Lipoprotein Lipase S447X
A Naturally Occurring Gain-of-Function Mutation

Jaap Rip, Melchior C. Nierman, Colin J. Ross, Jan Wouter Jukema, Michael R. Hayden,
John J.P. Kastelein, Erik S.G. Stroes, Jan Albert Kuivenhoven

Abstract—Lipoprotein lipase (LPL) hydrolyzes triglycerides in the circulation and promotes the hepatic uptake of remnant lipoproteins. Since the gene was cloned in 1989, more than 100 LPL gene mutations have been identified, the majority of which cause loss of enzymatic function. In contrast to this, the naturally occurring LPLS447X variant is associated with increased lipolytic function and an anti-atherogenic lipid profile and can therefore be regarded as a gain-of-function mutation. This notion combined with the facts that 20% of the general population carries this prematurely truncated LPL and that it may protect against cardiovascular disease has led to extensive clinical and basic research into this frequent LPL mutant. It is only until recently that we begin to understand the molecular mechanisms that underlie the beneficial effects associated with LPLS447X. This review summarizes the current literature on this interesting LPL variant.

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Key Words: cardiovascular disease  ■  lipids  ■  lipoprotein lipase  ■  lipoproteins  ■  S447X

Lipoprotein lipase (LPL) plays a central role in human lipid homeostasis and energy metabolism.1 The main function of this enzyme is the hydrolysis of plasma triglycerides (TGs) that are packaged in apolipoprotein (apo) B containing lipoproteins. It furthermore mediates the clearance of atherogenic remnant lipoproteins from the circulation.2 The gene encoding for LPL is located on chromosome 8 and is expressed mainly in skeletal muscle, adipose tissue, and heart muscle. Homozygosity or compound heterozygosity for missense, nonsense mutations, deletion, or insertions in the LPL gene, resulting in complete loss of enzyme function,3,4 cause the accumulation of chylomicrons in the circulation, a phenotype known as type I hyperlipoproteinemia. This rare autosomal recessive disorder can be lethal because of (recurrent) hemorrhagic pancreatitis.3

The LPL gene locus is highly polymorphic and many single nucleotide polymorphisms (SNP) in both coding and noncoding regions have been used to study associations with lipids, lipoproteins, and risk for atherosclerosis. Most of these SNPs have only mild detrimental effects on LPL function or are mere markers for genetic variation elsewhere in the genome.5 Two SNP in the coding DNA (cSNPs) that have been studied extensively concerning point mutations in exon 2 and 6, causing the substitution of an aspartic acid to an asparagine residue at position 291 (N291S), respectively. These mutations occur at high frequencies in the general population (up to 5%) and are associated with elevated TGs, decreased high-density lipoprotein (HDL) cholesterol levels, and concomitantly with a higher incidence of cardiovascular disease (CVD).6–13 compared with noncarriers. Several in vivo and in vitro studies have shown that both LPLD9N and LPLN291S have decreased lipolytic activity compared with LPLWT.8,12,14–16 For LPLD9N this was reported to relate to decreased cellular secretion,6 whereas LPLN291S was shown to be less stable compared with LPLWT.17 In a more recent study, Fisher et al showed that LPLD9N causes enhanced low-density lipoprotein (LDL) binding and monocyte adhesion compared with LPLWT and was thus suggested to enhance foam cell formation in the vascular wall.18

A third frequently occurring cSNP concerns a C to G mutation in exon 9 at position 1595. This nucleotide change introduces a premature stop codon at position 447, resulting in a mature protein that lacks the C-terminal serine and glycine, from now on denoted as LPLS447X. In contrast to all other LPL variants, this mutation is associated with beneficial effects on lipid homeostasis and atheroprotection.5 Such gain-of-function as the result of a mutation in genomic DNA has rarely been reported in the literature,19,20 but, interestingly, most are associated with protection against CVD.21–23 These mutations may be especially favorable in modern times now that people live longer and are subject to a much higher risk for development of CVD because of a poor lifestyle. The molecular event that underlies the appearance of LPLS447X occurred before the Indo-German divi-

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Plasma Lipids and Lipoproteins

Table 1 provides an overview of all studies on LPLS447X and the main findings that have been published thus far.

Focusing on lipid metabolism, several studies have shown significantly lower plasma TG levels and higher plasma HDL cholesterol levels in 447X carriers compared with noncarriers.5,16,24,25,27–49 In some reports, a clear allele dosage effect was observed, indicative of a biological relationship these parameters.24,30 In addition, most investigators reported that carriers of the mutation did not exhibit changes in total cholesterol and low-density lipoprotein cholesterol levels compared with noncarriers.41–44,46,50–52

Interestingly, the mutation appears to especially lower plasma TG levels in smoking and drinking females,44,46,53 in obese subjects,40 in carriers of deleterious apoCIII polymorphisms,44,53 and in subjects with the apoE4 allele.44,46,50 Thus, it appears that LPLS447X moderates the effects of risk factors for CVD but the mechanisms that underlies these observations are unclear.

The lipid measurements in the majority of studies have been performed in the fasted state. However, LPL action is especially required under postprandial conditions in which dietary lipids transported in chylomicrons need to be catabolized to enable uptake of free fatty acids by skeletal/heart muscle and adipose tissue. Five studies have thus far addressed the question whether LPLS447X has an impact on postprandial TG metabolism.24,54–57 In an initial report, Humphries et al showed in 332 offspring of fathers with essential hypertension, carriers were shown to exhibit moderately increased blood pressure.51 In contrast, haplotype analysis in 501 normotensive and 497 hypertensive Chinese subjects showed that the mutation was more frequent in the hypertensive group, in fact suggesting a protective effect of LPLS447X.64

In a second report, others did not observe significant differences in TG clearance after infusion of chylomicron-like emulsions in a small mixed population of 7 male and 5 female heterozygotes versus 6 male and 7 female controls.55 In a third study it was found that healthy male heterozygotes (n=15) had an increased postprandial clearance of triglyceride-rich lipoproteins (TRL) compared with noncarriers (n=36).56 In a recent study by our group, 15 healthy male volunteers, heterozygous for 447X, showed an increased postprandial apoB48 clearance compared with noncarriers after a standardized oral fat load when compared with controls matched for gender, age, alcohol use, body mass index, and smoking. We also found that carriers of the mutation have a higher LPL concentration in preheparin serum (further discussed later). With these findings, we set out to test the hypothesis that LPLS447X enhances apoB100 catabolism.57 In summary, 5 healthy male homozygotes for 447X and 5 male controls were continuously fed and received continuous infusion of a stable isotope. Compared with controls, carriers presented with a 2-fold enhanced conversion of TRL in addition to an enhanced LDL removal. In conclusion, 4 of 5 studies indicate that carriers of the 447X mutation have an enhanced capacity to lower postprandial TG levels when compared with noncarriers.

Cardiovascular Disease, Blood Pressure, Alzheimer Disease, and Cancer

Cardiovascular Disease

A considerable number of studies have suggested that 447X carriers have a lower CVD risk,5,24,27,30,39,58 but this was not confirmed by other investigators.28,37,52,59–61 Wittrup et al were the first to conduct a meta-analysis on the associations between several LPL gene variants and risk of ischemic heart disease (using 8 of these studies) and calculated a 17% decreased risk in carriers of LPLS447X. In a second meta-analysis, the same investigators noted that the protective effect was gender-specific, providing benefit only to males with 18% reduced risk of future CVD.30 In a review, Hokanson et al, however, reported a 19% risk reduction in both sexes.62 Taken together, it appears that LPLS447X is associated with protection against CVD in accordance with the beneficial changes it confers to the lipid profile.

Blood Pressure, Alzheimer Disease, Cancer

This paragraph summarizes a small number of reports on the relation between LPLS447X and blood pressure, Alzheimer disease, and cancer.

The association between the LPLS447X variant and hypertension was assessed in highly diverse study cohorts. In healthy volunteers (n=696), 447X was associated with decreased systolic and diastolic blood pressure levels, but only in women (n=337).63 In individuals with familial hypercholesterolemia, a decreased diastolic blood pressure and a trend toward decreased systolic blood pressure was found in 128 both male and female LPLS447X carriers compared with 488 controls.62 In contrast, in dyslipidemic Chinese patients with essential hypertension, carriers were shown to exhibit moderately increased blood pressure.51 In contrast, haplotype analysis in 501 normotensive and 497 hypertensive Chinese subjects showed that the mutation was more frequent in the normotensive group, in fact suggesting a protective effect of LPLS447X.64

LPL also plays a central role in cholesterol metabolism in the brain.65,66 The highest LPL activity is found in the hippocampus and the presence of LPL is thought to have a favorable effect on the survival and regeneration of neurons. LPL could therefore putatively affect the development of Alzheimer disease. Supporting this line of thought, a lower incidence of Alzheimer disease in 447X carriers was recently shown in 3 studies.67–69 In contrast, 2 other studies could not show a relationship between LPLS447X and Alzheimer disease.70,71

Because prostate cancer is associated with increased dietary fat intake,72 genetic factors that influence lipid metabolism may also be linked to the development of prostate cancer. A possible role of LPL in the development of prostate cancer was shown in only 1 study with 273 Japanese prostate cancer patients, 205 benign prostatic hyperplasia patients, and 230 male controls. In this study, LPLS447X was found associated with an increased risk for prostate cancer,73 which was attributed to an increased availability of free fatty acids, released by LPL activity.74,75
TABLE 1. Publications in Which the Associations Between LPL<sup>447X</sup> and Plasma Lipid Levels and/or Cardiovascular Disease Was Investigated

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plasma TG&lt;sup&gt;++/-&lt;/sup&gt; and&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>Plasma HDL-C&lt;sup&gt;++/-&lt;/sup&gt; and&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>Cardiovascular Disease&lt;sup&gt;++/-&lt;/sup&gt; and&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>Subjects Male/Female (Carriers/Noncarriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nierman et al, 2005&lt;sup&gt;57&lt;/sup&gt;</td>
<td>No diff.</td>
<td>No diff.</td>
<td>M&lt;sup&gt;6/-/-&lt;/sup&gt;/6 Netherlands</td>
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<td>Nierman et al, 2005&lt;sup&gt;54&lt;/sup&gt;</td>
<td>↓14%</td>
<td>N.S.</td>
<td>No diff.</td>
<td>M&lt;sup&gt;15/-/-/15&lt;/sup&gt; Netherlands</td>
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<tr>
<td>Goodarzi et al, 2002&lt;sup&gt;44&lt;/sup&gt;</td>
<td>↓12%</td>
<td>N.S.</td>
<td>↑2.8%&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lopez-Miranda et al, 2004&lt;sup&gt;46&lt;/sup&gt;</td>
<td>No diff.</td>
<td>No diff.</td>
<td>M (26/25) Spain</td>
<td></td>
</tr>
<tr>
<td>Lee et al, 2004&lt;sup&gt;46&lt;/sup&gt;</td>
<td>↓10.6%&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.057</td>
<td>↑5%&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Almeida et al, 2003&lt;sup&gt;55&lt;/sup&gt;</td>
<td>No diff.</td>
<td>No diff.</td>
<td>M+F (13/12) Brazil</td>
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<tr>
<td>Skoglund-Andersson et al, 2003&lt;sup&gt;49&lt;/sup&gt;</td>
<td>↓16.6%&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt;0.05</td>
<td>↑7.4%&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>Morabia et al, 2003&lt;sup&gt;55&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>Protected against CVD (odds ratio 0.39; &lt;0.05)</td>
<td></td>
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<tr>
<td>Wittrup et al, 2002&lt;sup&gt;20&lt;/sup&gt;</td>
<td>↓6.5%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.001</td>
<td>↑4.2%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.001</td>
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<td>Corella et al, 2002&lt;sup&gt;44&lt;/sup&gt;</td>
<td>↓10%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.37</td>
<td>↑2%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.99</td>
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<tr>
<td>Talmud et al, 2002&lt;sup&gt;21&lt;/sup&gt;</td>
<td>↓9.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>↑3.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<td>Shimo-Nakanishi et al, 2001&lt;sup&gt;26&lt;/sup&gt;</td>
<td>↓18%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.002</td>
<td>↑11%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.001</td>
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<td>McCladdery et al, 2001&lt;sup&gt;21&lt;/sup&gt;</td>
<td>↓11.1%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
<td>↑7.7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<td>Clee et al, 2001&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>No diff.</td>
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<td>Chen et al, 2001&lt;sup&gt;27&lt;/sup&gt;</td>
<td>↓18.7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑2.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<td>Garenc et al, 2000&lt;sup&gt;45&lt;/sup&gt;</td>
<td>↓21.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑4.4%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
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<td>Arca et al, 2000&lt;sup&gt;22&lt;/sup&gt;</td>
<td>↓21%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.06</td>
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<td>Sass et al, 2000&lt;sup&gt;63&lt;/sup&gt;</td>
<td>↓13.3%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑4%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
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<tr>
<td>Gagne et al, 1999&lt;sup&gt;19&lt;/sup&gt;</td>
<td>↓14%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.02</td>
<td>↑5.3%&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<tr>
<td>Hallman et al, 1999&lt;sup&gt;12&lt;/sup&gt;</td>
<td>↓15%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.05</td>
<td>↑3.7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<td>Humphries et al, 1998&lt;sup&gt;24&lt;/sup&gt;</td>
<td>↓10.6%&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<td>Kuivenhoven et al, 1997&lt;sup&gt;28&lt;/sup&gt;</td>
<td>↑</td>
<td>&lt;0.001</td>
<td>M (50/191) Netherlands, high, medium and low HDL groups</td>
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<td>Wittrup et al, 2002&lt;sup&gt;20&lt;/sup&gt;</td>
<td>↓5%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.001</td>
<td>↑2%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.99</td>
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<td>Wittrup et al, 2002&lt;sup&gt;20&lt;/sup&gt;</td>
<td>↓9.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>↑3.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.02</td>
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<td>Talmud et al, 2002&lt;sup&gt;21&lt;/sup&gt;</td>
<td>↓13%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.002</td>
<td>↑4.2%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>Shimo-Nakanishi et al, 2001&lt;sup&gt;26&lt;/sup&gt;</td>
<td>↓10%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
<td>↑3%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
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<tr>
<td>McGladdery et al, 2001&lt;sup&gt;21&lt;/sup&gt;</td>
<td>↓11.1%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
<td>↑7.7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.055</td>
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<tr>
<td>Clee et al, 2001&lt;sup&gt;12&lt;/sup&gt;</td>
<td>↓20.4%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>No diff.</td>
<td>Trend of reduced vascular disease (odds ratio: 0.61; P=0.10)</td>
</tr>
<tr>
<td>Chen et al, 2001&lt;sup&gt;27&lt;/sup&gt;</td>
<td>↓18.7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑2.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<td>Garenc et al, 2000&lt;sup&gt;45&lt;/sup&gt;</td>
<td>↓21.8%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑4.4%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
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<tr>
<td>Arca et al, 2000&lt;sup&gt;22&lt;/sup&gt;</td>
<td>↓21%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑7%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0.06</td>
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<tr>
<td>Sass et al, 2000&lt;sup&gt;63&lt;/sup&gt;</td>
<td>↓13.3%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>↑4%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>N.S.</td>
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<tr>
<td>Humphries et al, 1998&lt;sup&gt;24&lt;/sup&gt;</td>
<td>↓10.6%&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>&lt;0.05</td>
<td>No diff.</td>
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<td>Kuivenhoven et al, 1997&lt;sup&gt;28&lt;/sup&gt;</td>
<td>↑</td>
<td>&lt;0.001</td>
<td>M (50/191) Netherlands, high, medium and low HDL groups</td>
<td></td>
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</table>
(Continued)
Unequivocal data regarding the association between LPLS447X, cancer, blood pressure, and Alzheimer disease are likely hampered by small sample size, differences in genetic background, and different inclusion/exclusion criteria urging for careful interpretation. In general, genetic associations studies to study biological relationships need the use of very large population samples as recently reviewed and commented by Hattersley et al and Cordell et al.76,77

### Mechanism Underlying the Beneficial Effects of the S447X Variant

The LPLS447X variant is thus associated with changes in lipid and lipoprotein metabolism and cardiovascular protection, but what molecular mechanisms are responsible for these beneficial effects? This question is not easily answered when one considers that the effects of this mutant LPL are only appreciated when studied in large groups of individuals indicating that the effects are mild in nature. It is possible that LPLS447X acts through one mechanism but maybe this LPL mutant has direct effects on multiple pathways in LPL’s complex biology. It can also be imagined that, eg, a slight increase LPL concentration through improved secretion of the mutant protein from parenchymal tissues has only mild effects on total LPL activity, LPL levels, and lipoprotein clearance from the circulation, but when combined render the protective effects observed. In the next paragraphs, we discuss specific aspects of LPL biology that may be altered if LPL’s monomers lack the 2 C-terminal amino acids. We focus on LPL activity and LPL concentration in the circulation, on the stability of LPL and its binding to heparin sulfate (HS) containing proteoglycans, on the LPL-mediated clearance of (remnant) lipoproteins by the liver, and, finally, on the expression of LPL and uptake of lipoproteins by macrophages (Figure).

### LPL Activity and LPL Concentration

#### Catalytic Activity

Increased LPL activity results in lower plasma TG levels and higher HDL cholesterol levels.78

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TABLE 1. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plasma TG (+/−)</th>
<th>Plasma HDL-C (+/−)</th>
<th>Cardiovascular Disease (+/−)</th>
<th>Subjects Male/Female (Carriers/Noncarriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groenemeijer et al, 1997²⁷</td>
<td>↓ 8% 0.044</td>
<td>↑ 4.4% 0.013</td>
<td>Protected against CAD (odds ratio 0.73; (P&lt;0.05))</td>
<td>M (149/662) REGRESS study, CAD patients</td>
</tr>
<tr>
<td>Salah et al, 1997⁵⁰</td>
<td>↓ 19% 0.01</td>
<td>↑ 4.4% N.S.</td>
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<td>M+F (242/831) France, Stanislas cohort</td>
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<tr>
<td>Peacock et al, 1997³³</td>
<td>No diff.</td>
<td>No diff.</td>
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<td>M+F (315) Iceland</td>
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<td>Knudsen et al, 1997³⁴</td>
<td>No diff.</td>
<td>No diff.</td>
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<td>M+F Finland, 99 hyperTG patients + 75 controls</td>
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<tr>
<td>Galton et al, 1996¹⁸</td>
<td>↓ &lt;0.04</td>
<td>↑ 0.002</td>
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<td>M (211/997) Caerphilly Prospective Heart Disease Study</td>
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<tr>
<td>Hegele et al, 1996⁶⁷</td>
<td>No diff.</td>
<td>No diff.</td>
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<td>M+F (45/717) Canada, Huterite population</td>
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<td>Zhang et al, 1995⁵⁰</td>
<td>↓ &lt;0.05</td>
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<td></td>
<td>M (63/257) UK, CAD patients</td>
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<td>Jemaa et al, 1995⁵⁶</td>
<td>↓ 10.1% 0.01</td>
<td>↑ 4.5% N.S.</td>
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<td>M (165/556) France, Ireland, ECTIM study</td>
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<tr>
<td>Mattu et al, 1994⁵⁹</td>
<td>↓ 3% N.S.</td>
<td>↑ 3% N.S.</td>
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<td>M (22/101) Welsh, CAD patients and controls</td>
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<tr>
<td>Peacock et al, 1992⁴⁸</td>
<td>N.S.</td>
<td>↑ 4.8% N.S.</td>
<td></td>
<td>M (18/155) Sweden, MI survivors</td>
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<td>Stocks et al, 1992⁴⁶</td>
<td>↓ 4.5% N.S.</td>
<td>↑ 4.8% N.S.</td>
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<td>M (29/120) UK</td>
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<td>Hata et al, 1990⁴⁶</td>
<td>N.S.</td>
<td></td>
<td></td>
<td>M+F (31/121) hyperTG patients and controls</td>
</tr>
<tr>
<td>Meta-Analyses</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wittrup et al, 2002⁵⁰</td>
<td>↓ 10%&lt;sup&gt;n&lt;/sup&gt; 0.001</td>
<td>↑ 4%&lt;sup&gt;n&lt;/sup&gt; 0.001</td>
<td>Protected against heart disease in men (odds ratio 0.83; (P=0.01))</td>
<td></td>
</tr>
<tr>
<td>Hokanson et al, 1999⁵²</td>
<td></td>
<td></td>
<td>Protected against CVD (odds ratio 0.81)</td>
<td></td>
</tr>
<tr>
<td>Wittrup et al, 1999⁵</td>
<td>↓ 8%</td>
<td>↑ 4.4%</td>
<td>Protected against CHD (odds ratio 0.8)</td>
<td></td>
</tr>
</tbody>
</table>

M indicates males; F, females; \(+/−\), heterozygous S447X carriers; \(−/−\), homozygous S447X carriers; ND, not determined; NS, nonsignificant.
is characteristic for 447X carriers, one may hypothesize that LPL S447X simply has enhanced lipolytic capacity compared with wild-type LPL. Reviewing the literature on this topic, however, reveals unequivocal results. In direct comparisons (in vitro) with LPL WT, LPL S447X has been reported to exert increased (H11001 85%),79 unchanged,17,80,81 and even reduced catalytic activity (H11002 30%).82 These discrepancies may relate to the type of cells used and how the culture media was harvested (in presence or absence of heparin) and handled. Irrespective of these results, data on LPL activity in carriers of the mutation suggest overall that LPL S447X has increased lipolytic potential over LPL WT. Postheparin LPL activity has been measured in at least 8 studies, summarized in Table 2. In 2 initial studies in Swedish myocardial infarction survivors (n=173) and in hypertriglyceridemic patients (n=174) from Finland, postheparin LPL activity was shown to be similar in patients that did or did not have the mutation.34,48 Using larger population samples, 2 studies29,40 (475 and 397 subjects, respectively), however, showed significant 18% to 36% increases in postheparin LPL activity in carriers compared with noncarriers. Our group previously genotyped and assessed postheparin LPL activity levels in 804 males with established coronary atherosclerosis. In this cohort, we identified an overrepresentation of 447X carriers in the highest quartile of LPL activity compared with the lowest quartile (18.3% versus 11.5%; P<0.006).16 Unpublished thus far, Table 3 presents that postheparin LPL activity levels were significantly higher in heterozygote carriers (n=118) but not in the small number of homozygotes (n=6) compared with noncarriers (n=539). In 2 subsequent studies concerning only 15 heterozygotes (compared with 15 controls)54 and 6 homozygotes (compared with 6 controls),57 we did not find a differences in postheparin LPL activity likely because of the very small sample sizes. Taken together, the published

Different pathways by which LPL S447X may exert its beneficial effects include: (1) increased lipolytic activity and/or concentration in the circulation; (2) increased stability of LPL dimers and better binding to heparan sulfate containing proteoglycans and lipoproteins; (3) promotion of hepatic uptake of lipoproteins; and (4) reduced LPL-mediated uptake of modified lipoproteins by macrophages. LPL indicates lipoprotein lipase; TG, triglycerides; FFA, free fatty acids; HSPG, heparan sulfate proteoglycans; CM, chylomicron; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; CMr, chylomicron remnant; MC, macrophages; SMC, smooth muscle cells.

**TABLE 2. List of Studies in Which the Plasma LPL Concentration and/or Activity of S447X Carriers Was Assessed and Compared With Noncarriers**

<table>
<thead>
<tr>
<th>Reference</th>
<th>LPL Activity and Concentration</th>
<th>Subjects M/F* (Carriers/Noncarriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peacock et al, 1992†</td>
<td>Post-heparin LPL activity not different</td>
<td>M (18/155) Sweden, MI survivors</td>
</tr>
<tr>
<td>Knudsen et al, 1997†</td>
<td>Post-heparin LPL activity not different</td>
<td>M/F Finland, 99 HyperTG + 75 controls</td>
</tr>
<tr>
<td>Garenc et al, 2000††</td>
<td>↑ Post-heparin LPL Activity (+18.8%, P&lt;0.05) in men only</td>
<td>M (40/188)+F (43/204) HERITAGE family study</td>
</tr>
<tr>
<td>Goodarzi et al, 2005††</td>
<td>↑ Post-heparin LPL Activity (+35.9%, P&lt;0.05)</td>
<td>M+F (44/353) Mexican-Americans</td>
</tr>
<tr>
<td>Henderson et al, 1999†</td>
<td>↑ Post-heparin LPL activity (P&lt;0.05)</td>
<td>M (118/613) REGRESS study</td>
</tr>
<tr>
<td>Nierman et al, 2005††</td>
<td>↑ Pre-heparin LPL concentration (4-fold, P=0.01)</td>
<td>M (6/6) Netherlands</td>
</tr>
<tr>
<td>Nierman et al, 2005††</td>
<td>↑ Pre-heparin LPL concentration (2.4-fold, P&lt;0.0001)</td>
<td>M (15/15) Netherlands</td>
</tr>
<tr>
<td>Skoglund-Andersson et al, 2003†</td>
<td>↑ Pre-heparin LPL activity (+58.8%; P=0.001)</td>
<td>M (74/303) North European, 50 years old</td>
</tr>
</tbody>
</table>

*M indicates male; F, female.
literature suggests enhanced postheparin LPL activity in 447X carriers compared with controls but large numbers of individuals are required to unmask this effect. In all of these studies, heparin was used to release LPL from the endothelium to run the usual assays for LPL activity. But to what extent does this methodology reflect the actual LPL-mediated TG hydrolysis in vivo? Some investigators have shown that it is also possible to measure LPL activity levels in nonheparinized plasma, although the activity levels are very low.83 Using a very sensitive activity assay, Skoglund-Andersson et al identified a 60% increase in preheparin LPL activity in 18 carriers of the mutation compared with noncarriers.59 These investigators postulated that this increase could indeed be responsible for the slightly decreased TG levels and increased HDL cholesterol levels. Further indirect supporting evidence that LPLS447X has superior lipolytic activity over LPLWT was given by the higher apoB100 turnover rates of TRL in 447X carriers as already discussed. Because TRL conversion in plasma is almost entirely attributable to LPL-mediated TG hydrolysis,84 this suggests increased lipolytic activity of the mutant enzyme. Furthermore, a recent study in LPL knockout mice showed 2-fold higher LPL activity after adenoviral gene transfer of cDNA encoding for LPLS447X compared with transfer of the wild-type LPL cDNA.85 This study also demonstrated that expression of the LPLS447X variant is a more potent triglyceride-lowering strategy than a similar one using LPLWT.

### Stability of LPL Binding to Heparan Sulfate Containing Proteoglycans and Lipoproteins

In the circulation, LPL is normally bound to HS-containing proteoglycans and lipoproteins, which mediate LPL binding to endothelium and primarily active as a dimer.

**Assessment of LPL binding to proteoglycans**

Using various ELISA methods, we have chosen to refrain from reviewing the literature in this respect but wish to mention that potential compared with LPLWT and is present at higher concentrations in preheparin plasma. These findings may explain the beneficial effects of LPLS447X on lipid profiles and CVD.

### In summary

The afterthought analyses performed to date have been unable to provide a convincing explanation for the increased LPL activity and LPL concentrations (the latter in preheparin plasma) observed in carriers of the mutation.

**TABLE 3. Post-Heparin LPL Activity Levels and Heterozygosity and Homozygosity for S447X in Males With Established Coronary Atherosclerosis From the Regress Study.**

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Noncarriers</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL activity (mU/ml)</td>
<td>107±43</td>
<td>121±54*</td>
<td>108±32†</td>
</tr>
</tbody>
</table>

*P<0.01 vs. +/+ group, †P<0.05 vs. +/+ group, ‡P<0.01 vs. group, all adjusted for BMI, age, NYHA class, systolic blood pressure and medication.

The prospective “European Prospective Investigation into Cancer and Nutrition” Norfolk cohort.88 The 1006 CAD cases and 1980 matched controls studied here are, however, not yet genotyped for the SNP underlying LPLS447X, but these results are anticipated soon.

It may be noted that the quantification of LPL levels in plasma is dependent on the antibodies used and is moreover complicated by the differences in avidity for LPL monomers and dimers. For the current review, this aspect is even more complicated when considering the theoretical mix of 3 types of dimeric LPL (S447 and 447X, and chimeric dimers) and 2 monomeric LPL species in postheparin plasma of heterozygotes for 447X. Taken this complexity, and the lack of direct comparisons of data generated by various ELISAs using an identical set of clinical samples, we have chosen to refrain from reviewing the literature in this respect but wish to underline that this issue may need more attention in the future.

In summary, the published literature gives strong support for the notion that the LPLS447X variant exerts higher lipolytic potential compared with LPLWT and is present at higher concentrations in preheparin plasma. These findings may explain the beneficial effects of LPLS447X on lipid profiles and CVD.

**Stability of LPL Binding to Heparan Sulfate Containing Proteoglycans and Lipoproteins**

In the circulation, LPL is normally bound to HS-containing proteoglycans and lipoproteins, which mediate LPL binding to endothelium and primarily active as a dimer. The affinity of the dimers for HS is higher compared with (inactive) monomeric LPL and, moreover, LPL dimers are stabilized by HS binding. Thus, the differences found in preheparin plasma LPL concentration and activity, and postheparin LPL activity may derive from differences in LPL dimer stability (or the stability of chimeric heterodimers in heterozygotes). Zhang et al showed, however, that LPLWT and LPLS447X as produced by transiently transfected COS cells had similar similarities as tested by measuring catalytic activities after incubations at 37°C.17 We recently confirmed this by measuring catalytic activities of recombinant LPLWT and LPLS447X after prolonged incubations at 37°C and in the presence of 0 to 0.5 mmol/L guanidine chloride.85 However, the increased concentration of LPLS447X in preheparin plasma may also be caused by decreased affinity of LPLS447X for HS-proteoglycans compared with LPLWT. Zhang et al tested this for the 2 variants using heparin Sepharose columns but found similar affinities for both (monomers and dimers).17 It could also be hypothesized that LPLS447X has higher affinity for lipoproteins in the circulation compared with LPLWT. Further indirect supporting evidence that LPLS447X has superior lipolytic activity over LPLWT was given by the higher apoB100 turnover rates of TRL in 447X carriers as already discussed. Because TRL conversion in plasma is almost entirely attributable to LPL-mediated TG hydrolysis,84 this suggests increased lipolytic activity of the mutant enzyme. Furthermore, a recent study in LPL knockout mice showed 2-fold higher LPL activity after adenoviral gene transfer of cDNA encoding for LPLS447X compared with transfer of the wild-type LPL cDNA.85 This study also demonstrated that expression of the LPLS447X variant is a more potent triglyceride-lowering strategy than a similar one using LPLWT.
Clearance of Lipoproteins by the Liver
It is already mentioned that LPL promotes the uptake of atherogenic lipoproteins by the liver via the very-low-density lipoprotein and LDL receptors through acting as a ligand and/or a molecular bridge. Although this action of LPL has long been shown to occur in vitro and in animal, Zheng et al were recently the first to our knowledge to report that the enhanced clearance of apoB-containing lipoproteins by LPL also occurs in humans. These authors furthermore state that this mechanism may be particularly important for clearing intestinal lipoproteins in the postprandial state. Thus, it may be hypothesized that a better clearance of atherogenic remnant lipoproteins in 447X carriers underlies the observed reduced risk of atherosclerosis. However, Salinelli et al showed that the binding, uptake, and degradation of very-low-density lipoprotein in LPL-S447X producing COS cells was not different from LPL-WT producing cells. Also, the hepatic clearance of a radioactive-labeled chylomicron-like emulsion in a small number of 447X carriers was found comparable to controls. However, we recently showed that homozygotes and heterozygotes for 447X have enhanced LDL57 and apoB 48 clearance rates, respectively, supporting the idea of an increased bridging function for the LPL-S447X variant when considering increased levels of freely circulating LPL in these subjects compared with controls.

Uptake of Lipoproteins by Macrophages
It has been generally acknowledged that LPL in addition to skeletal, heart, and adipose tissue is also produced by monocye-derived cells in the subendothelial space and that this leads to foam cell formation, a key event in atherogenesis. Clee et al provided evidence that LPL in the vascular wall was indeed a proatherogenic factor, albeit in a mouse model for atherosclerosis. This hypothesis finds support in studies of LPL overexpression in macrophages leading to increased atherosclerosis in the aorta of rabbits. Thus, it could be hypothesized that the atheroprotective effects of LPL-S447X may derive from reduced expression of LPL by macrophages but more likely by reduced uptake of (modified) LDL in subendothelial macrophages in carriers of the mutation. Such an effect would provide a straightforward explanation of the anti-atherogenic effects that are associated with LPL-S447X.

Conclusions
The bulk of evidence summarized shows that carriers of the 447X mutation have lower TG levels and increased HDL cholesterol levels with a concomitant lower incidence of CVD compared with noncarriers. These finding support the notion that it concerns a gain-of-function mutation, the very reason for the use of LPL-S447X in the development of gene therapy for human LPL deficiency. The unraveling of the molecular mechanisms responsible for these beneficial effects has, however, proven difficult. Most studies in humans indicate that the beneficial effects are associated with enhanced TG-lowering capacity mainly attributed to increased lipolytic function. However, the noted differences were rather small and as a result mainly identified in studies with larger groups of individuals. The idea that LPL-WT and LPL-S447X are only slightly different and may impact simultaneously numerous aspects of LPL biology (with cumulative, synergistic, or opposing effects) in vivo may underlie the fact that many molecular (in vitro) studies did not identify differences between LPL-WT and LPL-S447X regarding catalytic activity, stability of the protein, affinity for heparin Sepharose, or capacity to mediate uptake of lipoproteins.

Future Research
Additional insight into the molecular mechanisms how LPL-S447X exerts its beneficial effects may come from studies on the affinity of this mutant for circulating lipoproteins. Also, a comparison of LPL-WT and LPL-S447X in the processes of foam cell formation, intracellular trafficking, cellular secretion, and translocation (over the endothelium) may be warranted, but chances to find marked differences may be slim for the reasons indicated. The need for heparin injections to assess LPL function in humans, which likely kept many investigators from studying LPL in their clinical studies, has unfortunately limited our knowledge on how LPL is related to (patho)physiological conditions. Maybe the use of sensitive ELISAs or the use of minor amounts of catalytically active LPX on circulating lipoproteins may bring relief for future studies on LPL and its natural variants. Furthermore, studies on the interactions of both LPL variants with its activators apoCI99 and apoAV and with negative regulators such as apoCIII, angptl3, and angptl4 have not been published thus far. Adding to the complexity, Karpe et al have furthermore provided evidence for differential regulation of the secretion (and uptake) of active and inactive LPL in adipose tissue and skeletal muscle in humans, which may be explained by local differences in LPL affinity for endothelial cells. These intriguing and poorly understood aspects of LPL biology may also need to be accounted for when comparing the actions of LPL and its natural mutants.

Acknowledgments
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Lipoprotein Lipase S447X: A Naturally Occurring Gain-of-Function Mutation
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