Interaction of the lipoprotein lipase asparagine 291 → serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults

Rachel M. Fisher,[1,]* France Mailly,* Rachel E. Peacock,* Anders Hamsten,† Mary Seed,§ John S. Yudkin, Ulrike Beisiegel,†† Giso Feussner,§§ George Miller,*** Steve E. Humphries,* and Philippa J. Talmud***

Division of Cardiovascular Genetics,* Department of Medicine, University College London Medical School, Rayne Institute, University Street, London WC1E 6JJ, UK; The Atherosclerosis Research Unit,† King Gustaf V Research Institute, S-17176 Stockholm, Sweden; Department of Medicine,§ Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK; Academic Division of Medicine,** Department of Medicine, University College London Medical School, Whittington Hospital, London N19 3UA, UK; Medizinische Klinik,†† Universitäts-krankenhaus Eppendorf, D-20246 Hamburg, Germany; Department of Internal Medicine I,§§ Endocrinology/Metabolism, Bergheimer Strasse 58, D-69115 Heidelberg, Germany; and MRC Epidemiology and Medical Care Unit,*** Wolfson Institute of Preventive Medicine, The Medical College of St. Bartholemew's Hospital, Charterhouse Square, London EC1M 6BQ, UK

Abstract: A mutation in the lipoprotein lipase (LPL) gene, resulting in the substitution of asparagine by serine at residue 291 (LPL-S291), was found to occur in young survivors of a myocardial infarction from Sweden, combined hyperlipidemic subjects from the United Kingdom, and type III hyperlipidemic subjects from Germany at allelic carrier frequencies no different from those found in companion healthy control subjects (3.63 vs. 3.37; 1.85 vs. 1.60; and 2.00 vs. 1.56%, respectively). In a group of 620 healthy middle-aged men from the United Kingdom with baseline and three subsequent annual lipid measurements, mean plasma triacylglycerol (TG), (but not plasma cholesterol) concentrations in carriers of the mutation were significantly elevated over non-carriers (1.95 vs. 1.61 mmol/l, $P = 0.05$, and 5.83 vs. 5.65 mmol/l, $P = 0.29$, respectively). When these healthy control subjects were divided according to tertiles of body mass index (BMI), as expected, non-carriers whose BMI was in the upper two tertiles (BMI ≥ 25.0 kg/m^2) had higher plasma TG concentrations than those in the lowest tertile (1.90 vs. 1.54 mmol/l), but this difference was much greater in LPL-S291 carriers (2.33 vs. 1.36 mmol/l, $P = 0.01$, BMI × genotype interaction, $P = 0.02$). To confirm this effect, a second group of 319 healthy subjects from the United Kingdom was screened for LPL-S291. The allelic frequency of the mutation was found to be 1.88% and the effect on plasma lipid concentrations was very similar to that observed in the first control group (plasma TG, 2.31 vs. 1.27 mmol/l, $P < 0.001$ for LPL-S291 carriers vs. non-carriers, respectively). As before, those carriers whose BMI was in the top two tertiles for this sample (BMI ≥ 23.3 kg/m^2) had higher plasma TG concentrations than non-carriers (2.31 vs. 1.42 mmol/l). Thus, the LPL-S291 variant may predispose individuals to elevated plasma TG concentrations under conditions such as increased BMI.—**Fisher, R. M., F. Mailly, R. E. Peacock, A. Hamsten, M. Seed, J. S. Yudkin, U. Beisiegel, G. Feussner, G. Miller, S. E. Humphries, and P. J. Talmud.** Interaction of the lipoprotein lipase asparagine 291 → serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults. *J. Lipid Res.* 1995. **36:** 2104–2112.

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Several studies have demonstrated a positive relationship between plasma triacylglycerol (TG) concentration and the risk of coronary artery disease (CAD) (1–5), and thus subjects with disorders resulting in elevated plasma

Abbreviations: ASO, allele specific oligonucleotide; apo, apolipoprotein; BMI, body mass index; Chol, cholesterol; CAD, coronary artery disease; FCHL, familial combined hyperlipidemia; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; MI, myocardial infarction; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; TG, triacylglycerol; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

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TG concentrations may be at increased risk of CAD. A key enzyme in the metabolism of the TG-rich lipoproteins is lipoprotein lipase (LPL). LPL hydrolyzes the TG in the core of TG-rich lipoproteins (mainly chylomicrons and very low density lipoproteins (VLDL)) to produce smaller, relatively TG-depleted remnant lipoprotein particles. Patients who are homozygous, or compoundly heterozygous, for mutations in the LPL gene that cause complete LPL deficiency (type I hyperlipoproteinemia) and fasting chylomicronemia (6), occur at a frequency of roughly one per million. Carriers of these mutations, which drastically alter LPL activity or mass, may be as frequent as one per 500. The study of a large type I kindred has shown that some (but not all) relatives who are heterozygous for LPL deficiency have high plasma TG and/or cholesterol concentrations, and that it is most marked in individuals over 40 years in age (7). In addition, it is possible that mutations giving rise to partial LPL deficiency may underlie elevated plasma TG concentrations and hence an increased risk of CAD.

Familial combined hyperlipidemia (FCHL) is a disorder characterized by elevated concentrations of plasma cholesterol, TG, or both in the proband and at least one relative (8–11). The common feature in FCHL appears to be overproduction of TG-rich lipoproteins (11–13), but the different genetic defects causing this heterogeneous disorder remain unknown. A recent study of 55 families with FCHL (14) found evidence (by segregation analysis) for a major gene primarily affecting TG concentrations. One third of cases with FCHL have been shown to have decreased LPL activity (15, 16), an observation that suggests that mutations in the gene for LPL in some individuals may result in partial defects in LPL catalytic activity, and that this may be related to the elevated plasma lipid concentrations observed in FCHL. Another disorder associated with hypertriglyceridemia and increased risk of CAD is type III hyperlipoproteinemia, which is characterized by an accumulation of TG-rich lipoprotein remnants in combination with a receptor binding defective isoform of apolipoprotein (apo) E, apoE2 (17). Whereas 90% of cases of type III hyperlipoproteinemia have the E2/E2 phenotype, fewer than 1% of individuals of E2/E2 phenotype have type III hyperlipoproteinemia. Thus, it appears that a secondary defect is required for the disorder to be manifest and low LPL activity or mass may be such a factor.

Three recent studies have screened the LPL gene for mutations, either in FCHL subjects with reduced LPL activity and mass from the United States (18), in subjects with FCHL of French Canadian origin (19), or in patients with combined hyperlipidemia from the United Kingdom, Sweden, and Holland (20). Of the mutations identified, nucleotide changes were found in exons 2, 5, and 9, and "silent" substitutions were identified in exons 3, 4, and 8. As these same mutations also occur in healthy people, it can be concluded that these mutations may predispose to hyperlipidemia or FCHL, but are not sufficient for hyperlipidemia to develop.

The aim of this study was to investigate the frequency and functional role of a point mutation in exon 6 of the LPL gene. This mutation (asparagine 291 → serine, abbreviated to LPL-S291) has previously been reported to play a role in the development of type III hyperlipoproteinemia in E2/2 homozygotes, but not to occur in the general population (21) or to occur at a much lower frequency in control subjects than in subjects with FCHL or type III hyperlipoproteinemia (22). It has also been identified in a patient with pregnancy-induced chylomicronemia (23), and in a group of French Canadian hyperlipidemic subjects (24). A study published after initial submission of this manuscript found that this mutation occurred in approximately 1 in 20 males with proven atherosclerosis, to be associated with significantly reduced high density lipoprotein (HDL)-cholesterol concentrations but not with significantly elevated plasma TG concentrations (25). Independently of these reports, the LPL-S291 variant was identified in our laboratory, and here we report on the frequency of this mutation in FCHL and type III hyperlipidemia patients and healthy individuals from the United Kingdom, Sweden, and Germany.

METHODS

Subjects

Most of the groups of individuals examined in this study have been described in detail elsewhere. The initial search for mutations using single-strand conformation polymorphism (SSCP) screening was carried out on individuals selected from 85 Swedish patients and 89 controls (described below) on the basis of their postheparin LPL activity, either above 111 mU/ml, or below 65 mU/ml, the mean LPL activity of the Swedish subjects being 85.6 ± 3.8 mU/ml.

Specific screening for the LPL-S291 mutation was undertaken in DNA samples obtained through six separate studies: *a)* 85 patients and 89 matched controls taking part in a Swedish study of myocardial infarction (MI) before age 45 (26, 27); *b)* 41 consecutive patients with combined hyperlipidemia from the Lipid Clinic of Charing Cross Hospital, 93% of whom had a family history of hyperlipidemia (16); *c)* 67 combined hyperlipidemic men recruited from the lipid clinic of St. Thomas' Hospital (28); *d)* 360 male subjects aged 40 to 64 years attending a general practice in Southern England and 389 male subjects from a general practice in Scotland, all of whom had been recruited as part of the

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Northwick Park Heart Study II (NPHS II) project and were free of CAD at the time of entry into the study, as assessed by questionnaire and electrocardiography (29); *e)* 319 healthy subjects (parents (244 subjects) aged 40–75 years and offspring (75 subjects) aged 15–40 years) recruited from a general practice in north London as part of the Goodinge Study, designed to investigate associations of urinary albumin excretion rate with cardiovascular disease in non-diabetic subjects (30, 31); *f)* 75 German patients with type III hyperlipoproteinemia and homozygosity for apoE2 attending the lipid clinics at the Department of Internal Medicine of the University of Heidelberg (32, 33); and *f)* 96 German controls participating in the European Atherosclerosis Research Study (34).

Biochemical analysis

Cholesterol and TG concentrations were determined by standard colorimetric methods (16). Biometrical data previously obtained for each of the studies were used for comparison of carriers with non-carriers.

DNA analysis

Blood was collected in 10-ml Na-EDTA tubes and kept frozen at -20°C. DNA was extracted by the salting-out method (35) or as previously described (36). Polymerase chain reaction (PCR) amplification of LPL exon 6, yielding a 335 base pair product, was carried out on an OmniGene Temperature Cycler (Hybaid Ltd., Middlesex, UK) using a "touchdown" program (one in which the annealing temperature is gradually decreased over a series of steps) and simulated tube temperature. The primers (from Genosys, UK) on either side of exon 6 (sequence from Oka et al. (37)) had the following sequence:

Left hand 5′-CTGCCGAGATACAATCTTG-3′
Right hand 5′-GCATGATGAAATAGGACTCC-3′

After denaturation at 98°C for 1 min (except for an initial denaturation for 5 min at 97°C), the annealing temperature was decreased from 70 to 55°C in five steps (70°, 65°, 61°, 58°, 55°C) over eight cycles, keeping extension conditions constant at 72°C for 1.5 min. PCR reactions (in a volume of 30 μl) were carried out in a standard buffer as recommended by Gibco-BRL (10 × = 500 mmol/l KCl, 100 mmol/l Tris-HCl, pH 8.3, 2 mmol/l each dNTP, 0.01% gelatin) with 300 ng each primer per reaction, and final concentrations of 5% W-1 detergent, and 1.5 mmol/l $MgCl_2$. *Taq* polymerase (Gibco-BRL, UK) was used at a concentration of 1.0 U per 100 μl PCR cocktail.

For SSCP analysis, amplification was performed as described above except that 0.2 μl [α-^{32}P]dCTP at 10 μCi/μl, 3000 mCi/mmol (Amersham, UK) was added to each sample. An aliquot of the PCR product was then diluted 5-fold in 0.1% SDS/10 mmol/l sodium-EDTA and kept frozen until used. The DNA single strands were separated as described by Orita et al. (38) with minor modifications. Samples were denatured by boiling and separated by gel electrophoresis for 18 h on a 10% glycerol, 7.5% polyacrylamide gel (40 cm, 0.4 mm thick, with a 50:1 acrylamide/bis ratio) at 15 mA constant current. Direct sequencing of variants detected by SSCP was carried out using the same primers as used in the amplification reaction, but with one 5′ biotin labeled. The PCR product was purified using the Geneclean II kit (Bio101, La Jolla, CA) and then sequenced by the dideoxy method as described by Green et al. (39) using modified T7 polymerase (Sequenase, United States Biochemical Corp.) (results not shown).

Screening for the LPL-S291 was performed by allele specific oligonucleotide (ASO) hybridization of amplified DNA. Exon 6 was amplified by PCR and the agarose checking gel was double Southern blotted onto nylon membranes (Hybond N$^+$, Amersham). Oligonucleotides (from Genosys, UK) used for the ASO were homologous to the common (Asn) and variant sequences (Ser). They were end-labeled with [γ-^{32}P]ATP and had the following sequence:

Asn291 5′-TGACTTTATTGATCTCA-3′
Ser291 5′-GACTTTACTGATCTCA-3′

Hybridization was performed by incubating the labeled oligonucleotides with the membranes for 3 h at 33°C and then washing for 2 min at room temperature in 2 × saline–sodium phosphate–EDTA (SSPE) and 0.1% SDS, and for 5 min at 42°C in 5 × SSPE and 0.1% SDS. Membranes were exposed to film for 72 h at -70°C.

Statistical analysis

The gene-counting method with a χ^2 test with Yates correction was used to compare the frequency of the LPL-S291 variant allele among the different groups. All other tests and transformations were performed using the SPSS/PC + statistical package. Mann-Whitney non-parametric test and *t*-test were used to compare concentrations of plasma lipids between carriers and non-carriers of the LPL-S291 variant. To test differences in TG concentrations, values were log-transformed prior to statistical analysis. Statistical significance was considered to be at the 0.05 level. For all statistical comparisons of the effects on plasma lipids, carriers of the LPL-N9 variant were excluded (approximately 3% of the subjects), none of which were also LPL-S291 carriers.

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RESULTS

Exons 2–9 of the LPL gene were screened for mutations using SSCP in 27 of the subjects from Sweden, selected from the whole group of young MI survivors and healthy controls on the basis of having either low or high LPL activity. A number of SSCPs were identified; here we present an SSCP found in two individuals in exon 6. Direct sequencing of exon 6 revealed an A to G transition at position 1127, resulting in the substitution of a serine for an asparagine residue at amino acid 291. The base change creating the LPL-S291 mutation does not introduce a restriction enzyme site, and to screen samples for the presence of the LPL-S291 mutation, ASO hybridization was used. The frequency of this mutation was investigated in a group of young MI survivors from Sweden, combined hyperlipidemic subjects from the United Kingdom, type III hyperlipidemic subjects from Germany, and the corresponding general population control subjects for each group, as shown in **Table 1.** The characteristics of these groups have been published previously (16, 26–34). In none of the patient groups was there statistical evidence for a difference in the frequency of the allele coding for LPL-S291 compared to the control subjects. In the control groups, the allelic frequency of LPL-S291 ranged from 1.6% in Germany and the United Kingdom to 3.4% in Sweden but these differences were not statistically significant in these relatively small samples.

When the LPL-S291 carriers were compared within the different patient groups, there were no significant differences in age, BMI, or plasma lipid and apolipoprotein concentrations from the non-carriers. Postheparin LPL activity measurements available for 101 of the Swedish individuals (27) showed no difference in activity between carriers and non-carriers of LPL-S291. For none of the subjects were measurements of LPL mass available.

The absence of any difference in LPL-S291 frequency between the patient and control groups and the above results left the unanswered question; what effect, if any, does the LPL-S291 mutation have? To address this issue, the effect of the LPL-S291 variant was investigated further in the control subjects from the United Kingdom participating in the NPHS II project. The plasma TG concentration was measured in the subjects on entry into the study and at yearly intervals for up to 3 years. The carriers of the LPL-S291 mutation had higher plasma TG concentrations at base-line than the non-carriers (**Fig. 1**), and this difference was maintained over the 3-year time period: overall, mean plasma TG concentrations were 21% higher in LPL-S291 carriers than in non-carriers ($P = 0.05$). At no time over the 3 years was there a difference in BMI between carriers and non-carriers of LPL-S291. When subjects receiving treatment for hypertension were excluded from the analysis (63 non-carriers and 2 carriers), this difference in plasma TG concentrations was unchanged.

To examine the effect of obesity on the expression of the LPL-S291 variant, the relationship of BMI (divided according to tertiles) with plasma TG concentration in carriers and non-carriers of LPL-S291 was investigated (**Fig. 2a**). As expected, in the non-carriers there was a consistent graded increase in plasma TG across the BMI tertiles. However, in carriers of LPL-S291 there was a much larger increase in plasma TG concentration in the upper two BMI tertile groups compared to the lowest BMI tertile group, with a significant BMI × genotype interaction ($P = 0.02$). There was no effect of the LPL-S291 mutation on baseline plasma cholesterol or apoA-I concentrations (**Table 2**).

To determine whether a similar relationship between LPL-S291, TG concentrations, and BMI existed in an independent sample, the LPL-S291 mutation was investigated in an additional group of 319 healthy subjects from the United Kingdom participating in the Goodinge Study. By contrast with the NPHS II group, these subjects were approximately 50% female and were of a larger age range (parents 40–75 years, offspring 15–40 years). As shown in Table 2, LPL-S291 carriers had significantly higher fasting plasma TG concentrations than non-carriers, but total plasma and HDL-cholesterol concentrations were not significantly different. When plasma TG concentrations were examined with respect to BMI, the genotype × BMI interaction was $P = 0.14$ (Fig. 2b) and this interaction was maintained when TG

TABLE 1. Frequency of LPL-S 291 carriers in patients and controls (95% confidence interval)

	Patients		Companion Control Group	
Patient Group, Origin	Carriers:Non-Carriers of S291	Allele Frequency (95% CI)	Carriers:Non-Carriers of S291	Allele Frequency (95% CI)
		%		%
Young MI survivors, Sweden	6:79	3.63 (0.76–6.30)	6:83	3.37 (0.72–6.02)
Combined hyperlipidemic, UK	4:104	1.85 (0.05–3.65)	24:725[a]	1.60 (0.10–2.24)
			12:307[b]	1.88 (0.83–2.94)
Type III, Germany	3:72;	2.00 (-0.24–4.24)	3:93	1.56 (-0.19–3.32)

[a]UK control subjects participating in the Northwick Park Heart Study II project.
[b]UK control subjects participating in the Goodinge Study.

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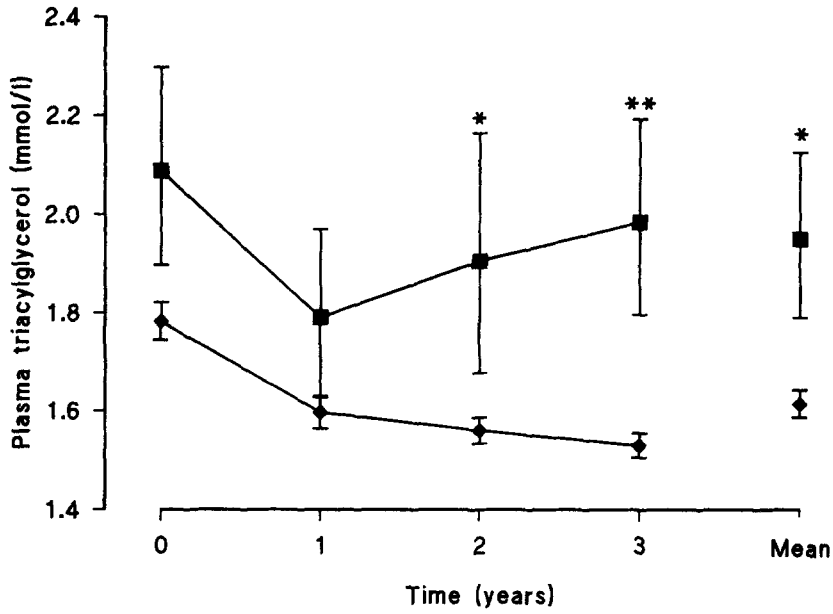

Fig. 1. Mean plasma TG concentrations (± SEM) in the United Kingdom male control subjects participating in the Northwich Park Heart Study II project measured at baseline and at yearly intervals up to 3 years in 599 non-carriers (◆) and 21 carriers (■) of the LPL-S291 mutation. The mean values of the four time points are also shown. Levels of statistical significance between the non-carriers and carriers of the LPL-S291 mutation are $*P \leq 0.05$, $**P < 0.005$. Samples taken for TG measurements were obtained from subjects who had been asked to eat only a light meal before venipuncture.

concentrations were corrected for age, sex, and alcohol consumption ($P = 0.12$). Although this interaction was not significant, the trend was similar to that observed in the NPHS II participants (Fig. 2a). When the Goodinge Study subjects were categorized according to tertiles of plasma TG concentrations, the distribution of LPL-S291 carriers within the tertiles was significantly different from that of non-carriers ($P < 0.007$), with 75% of the LPL-S291 carriers appearing in the highest tertile of plasma TG.

DISCUSSION

The functional significance of the LPL-S291 substitution has been assessed in vitro by site-directed mutagenesis of human LPL cDNA and transient expression in COS cells (21, 22, 25). These studies have found the LPL-S291 substitution results in a reduction of LPL activity of approximately 30–50% (21, 22, 25), a significant increase in LPL monomer, and a decreased LPL dimer to monomer ratio compared to wild type LPL (25). These in vitro effects of the LPL-S291 mutation have been confirmed in our laboratory (F. Mailly, S. E. Humphries, and P. J. Talmud, unpublished observations). LPL is only enzymatically active as a dimer (40, 41), thus a mutant protein that dimerizes less avidly will result in reduced catalytic activity. This is likely to be of particular importance when the lipolytic system becomes challenged by environmental factors such as pregnancy (23, 42), obesity (43), estrogen therapy (44), increasing age (7), and possibly alcohol intake or diabetes. LPL has been reported to act as a ligand for the hepatic LDL receptor-related protein (LRP), increasing the uptake of remnant lipoproteins, but this mechanism is thought to be independent of TG hydrolysis by LPL (45, 46). However, the possible effect of the LPL-S291 mutation on the hepatic re-uptake of nascent apoB-containing lipoproteins was not assessed in this study. A functional role of LPL-S291 is supported by the complete conservation of the N291 residue across species from guinea pig to humans (**Fig. 3**). The substitution of asparagine residue by serine changes an amide group to an hydroxyl group, a change likely to affect the confirmation of the protein. While no definite function of residue 291 of the LPL protein has been proved, a mutation substituting an alanine residue for an arginine at position 294 is known to impair heparin binding (47), thus implicating the importance of the neighbouring residue 291.

The frequency of the LPL-S291 variant has been studied by several groups. In the first report (21), there were no LPL-S291 carriers detected in a sample of 150 healthy Caucasian individuals; in contrast, the second

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report (22) found the mutation at a low frequency in controls (1.9%) and a frequency nearly five times as high in FCHL patients and more than five times as high in a group of 31 type III hyperlipoproteinemic patients. A study of 95 French Canadian patients with hypertriglyceridemia found five LPL-S291 carriers, but none in a group of over 150 normotriglyceridemic individuals (24). In the most recent report (25, published after submission of this manuscript), LPL-S291 was found at frequencies of 5.2% and 4.6% in patients with premature atherosclerosis and control subjects, respectively, but the frequency increased as subjects were categorized according to their HDL-cholesterol concentrations, with lower concentrations being associated with higher frequencies. The data presented here demonstrate the presence of LPL-S291 in subjects from three different countries: Sweden, the United Kingdom, and Germany. The frequency of LPL-S291 was similar in each patient group and the corresponding (country-specific) control subjects. In particular, we do not confirm the original report (21) that the LPL-S291 variant was more frequent in type III patients. We found an allelic frequency of 2.0% in type III hyperlipidemic subjects from Germany compared to 1.6% in a matched healthy group. Taken together, these data make it unlikely that this mutation plays a distinct causal role in the development of any of these dyslipidemias, unlike mutations in the gene for the low density lipoprotein (LDL)-receptor that cause familial hypercholesterolemia.

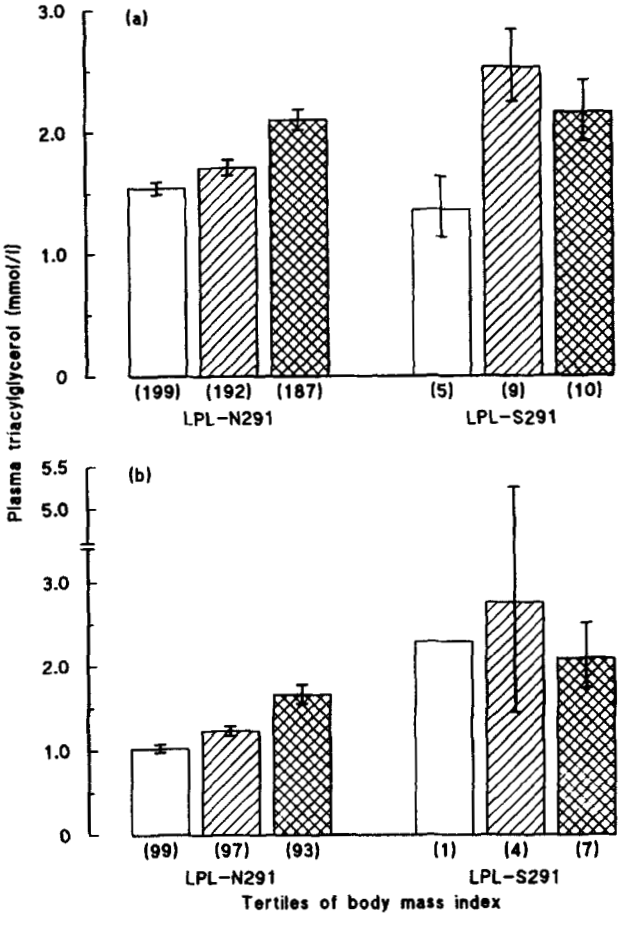

Fig. 2. (a) Baseline plasma TG concentrations (mean ± SEM) in the UK male control subjects participating in the Northwich Park Heart Study II project classified according to BMI tertiles: □ < 25.0; ▨ 25.0–27.7; and ▩ > 27.7 kg/m² in 578 non-carriers and 24 carriers of the LPL-S291 mutation. The test for interaction between LPL-S291 carriers/non-carriers and tertiles of BMI/plasma TG concentration was $P = 0.02$. The numbers in parentheses denote the number of subjects in each group. (b) Mean ± SEM plasma TG concentrations (measured after an overnight fast) in the UK control subjects participating in the Goodinge Study classified according to BMI tertiles: □ < 23.3; ▨ 23.3–26.4; and; ▩ > 26.4 kg/m² in 288 non-carriers and 12 carriers of the LPL-S291 mutation. The test for interaction between LPL-S291 carriers/non-carriers and tertiles of BMI/plasma TG concentration was $P = 0.14$ ($P = 0.16$ on exclusion of 68 offspring, 2 of whom were LPL-S291 carriers). The numbers in parentheses denote the number of subjects in each group.

Discrepancies between the present and previous studies could be due to the small sample sizes reported previously and the fact that the samples were drawn from different countries. They could also reflect the imposition of limits on plasma lipid concentrations for the selection of subjects. In one study (25), CAD patients were selected on the basis of plasma TG concentrations ≤ 4 mmol/l, and plasma cholesterol concentrations between 4 and 8 mmol/l. In another study (24), normal subjects were selected to have HDL-cholesterol concentrations between the 40th and 60th percentile. Such selection criteria were not utilized in this study and both the healthy groups studied are likely to be representative of the general populations from which they were drawn. As the LPL-S291 mutation is associated with increased plasma TG and decreased HDL-cholesterol concentrations, selecting subjects for screening on the basis of these criteria will inevitably lead to a bias in the observed LPL-S291 frequency and mask the true effect of the mutation.

In both groups of healthy individuals, the plasma TG concentrations in carriers of LPL-S291 were found to be higher than those in non-carriers, and the TG-raising effect of the mutation was maintained over time. Reymer et al. (25) also found that carriers of LPL-S291 tended to have higher plasma TG concentrations than non-carriers but reported that this effect was not significant (this may reflect the subject selection criteria, as discussed above). In the present study, the effect of adiposity on the manifestation of LPL-S291 in terms of plasma TG concentrations was investigated further by dividing subjects according to their BMI. In the NPHS II population-based sample of healthy men, plasma TG concentrations in carriers of LPL-S291 were significantly higher in the upper two tertiles of BMI compared to non-carriers. Unfortunately, waist:hip ratios, plasma

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TABLE 2. Plasma lipid concentrations in LPL-S291 carriers and non-carriers from the United Kingdom

	Northwick Park Heart Study II		Goodinge Study	
	N291/N291	N291/S291	N291/N291	N291/S291
Number	794	24	296	12
% Male	100	100	44.4	58.3
Age (yr)	55.8 ± 0.1	55.9 ± 0.6	48.0 ± 0.8	51.9 ± 4.4
TG[a] (mmol/l)	1.77	2.08	1.27	2.31[b]
	(1.74–1.82)	(1.90–2.30)	(1.24–1.31)	(1.85–2.89)
Cholesterol (mmol/l)	5.77 ± 0.04	6.10 ± 0.25	6.17 ± 0.09	6.68 ± 0.47
HDL-chol (mmol/l)			1.38 ± 0.02	1.28 ± 0.12
ApoA-I (mg/dl)	170 ± 2	167 ± 6		

Values are given as means ± SEM. Plasma lipid concentrations were measured in samples taken from the Northwick Park Heart Study II participants who had been asked to eat only a light meal before venipuncture, and in samples taken from Goodinge Study participants who had fasted overnight.

[a]Triglyceride concentrations were log transformed prior to analysis.

[b]N291/N291 versus N291/S291, $P < 0.001$ ($P < 0.001$ on exclusion of 71 offspring, 2 of whom were LPL-S291 carriers).

insulin concentrations, and measures of glucose tolerance were not available in these subjects, so no conclusions can be made as to the contribution of insulin resistance to the observed interaction. Although the interaction of LPL-S291 carrier status with BMI and plasma TG concentration was no longer statistically significant in the second group of population-based subjects investigated (healthy men and women from the Goodinge Study), the trend was clearly the same. This would implicate LPL-S291 in predisposing individuals to elevated plasma TG concentrations, most particularly in those subjects with higher BMI. That is, carriers of LPL-S291 who show an increase in BMI (known to be associated with increases in plasma TG concentration (43)) may experience greater increases in plasma TG concentrations than non-carriers showing a similar increase in BMI. This raises the possibility that moderately obese subjects who are also carriers of LPL-S291 may be at increased risk of CAD because of the development of a more atherogenic lipoprotein phenotype. The exact molecular mechanism of this interaction is unknown, and it is difficult to speculate on a mechanism because of the inability to distinguish between cause and effect from the currently available data.

The TG-raising effect of LPL-S291 reported here is very similar to that seen in carriers of the aspartic acid 9 → asparagine mutation (D9N) in exon 2 of LPL. In the same group of subjects as those analyzed in this present study (from the NPHS II), carriers of one or more LPL-N9 alleles (approximately 3% of the subjects) were reported to have significantly higher plasma TG concentrations than non-carriers (2.25 ± 0.34 vs. 1.82 ± 0.07 mmol/l, $P < 0.02$) but not plasma cholesterol concentrations (20). An interaction of LPL-N9 with BMI was also observed in these subjects: plasma TG concentrations in subjects with a BMI in the upper two tertiles

Fig. 3. Aligned amino acid sequence predicted from lipoprotein lipase cDNA clones for human, chicken, bovine, mouse, and guinea pig. The arrow shows the asparagine 291 residue; *indicates conserved residues relative to human.

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combined compared to those with a BMI in the lowest tertile were 117% higher in LPL-N9 carriers whilst only 26% higher in non-carriers (20). Thus the phenotypic expression of the LPL-N9 and LPL-S291 mutations is similar, but the mechanisms causing this phenotype may be different. It has been suggested that LPL-N9 substitution impairs secretion from the cell (20), and that LPL-S291 inhibits LPL dimer formation (25), but further work is required to determine the exact mechanisms.

Further studies are required to evaluate more fully the BMI effect of the LPL-S291 mutation on LPL action, and such studies are currently in progress in our laboratory. Elucidation of the impact of this mutation on lipid metabolism may help us to understand the clinical implications for subjects who are carriers for LPL-S291 and who may experience a large and clinically useful reduction in TG concentration for a moderate weight loss.

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