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# Carriers of the frequent lipoprotein lipase S447X variant exhibit enhanced postprandial apoprotein B-48 clearance

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**Abstract**

The frequent lipoprotein lipase S447X variant (LPLS447X) is firmly associated with a lower incidence of cardiovascular disease, the mechanisms for which remain to be established. To further unravel these beneficial effects, we studied the consequences of LPLS447X heterozygosity on LPL mass and activity, as well as on the postprandial lipoprotein profile. Fifteen male heterozygous LPLS447X carriers and 15 matched control subjects received an oral fat load (30 g/m$^2$). Lipid parameters were evaluated at baseline and 2, 3, 4, and 6 hours after fat loading. LPL concentration and activity were analyzed, and endothelial function was evaluated noninvasively as flow-mediated dilation of the brachial artery. Although baseline apoprotein B-48 (apoB48) levels were similar, the rise in apoB48 was attenuated in LPLS447X carriers with 25% lower peak values compared with controls ($P = .04$). In conjunction, LPLS447X carriers were characterized by a 2.4-fold increase in preheparin LPL mass ($P < .0001$). The decrease in postprandial flow-mediated dilation was comparable in both groups. LPLS447X carriers exhibit enhanced apoB48 clearance with concomitant increase in preheparin LPL mass, without changes in LPL activity. This combination might suggest a role for increased ligand action of LPL in LPLS447X carriers contributing to the cardiovascular protection in carriers of this mutation.


## 1. Introduction

Lipoprotein lipase (LPL) is a principal determinant in the metabolism of triglyceride (TG)-rich lipoproteins (TRLs). Although the enzymatic activity of LPL mediates hydrolysis of TGs from fasting (very-low-density lipoprotein) and postprandial TRLs, LPL also exhibits a ligand function that mediates hepatic clearance of TRLs [1]. Increased LPL activity in the circulation has been associated with a less atherogenic lipid profile [2]. Conversely, LPL located within the vessel wall gives rise to local release of proatherogenic substrates (free fatty acids and remnant particles) and may facilitate lipoprotein trapping within the subendothelial matrix [3], resulting in stimulated foam cell formation [4]. The presence of functional variants in the LPL protein has facilitated assessment of the role of LPL in the development of atherosclerotic vascular disease.

The lipoprotein lipase S447X (LPLS447X) variant, present in 18% to 22% of individuals in the general population, has been associated with increased postheparin LPL mass, whereas the LPL activity associated with this variant has created contrasting data [5,6]. The LPLS447X variant is associated with beneficial lipid profile changes, notably elevated high-density lipoprotein cholesterol (HDL-C) and lower TG levels, as well as a lower prevalence of cardiovascular disease (CVD) [7-10]. The mechanism for this cardiovascular protection is unknown. Enhanced "postprandial" lipoprotein clearance, as well as a direct effect on the arterial wall, has been put forward as a potential mechanism contributing to the antiatherogenic effects of LPLS447X [11,12]. In the present study, we evaluated the consequences of LPLS447X heterozygosity

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on LPL mass and activity, as well as on postprandial lipoprotein profile.

## 2. Methods

### 2.1. Participants

Fifteen male heterozygous LPLS447X carriers were selected from a database of the Research Lipid Clinic of the Academic Medical Center Amsterdam. The control group (n = 15), selected from the same database, was matched for sex, age, body mass index (BMI), smoking habits, and alcohol use. Subjects had no signs of overt CVD. All medications were stopped 2 weeks before the investigation. The study protocol was approved by the institutional review board of the Academic Medical Center, and all participants gave written informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki.

### 2.2. Genotyping

LPL and apolipoprotein E genotyping were performed as previously described [13,14].

### 2.3. Study design

All participants refrained from alcoholic beverages and smoking 24 hours before investigation. At the day of the study at 8:00 AM, fasting blood samples were drawn. Subsequently, all participants ingested an oral fat load ($t$ = 0), consisting of 30 g of fat per square meter of body surface area [15], administered as cream (35 g fat per 100 mL). Blood sampling was repeated at $t$ = 2, 3, 4, and 6 hours after fat load ingestion.

### 2.4. Biochemical measurements

Blood for lipid analysis was drawn in EDTA-coated tubes, and plasma was isolated by centrifugation at 3000 rpm at 4°C for 15 minutes and aliquoted for storage at −80°C. Baseline total cholesterol (TC) and TG levels were measured by standard enzymatic methods (CHOD-PAP and GPO-PAP, Roche Diagnostics, Mannheim, Germany). HDL-C was measured in the supernatant fraction after precipitation of apoprotein B-48 (apoB)–containing lipoproteins with dextran sulfate and magnesium chloride. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [16]. Baseline and postprandial TGs were measured using a commercially available kit (Triglyceride GPO-trinder, Sigma Diagnostics, Mannheim, Germany). Plasma apoB48 levels were measured by a sandwich enzyme-linked immunosorbent assay using antihuman apoB48 monoclonal antibodies as reported previously with minor modification [17]. Blood for preheparin and postheparin LPL concentration and activity measurements was collected in heparin-containing tubes before and 15 minutes after an intravenous injection of heparin (50 IU/kg body weight). LPL activity was analyzed as previously published [6]. LPL concentrations were measured using a commercially available kit (Markit-M LPL, Dainippon Pharmaceutical Co, Osaka, Japan).

### 2.5. Endothelial function

Endothelial function was assessed as flow-mediated dilation (FMD) of the brachial artery as described previously [18], at baseline and 2 and 6 hours after the fat load. Endothelium-independent vasodilation was assessed at baseline and 2 and 6 hours after the fat load, which was evoked by administering 0.3 mg nitroglycerin (NTG) sublingually.

### 2.6. Statistical analysis

Continuous (baseline) variables were compared between heterozygous LPLS447X carriers and control subjects using the Mann-Whitney $U$ test; $\chi^2$ test was applied for comparing distribution of dichotomous data. Longitudinal changes in TGs and apoB48 between the carriers and the controls were tested by analysis of repeated measures, using linear mixed models. In this model, first-order autoregression was used to specify the covariance structure for the residuals. The correlation coefficients were calculated using Spearman correlation. $P$ values of $<.05$ were considered statistically significant.

## 3. Results

### 3.1. Baseline characteristics

Baseline characteristics of heterozygous LPLS447X carriers and controls are shown in Table 1. Baseline TC, HDL-C, LDL-C, and TC were not significantly different between carriers and controls ($P$ = .94, .97, .65, and .31, respectively).

### 3.2. LPL mass and activity

In the carrier group, preheparin LPL mass was increased 2.4-fold compared with the matched controls (22.4 ± 8.3 vs 52.9 ± 20.8 ng/mL, $P < .0001$). In contrast, postheparin LPL mass (403.5 ± 97.2 vs 404.4 ± 155.3 ng/mL) and

Table 1
Baseline characteristics of controls and carriers

| | Controls (n = 15) | Carriers (n = 15) |
|---|---|---|
| Age | 50.1 ± 7.9 | 49.5 ± 8.3 |
| Smoking | 2 (13.3) | 2 (13.3) |
| BMI, kg/m$^2$ | 26.4 ± 3.5 | 25.4 ± 2.1 |
| SBP (mm Hg) | 127.7 ± 10.6 | 123.5 ± 7.9 |
| DPB (mm Hg) | 80.8 ± 5.7 | 78.4 ± 5.6 |
| Alcohol (U/wk) | 8.4 ± 7.1 | 10.2 ± 8.2 |
| TC (mmol/L) | 5.09 ± 0.69 | 5.06 ± 0.98 |
| HDL-C (mmol/L) | 1.21 ± 0.27 | 1.19 ± 0.31 |
| LDL-C (mmol/L) | 3.37 ± 0.58 | 3.38 ± 0.83 |
| TG (mmol/L) | 1.13 ± 0.35 | 1.07± 0.58 |

Carriers are heterozygous LPLS447X carriers. SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Data are presented as mean ± SD or as n (%).

activity (496.6 ± 126.5 vs 474.7 ± 102.9 mU/mL) were similar in controls and carriers ($P$ = .87 and .62, respectively).

### 3.3. Lipid metabolism after an oral fat load

In both groups, oral fat loading resulted in significant increases in mean TG levels ($P$ < .001), reaching maximum values 3 hours after the fat load (Fig. 1A). Although mean TG levels were not significantly different between the groups ($P$ = .93), peak TG levels tended to be lower in carriers (−14.8%, $P$ = .09). The area under the TG curve was 13% lower in carriers compared with matched controls, however, without significance ($P$ = .16). Because postprandial TGs are transported in apoB48-containing chylomicrons (CM), we subsequently focused on postprandial apoB48 levels. As expected, mean apoB48 levels increased significantly after fat loading in both groups ($P$ < .001; Fig. 1B). The mean increase in apoB48 levels was approximately 25% lower in carriers compared with controls ($P$ = .04). Notably, correction for baseline TG levels resulted in further increase of statistical significance ($P$ = .034). In addition, after the fat load, apoB48 reached its maximum in the carrier group 1 hour earlier compared with the controls. Six hours after the fat load, apoB48 levels returned toward baseline in both groups.

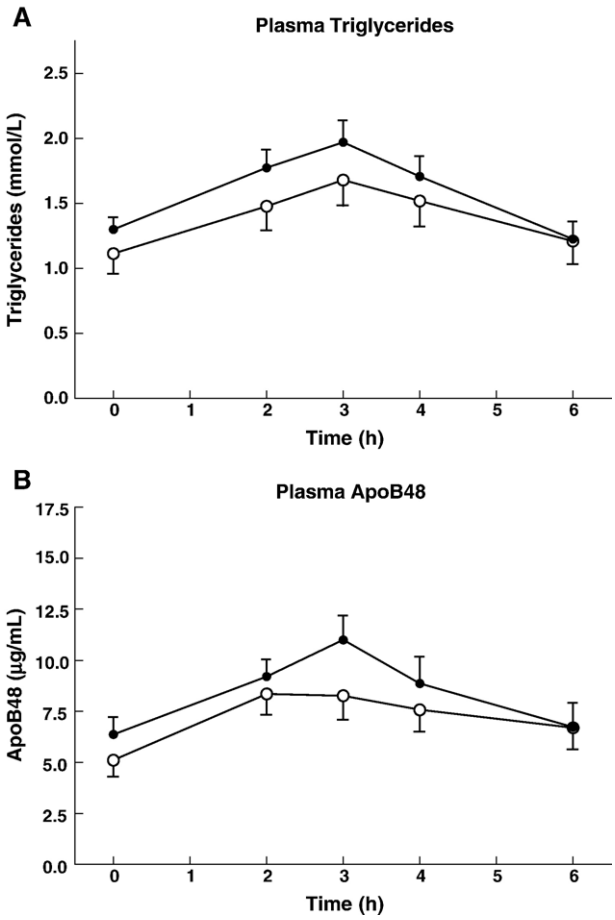


Fig. 1. A, Plasma TG levels. After fat loading, mean TG in both groups changed significantly over time ($P$ < .001). However, the response in both groups was similar ($P$ = .93). Peak TG level was found after 3 hours in both groups. B, Plasma apoB48 levels. ApoB48 increased significantly after fat loading ($P$ < .001) and significantly different over time ($P$ = .04). Baseline apoB48 levels were not statistically different in both groups ($P$ = .25). Carriers reached the apoB48 peak 1-hour before did controls, and their peak was 25% lower than that of controls. Open circles (○) represent the LPLS447X carriers, and closed circles (●) represent the matched controls. All data are presented as means ± SEM.

### 3.4. Endothelial function after an oral fat load

In both cohorts, fat loading induced a significant FMD impairment ($P$ < .001), reaching a maximum 2 hours after fat loading. The degree of impairment in FMD response was not significantly different between the 2 groups ($P$ = .57). At $t$ = 6 hours, FMD had returned toward baseline levels in both groups. Baseline NTG-induced vasodilation was not significantly different between the 2 groups ($P$ = .25). Fat loading had no effect on the NTG-induced vasodilation in any group ($P$ = ns).

## 4. Discussion

We show that heterozygous LPLS447X carriers exhibit an attenuated increase in apoB48 levels after ingestion of an oral fat load, whereas this enhanced clearance is not accompanied by aggravation of postprandial endothelial dysfunction. Concomitantly, carriers present with a significantly increased preheparin LPL mass. These data imply that enhanced apoB48 clearance in LPLS447X carriers may contribute to cardiovascular protection.

Because the major role of LPL is predominantly confined to the postprandial phase [19], we focused on postprandial lipid handling. Peak TG levels tended to be lower in carriers compared with controls, which fall in line with findings from the European Atherosclerosis Research Study [20]. Subsequently, we also assessed CM removal by measuring apoB48 levels. The peak apoB48 levels in carriers were 25% lower compared with controls, whereas peak levels were also reached earlier in carriers. These findings corroborate recent data showing that the LPLS447X variant was associated with enhanced postprandial clearance of TRLs [21].

To date, measurement of LPL in vivo has focused on postheparin values. More recently, the measurement of preheparin LPL data has been optimized. In our present investigation, we found a 2.4-fold increase in preheparin LPL mass in carriers. These data corroborate in vitro findings in which LPLS447X was shown to be associated with increased production of LPL monomers [5]. In vivo, LPL monomers are also present in the circulation [22], based on lower binding affinity of monomeric LPL to endothelial proteoglycans compared with dimeric LPL [23]. The LPLS447X carriers exhibited a 2-fold increase in preheparin LPL concentration compared with controls, whereas predominantly, preheparin LPL concentration has been shown to reflect monomeric LPL proteins [24]. The

finding of increased preheparin LPL mass in carriers corresponds to previously reported associations between increased preheparin LPL mass and HDL increase and TG decrease, which is a hallmark in LPLS447X carriers [22,25,26]. Interestingly, preheparin LPL mass has also been reported to be inversely correlated to CVD and its progression [27].

Inactive LPL has also been shown to mediate TRL removal from plasma in vivo [1,28]. With regard to the postprandial state, inactive LPL was shown to bind to CM in vitro [29], whereas binding of inactive LPL to CM was comparable to that of catalytically active LPL [29]. Consequently, in LPLS447X carriers, the increased pool of "enzymatically inactive" LPL might contribute to enhanced hepatic apoB48 clearance.

We have previously demonstrated that the site of enzymatic LPL activity determines its potential impact on vascular physiology. Although vessel wall LPL has predominantly proatherogenic consequences, circulating LPL appears to exert antiatherogenic effects [30]. Vessel wall LPL has been suggested to increase local exposure of proatherogenic substrates to the endothelial lining. In view of the predictive value of endothelial dysfunction for future CVD, increased clearance of TRL because of TRL entrapment in the vascular wall has been suggested to lead to enhanced endothelial dysfunction. Although we show clear attenuation of FMD upon fat loading in the present study, we do not find an aggravation of endothelial dysfunction in LPLS447X carriers in spite of the increased apoB48 removal rate. Hypothetically, these data may indicate increased removal of TRL particles through a mechanism other than TRL hydrolysis near the vascular endothelium in LPLS447X carriers.

### 4.1. Study limitations

Some aspects of our study deserve closer attention. The first aspect is that most studies have demonstrated that the LPLS447X variant is associated with an antiatherogenic lipid profile consisting of decreased TG levels and increased HDL-C levels [8]. The fact that we could not show significance in baseline TG levels has 2 explanations. Because our primary aim was to evaluate changes upon oral fat loading, we excluded subjects with obesity and carefully matched carriers and controls for sex, age, BMI, smoking habits, and alcohol use. Moreover, in view of the primary aim, we used a limited sample size. Reviewing the usually modest TG differences in carriers compared with controls, differences in TG levels in carriers of the frequent LPL variant have been reported in much larger numbers of carriers (n = 118-413) [31,32]. The second aspect is that, with regard to LPL activity, preheparin LPL activities were all below the detection limit. Although postheparin LPL activities were not different between carriers and controls, it should be noted that the latter reflects the activity of the total LPL pool, of which only a part is physiologically active [33]. Thus, based on data from the current study, we cannot exclude the effect of this frequent LPL variant on enzymatic activity of LPL.

### 4.2. Summary

We show attenuated increase in apoB48 levels upon fat loading in LPLS447X carriers. Concomitantly, carriers exhibited increased preheparin LPL mass. These findings of increased apoB48 clearance combined with increased circulating LPL mass might suggest a role for increased ligand action of LPL in LPLS447X carriers contributing to the lower prevalence of CVD. Additional kinetic studies are required to confirm these findings in LPLS447X carriers.

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