 TG levels in group 1, and the difference was significant compared with the population controls. By contrast, TG concentrations in IDL, LDL, HDL, or HDL_2 did not differ significantly among the groups (Table 2).

Serum cholesterol concentrations were similar among the study groups. However, VLDL cholesterol was markedly higher and LDL cholesterol lower in A291S heterozygotes than in the control subjects (Table 2). There were no differences in IDL cholesterol levels. HDL and HDL_2 cholesterol concentrations were significantly higher in the population control group than in either group 1 or group 2, whereas there was no difference between the A291S heterozygotes and the family control subjects.

Apolipoproteins. Serum apoA-I concentration was lower in groups 1 and 2 compared with group 3 (Table 2), but there were no differences in apoA-II levels. ApoB concentrations were highest in the heterozygote group, and the difference was significant compared with group 3. ApoC-II and apoC-III levels were markedly higher in group 1 than in either of the control groups.

Postheparin plasma lipase activities. **Figure 2** shows the individual and group mean postheparin plasma LPL activities. LPL activity was 205±63 mU/ml in group 1, 266 ±87 mU/ml in group 2, and 289±104 mU/ml in group 3. Overall, the between-group differences were significant in the crude ANOVA (P = 0.014) and in the sex- and age-adjusted ANOVA (P = 0.021). Tukey-Kramer-adjusted pairwise comparisons suggested a significant difference between groups 1 and 3 (P = 0.023), but only a borderline difference between the heterozygotes and the family controls (groups 1 and 2, P = 0.080), and no difference between groups 2 and 3 (P = 0.853). LPL protein concentrations were 1640±565 ng/ml (group 1), 1582±486 ng/ml (group 2), and 1833±496 ng/ml (group 3). The differences were not significant (ANOVA P = 0.313). Postheparin plasma HL activity was higher in group 1 (332 ± 162 mU/ml) than in group 2 (261 ± 147 mU/ml) or in group 3 (216 ± 99 mU/ml), but the differences were of only borderline significance by crude ANOVA (P = 0.051) and not significant in the adjusted ANOVA or in the pairwise comparisons (data not shown).

LDL particle size. The peak LDL particle sizes were 24.7 ± 1.3 nm (group 1), 25.9 ± 1.2 nm (group 2), and 26.5 ± 1.0 nm (group 3). The differences were highly significant by both crude (P = 0.0003) and adjusted (P = 0.0001) ANOVA. In pairwise comparisons, both group 2 (P = 0.026) and group 3 (P = 0.0002) had significantly larger LDL particles compared with group 1. In agreement, when the proportion of LDL particles larger than 25.5 nm in diameter was considered in each individual, the subjects in group 1 had significantly fewer such particles (37 ± 34%) compared with group 2 (70 ± 31%, P = 0.030) or with group 3 (81 ± 22%, P = 0.003).

Between-group comparisons in nonsmokers. To rule out smoking as a possible confounder, we repeated the between-group comparisons among the 12 and 11 nonsmokers in groups 1 and 2, respectively, and the 15 subjects in group 3 known to be nonsmokers. Overall, the results did not materially differ from those obtained by including all study subjects in the analyses. The differences in serum TG, VLDL TG, VLDL cholesterol, LDL cholesterol, HDL_2 cholesterol, apoC-II, apoC-III, and LDL particle size remained highly significant (data not shown). By contrast, the between-group differences in HDL_3 cholesterol, apoA-I, apoB, and LPL activity were not statistically significant when only nonsmokers were considered, possibly because of reduced statistical power.

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Multivariate regression analyses. To gain a better understanding of the determinants of TG-rich lipoprotein levels in our study population, we performed stepwise multivariate linear regression analyses with log VLDL TG and log VLDL cholesterol as dependent variables. **Table 3** shows the model selected to predict VLDL TG concentrations. The adjusted R^2 of the model was 0.829, i.e., the independent variables predicted 83% of the variation in VLDL TG levels. ApoB concentration was the most powerful predictor, followed by carrier state of the mutant allele A291S, serum insulin concentration, and postheparin plasma LPL activity. The model with VLDL cholesterol as the dependent variable had almost identical predictive power (82%), and the predictors, in order of decreasing power, were: apoB (standardized regression coefficient 0.637), LPL activity (-0.243), serum insulin (0.240), presence of A291S mutation (0.199), HL activity (-0.169), sex (-0.150, the sign indicating higher values of the dependent variable in men), and the presence of the apoE4 allele (0.127; i.e., a trend toward higher values in individuals carrying that allele).

We also examined the predictors of VLDL TG levels when apoB concentration was excluded from the potential predictor variables. **Table 4** shows that the presence of the mutant LPL allele and LPL activity measured in postheparin plasma both had a significant independent impact on the dependent variable, and the relative magnitude of this effect, indicated by the standardized regression coefficients, was virtually identical for these two predictors. BMI and the apoE4 allele also had a significant independent influence on VLDL TG concentrations, whereas insulin concentration and age, although selected into the model, did not reach conventional statistical significance. The predictive power of this model was 64%. A similar model with VLDL cholesterol as the dependent variable yielded a closely similar outcome (data not shown).

Because there were significant differences among the study groups in HDL cholesterol concentrations, we also explored the determinants of this variable by multivariate regression analysis. **Table 5** shows that apoA-I was by far the most important determinant of HDL cholesterol concentrations (standardized regression coefficient 0.943), and apoA-II (-0.191) and LPL activity (0.160) were also significant predictors. The A291S allele carrier state was not significantly related to HDL cholesterol levels (P = 0.123) in this multivariate model. The model explained 84% of the variation in the dependent variable.

As shown in **Table 6,** when apoA-I and apoA-II were not among the potential predictors of HDL cholesterol, sex was the most important determinant (female sex predicting higher levels, as expected). LPL activity (direct) and insulin concentration (inverse) were also significant predictors of HDL cholesterol level. The A291S mutant allele did not appear to have an independent influence on HDL cholesterol concentration and was therefore eliminated from the final model.

We also examined whether taking current smoking status into account would alter the results of the multivariate analyses. Smoking was not selected among the predictor variables of VLDL TG, whether apoB was among the potential independent variables or not. Likewise, the model to predict HDL cholesterol levels remained unaltered when apoA-I and apoA-II were in the model. Only when HDL cholesterol was the dependent variable and apoA-I and apoA-II were not among the predictor variables did smoking emerge as a negative predictor, but in accordance with the data in Table 6, female sex and LPL activity were still the significant positive predictors of HDL cholesterol levels.

Determinants of hypertriglyceridemia among A291S heterozygotes. **Table 7** lists selected characteristics of the 16 subjects heterozygous for the A291S mutation of the LPL gene. Nine of the subjects were hypertriglyceridemic (HTG) according to a predefined serum TG concentration cutoff point > 2.0 mmol/l, and the remaining 7 individuals were normotriglyceridemic (NTG). As shown in Table 7, hypertriglyceridemia ranged from moderate to massive; the highest serum TG concentration was found in the subject with untreated diabetes who had previously suffered three episodes of

TABLE 5. Multivariate linear regression model selected to predict HDL cholesterol concentrations

Variable	Coefficient	SE	Std Coeff[a]	P
ApoA-I	0.012	0.001	0.934	< 0.001
ApoA-II	-0.011	0.004	-0.191	0.016
LPL activity[b]	0.233	0.093	0.160	0.016
A291S[c]	-0.098	0.062	-0.103	0.123

The model was selected by automated stepwise regression analyses (see Methods for details). Apo indicates apolipoprotein; and LPL, lipoprotein lipase.
[a]Standardized regression coefficient.
[b]Transformed to natural logarithms.
[c]Variable indicating the presence or absence of the mutant $Asn^{291} \rightarrow Ser$ allele of the LPL gene.

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TABLE 6. Multivariate linear regression model selected to predict HDL cholesterol concentrations when apolipoprotein A-I and A-II concentrations were not among the predictor variables

Variable	Coefficient	SE	Std Coeff[a]	P
Sex	0.396	0.088	0.438	< 0.001
LPL activity[b]	0.429	0.111	0.381	< 0.001
Insulin[b]	-0.257	0.085	-0.297	0.004
ApoE4[c]	-0.152	0.089	-0.168	0.095
Age	0.005	0.003	0.155	0.116

The model was selected by automated stepwise regression analyses (see Methods for details). LPL indicates lipoprotein lipase; and apo, apolipoprotein.
[a]Standardized regression coefficient.
[b]Transformed to natural logarithms.
[c]Variable indicating the presence or absence of the ε4 allele of the apoE gene.

pancreatitis. All HTG subjects except two were hyperinsulinemic (defined a priori as serum insulin concentration > 10.0 mU/l). These two individuals (subjects 1.1.4 and 1.2.7) both had diabetes. Thus, if hyperinsulinemia and diabetes are regarded as markers of insulin resistance, all HTG subjects were insulin resistant, whereas none of the NTG subjects were ($P < 0.0001$).

By contrast, adiposity did not predict whether a carrier of the mutant allele was hypertriglyceridemic. Whether the cutoff point for obesity was BMI > 25 kg/m^2 or > 27 kg/m^2, obesity was not significantly related to hypertriglyceridemia among the A291S heterozygotes ($P = 0.596$ and $P = 0.302$, respectively). The mean ages of the HTG and NTG heterozygotes were also similar (39 and 40 years, respectively, $P = 0.950$). LPL activities were also unable to separate the HTG and NTG groups (209 and 199 mU/ml, respectively, $P = 0.838$).

Subject 1.2.7 was of special interest because of his extremely low postheparin plasma LPL activity, 35.2 mU/ml (Fig. 2). Because he had newly diagnosed diabetes at the time of the study, postheparin plasma lipase measurements were repeated about six months later during insulin and gemfibrozil therapy. LPL activity was found to be 83.4 mU/ml, i.e., still lower than in any other carrier of the A291S mutation. His serum TG concentrations were 145 and 31 mmol/l, apoC-II concentrations were 66 and 16 mg/dl, and apoC-III concentrations were 258 and 58 mg/dl before and after therapy, respectively. ApoC-II and apoC-III median values in group 1 were 6.4 and 13.8 mg/dl, respectively.

SSCP analysis and sequencing of exon 6 of the LPL gene. SSCP analysis revealed an aberrant electrophoretic migration of the PCR product from exon 6 in the proband. Sequencing of the PCR product derived from exon 6 revealed a single nucleotide mutation A → G resulting in a substitution of asparagine for serine at the amino acid 291 in the mature protein. Sequencing of all the 9 exons of subject 1.2.7 revealed a heterozygote state for the A291S mutation but no other mutations.

Solid phase minisequencing. To search for the A291S mutation in other family members, a solid phase minisequencing was carried out. This mutation was found in 11 individuals in family # 1 and in 5 in family # 2. All the affected subjects were heterozygotes for this mutation. The minisequencing results, therefore, confirmed that the propositi and their affected relatives were carriers for A to G transition at the second base of the codon 291.

Expression of the wild-type and mutant cDNAs in COS-7 cells. The construct containing the A291S mutation was transiently expressed in COS-7 cells and the culture media, and cell homogenates were analyzed for LPL activity and protein mass. The results showed that the A291S mutant construct produced a protein with only 50% activity compared with that of the wild type, in good agreement with previously published in vitro expression studies of the A291S mutation (15, 16, 36).

DISCUSSION

We studied 16 subjects heterozygous for the Asn^{291} → Ser mutation of the LPL gene. We found that, as a group, the carriers had on average 23% lower postheparin plasma LPL activities than a control group consisting of unaffected relatives and spouses and 29% lower activities than a group of unrelated healthy control subjects. The latter difference was statistically significant, but the difference between the carriers and the family controls did not reach conventional statistical significance. Only one affected subject (1.2.7) had markedly decreased LPL activity. At the time of the studies he had untreated diabetes and gross hyperlipidemia (Table 7). Sequencing of the coding regions of the LPL gene did not reveal other mutations than A291S. Regulatory factors of LPL gene expression, such as insulin deficiency, or changes in other proteins, such as high apoC-III levels, might have partially accounted for his exceptionally low LPL activity. The remaining affected subjects had LPL activities in the low-normal range, and

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a graphical presentation of the data (Fig. 2) suggests that the between-group differences were mainly due to absence of high LPL activities in the heterozygote group. The data on in vitro expression studies showing that the A291S mutant protein exhibits only 50% activity of wild-type LPL agree well with the clinical observations. Similar LPL protein concentrations among the study groups are also in line with the expression studies indicating that a normal amount of LPL protein was secreted by cells transfected with the A291S-mutant construct. Our results regarding the influence of the A291S mutation on LPL activity in vivo and in vitro agree well with recent data by Reymer et al. (18).

We found large differences among the groups in VLDL TG and VLDL cholesterol concentrations. Serum apoB concentrations were also high among the carriers, albeit to a lesser degree than the lipid levels. Because the relation between serum TG levels, the presence of an abnormal LPL, and LPL activity were not straightforward, we explored the determinants of VLDL TG and other metabolic variables in a multivariate regression analysis. We found that in addition to the apoB level, heterozygosity for the A291S mutation, serum insulin concentration, and LPL activity (inversely) all made a significant independent contribution and had considerable power to predict VLDL TG levels (Table 3).

Because apoB is a constituent of VLDL it was also of interest to study the determinants of VLDL TG without apoB among the predictor variables (Table 4). In agreement with the previous analysis, we found that the presence of the mutant allele was a strong positive determinant of VLDL TG levels whereas LPL activity had an equally strong and independent but inverse association. Thus, it seems that the presence of the mutation in our study subjects not only caused somewhat lower LPL activities but also was associated with some other characteristic that results in hypertriglyceridemia.

To further elucidate what determines hypertriglyceridemia among carriers of the A291S mutation, we compared several variables in the HTG carriers to those of NTG carriers. LPL activity, age, or BMI did not significantly differ among these two groups. In contrast, hyperinsulinemia, defined as serum insulin > 10 mU/l, or the presence of NIDDM strongly predicted the presence of hypertriglyceridemia in this population. Because hyperinsulinemia correlates reasonably well with insulin resistance among nondiabetic subjects (37) and because NIDDM is an insulin-resistant state (38), we suggest that insulin resistance is the primary underlying factor of hypertriglyceridemia among the A291S heterozygotes now studied. This is in line with a previous observation of at times gross hypertriglyceridemia in a NIDDM patient with the $His^{183} \rightarrow Gln$ substitution in the LPL gene (26).

The underlying metabolic mechanism that causes hypertriglyceridemia among mildly LPL-deficient heterozygotes is most likely overproduction of VLDL, a

TABLE 7. Characteristics of the subjects heterozygous for the $Asn^{291} \rightarrow Ser$ mutation of the lipoprotein lipase gene

Subject/Sex	Age	BMI	TG	Cholesterol	LPL	Insulin
	yr	kg/m^2	*mmol/l*		*mU/ml*	*mU/l*
1.1.1/M	60	26.7	1.6	5.0	255	9.3
1.1.2/M	66	28.5	13.3	9.4	259	11.6
1.1.4/F	64	24.0	7.0	6.5	259	3.3
1.2.1/F	27	32.0	2.2	5.6	228	22,8
1.2.2/M	39	28.1	4.0	4.4	178	12.4
1.2.3/M	36	29.3	3.6	6.6	196	10.1
1.2.5/F	33	23.9	1.5	4.8	156	4.9
1.2.7/M	37	27.4	145.0	67.0	35	4.0
1.2.8/F	40	29.8	18.0	7.3	175	14.7
1.3.1/F	13	20.9	1.1	4.6	193	5.6
1.3.2/M	13	24.9	12.0	6.3	294	15.8
2.1.1/M	54	27.0	1.4	5.1	193	5.1
2.1.3/F	54	27.8	1.5	5.6	248	5.0
2.2.1/M	30	21.0	0.8	2.8	136	6.4
2.2.2/F	28	36.3	4.8	5.1	259	10.5
2.2.3/M	31	33.5	1.5	4.4	214	8.1

BMI indicates body mass index; TG, fasting serum triglyceride concentration; Cholesterol, fasting serum cholesterol concentration; LPL, postheparin plasma lipoprotein lipase activity; and Insulin, fasting serum insulin concentration.

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well-known feature of NIDDM (39, 40). In insulin resistance and hyperinsulinemia, the lipolysis-inhibiting effect of insulin in adipose tissue is blunted (41), resulting in an increased flux of free fatty acids to the liver and in substrate-driven VLDL overproduction (41, 42). It is also possible that insulin normally directly inhibits hepatic VLDL production and that the liver can become resistant to this effect in insulin-resistant states (43).

Disturbed metabolism of TG-rich lipoproteins has multiple effects on other lipoprotein classes (44). Accordingly, we found a strong preponderance of small dense LDL among the carriers of the A291S mutation. We also found TG enrichment of HDL_3 in the carrier group and a similar trend for IDL. Low HDL cholesterol, mainly HDL_2 cholesterol, was also a characteristic of the A291S heterozygotes. It is well known that retarded catabolism of TG-rich lipoproteins is associated with low levels of HDL_2 (45), and recent data suggest that the A291S mutation may be associated with low HDL cholesterol levels (18). However, in our study also the unaffected relatives had low HDL cholesterol and apoA-I concentrations compared with the population control group, suggesting that the low HDL levels among the heterozygotes were partly due to other features.

In conclusion, the $Asn^{291} \rightarrow$ Ser mutation of the LPL gene is mostly associated only with a mildly depressed postheparin plasma LPL activity and does not necessarily lead to manifest hyperlipidemia. This observation is in line with data on heterozygous carriers of other LPL mutations, as reviewed by Lalouel, Wilson, and Iverius (46). However, in some individuals a moderate to massive hypertriglyceridemia ensues, apparently regardless of age, body weight, or measured LPL activity. Thus, with regard to hypertriglyceridemia, the mutation appears to have only a permissive role; concomitant abnormalities are required to render the metabolic abnormality manifest. In the two kindreds now studied, insulin resistance, presenting either as NIDDM or as hyperinsulinemia, seems to be the most important factor in determining VLDL production and thereby the development of hyperlipidemia. Our results suggest that VLDL overproduction, linked to a subtle derangement of VLDL catabolism, may often underlie hypertriglyceridemia.

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REFERENCES

1. Eckel, R. H. 1989. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* **320:** 1060–1068.
2. Taskinen, M-R., and T. Kuusi. 1987. Enzymes involved in triglyceride hydrolysis. *Baillieres Clin. Endocrinol. Metabolism.* **1:** 639–666.
3. Braun, J. E. A., and D. L. Severson. 1992. Regulation of the synthesis, processing and translocation of lipoprotein lipase. *Biochem. J.* **287:** 337–347.
4. Olivecrona, T., and G. Bengtsson-Olivecrona. 1990. Lipases involved in lipoprotein metabolism. *Curr. Opin. Lipidol.* **1:** 116–122.
5. Wang, C-S., J. Hartsuck, and W. J. McConalthy. 1992. Structure and functional properties of lipoprotein lipase. *Biochim. Biophys. Acta.* **1123:** 1–17.
6. Santamarina-Fojo, S. 1992. Genetic dyslipoproteinemias: role of lipoprotein lipase and apolipoprotein C-II. *Curr. Opin. Lipidol.* **3:** 186–195.
7. Krauss, R. M., P. N. Herbert, R. I. Levy, and D. S. Fredrickson. 1973. Further observations on the activation and inhibition of lipoprotein lipase by apolipoproteins. *Circ. Res.* **33:** 403–411.
8. McConathy, W. J., J. C. Gesquiere, H. Bass, A. Tartar, J-C. Fruchart, and C-S. Wang. 1992. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *J. Lipid Res.* **33:** 995–1003.
9. Huttunen, J. K., C. Ehnholm, P. K. J. Kinnunen, and E. A. Nikkilä. 1975. An immunochemical method for the selective measurement of two triglyceride lipases in human postheparin plasma. *Clin. Chim. Acta.* **63:** 335–347.
10. Sparkes, R. S., S. Zollman, I. Klisak, T. G. Kirchgessner, M. C. Komaromy, T. Mohandas, M. C. Schotz, and A. J. Lusis. 1987. 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.
11. Kirchgessner, T. G., K. L. Svenson, A. J. Lusis, and M. C. Schotz. 1987. The sequence of cDNA encoding lipoprotein lipase: a member of a lipase gene family. *J. Biol. Chem.* **262:** 8463–8466.
12. Deeb, S. S., and R. Peng. 1989. Structure of the human lipoprotein lipase gene. *Biochemistry.* **28:** 4131–4135.
13. Auwerx, J., P. Leroy, and K. Schoonjans. 1992. Lipoprotein lipase: recent contributions from molecular biology. *Crit. Rev. Clin. Lab. Sci.* **29:** 243–268.
14. Antikainen, M., H. Tenkanen, M-R. Taskinen, and C. Ehnholm. 1995. Mutations affecting human lipoprotein lipase gene. *Z. Gastroenterol.* **(Suppl. 3):** 1–3.
15. Ma, Y., H. Zhang, M-S. Liu, J. Frohlich, J. D. Brunzell, and M. R. Hayden. 1993. Type III hyperlipoproteinemia in apoE2/2 homozygotes: possible role of mutations in the lipoprotein lipase gene. *Circulation* **88[part2]:** I-179 (abstract).
16. Zhang, H., P. W. A. Reymer, M-S. Liu, I. J. Forsythe, B. E. Groenemeyer, J. Frohlich, J. D. Brunzell, J. J. P. Kastelein, M. R. Hayden, and Y. Ma. 1995. Patients with apoE3 deficiency (E2/2, E3/2, and E4/2) who manifest with hyperlipidemia have increased frequency of an Asn 291 → Ser mutation in the human LPL gene. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1695–1703.
17. Minnich, A., A. Kessling, M. Roy, C. Giry, G. DeLangavant, J. Lavigne, S. Lussier-Cacan, and J. Davignon. 1995. Prevalence of alleles encoding defective lipoprotein

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lipase in hypertriglyceridemic patients of French Canadian descent. *J. Lipid Res.* **36:** 117–124.
18. Reymer, P. W. A., E. Gagné, B. E. Groenemeyer, H. Zhang, I. Forsyth, H. Jansen, J. C. Seidell, D. Kromhaut, K. E. Lie, J. Kastelein, and M. R. Heyden. 1995. A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nat. Genet.* **10:** 28–34.
19. Reymer, P. W. A., B. E. Groenemeyer, E. Gagné, L. Miao, E. E. G. Appleman, J. C. Seidel, D. Kromhout, S. M. Bijvoet, K. van de Oever, T. Bruin, M. R. Hayden, and J. J. P. Kastelein. 1995. A frequently occurring mutation in the lipoprotein lipase gene (Asn291Ser) contributes to the expression of familial combined hyperlipidemia. *Hum. Mol. Genet.* **4:** 1543–1549.
20. Havekes, L., P. de Knijeff, U. Beisiegel, J. Havinga, M. Smit, and E. Klasen. 1987. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J. Lipid Res.* **28:** 455–463.
21. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417–431.
22. Lahdenperä, S., J. Puolakka, T. Pyörälä, H. Luotola, and M-R. Taskinen. 1996. Effects of postmenopausal estrogen/progestin replacement therapy on LDL particles: comparison of transdermal and oral replacement regimens. *Atherosclerosis.* In press.
23. Antikainen, M., C. Holmberg, T. Olivecrona, G. Bengtsson-Olivecrona, C. Labeur, M. Rosseneu, and M-R. Taskinen. 1993. Changes in biological activity and immunoreactive mass of lipoprotein lipase in congenital nephrosis: relationship to hypertriglyceridaemia. *Eur. J. Clin. Invest.* **23:** 368–374.
24. Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* **155:** 335–350.
25. Beaucage, S. L., and M. H. Caruthers. 1982. Deoxynucleoside phosphoramidites—a new class of key intermediates to deoxypolynucleotide synthesis. *Tetrahedron Lett.* **22:** 1859–1862.
26. Tenkanen, H., M-R. Taskinen, M. Antikainen, I. Ulmanen, K. Kontula, and C. Ehnholm. 1994. A novel amino acid substitution (His183 → Gln) in exon 5 of the lipoprotein lipase gene results in loss of catalytic activity: phenotypic expression of the mutant gene in a heterozygous state. *J. Lipid Res.* **35:** 220–228.
27. Orita, M., Y. Suzuki, T. Sekiya, and K. Hayashi. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics.* **5:** 874–879.
28. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* **143:** 161–178.
29. Casanova, J-L., C. Pannetier, C. Jaulin, and P. Kourilsky. 1990. Optimal conditions for directly sequencing double-stranded PCR products with Sequenase. *Nucleic Acids Res.* **18:** 4028.
30. Syvänen, A-C., K. Aalto-Setälä, L. Harju, K. Kontula, and H. Söderlund. 1990. A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. *Genomics.* **8:** 684–692.
31. Bengtström, M., A. Jungell-Nortamo, and A-C. Syvänen. 1990. Biotinylation of oligonucleotides using a water-soluble biotin ester. *Nucleosides Nucleotides.* **9:** 123–127.
32. Wion, K. L., T. G. Kirchgessner, A. J. Lusis, M. C. Schotz, and R. M. Lawn. 1987. Human lipoprotein lipase complementary DNA sequence. *Science.* **235:** 1638–1641.
33. Taylor, J. W., J. Ott, and F. Eckstein. 1985. The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA. *Nucleic Acids Res.* **13:** 8765–8785.
34. Felgner, P. L., T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, and M. Danielsen. 1987. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA.* **84:** 7413–7417.
35. SYSTAT for Windows. 1992. Statistics, Version 5 Edition. Systat Inc., Evanston, IL. 210–379.
36. Buscà, R., J. Peinado, E. Vilella, J. Auwerx, S. S. Deeb, S. Vilaró, 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.
37. Laakso, M. 1993. How good a marker is insulin level for insulin resistance? *Am. J. Epidemiol.* **137:** 959–965.
38. DeFronzo, R. A., and E. Ferrannini. 1991. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* **14:** 173–194.
39. Howard, B. V. 1987. Lipoprotein metabolism in diabetes mellitus. *J. Lipid Res.* **28:** 613–628.
40. Taskinen, M-R. 1990. Hyperlipidaemia in diabetes. *Baillieres Clin. Endocrinol. Metab.* **4:** 743–775.
41. Coppack, S. W., M. D. Jensen, and J. M. Miles. 1994. In vivo regulation of lipolysis in humans. *J. Lipid Res.* **35:** 177–193.
42. Björntorp, P. 1994. Fatty acids, hyperinsulinemia, and insulin resistance: which comes first? *Curr. Opin. Lipidol.* **5:** 166–174.
43. Frayn, K. N. 1993. Insulin resistance and lipid metabolism. *Curr. Opin. Lipidol.* **4:** 197–204.
44. Grundy, S. M., and G. L. Vega. 1992. Two different views of the relationship of hypertriglyceridemia to coronary heart disease: implications for treatment. *Arch. Intern. Med.* **152:** 28–34.
45. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and G. Bengtsson-Olivecrona. 1984. Postprandial lipemia: a key for the conversion of high density $lipoprotein_2$ into high density $lipoprotein_3$ by hepatic lipase. *J. Clin. Invest.* **74:** 2017–2023.
46. Lalouel, J-M., D. E. Wilson, and P-H. Iverius. 1992. Lipoprotein lipase and hepatic triglyceride lipase: molecular and genetic aspects. *Curr. Opin. Lipidol.* **3:** 86–95.

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