Serum Lipoprotein Lipase Concentration and Risk for Future Coronary Artery Disease
The EPIC-Norfolk Prospective Population Study

Jaap Rip, Melchior C. Nierman, Nicholas J. Wareham, Robert Luben, Sheila A. Bingham, Nicholas E. Day, Joram N.I. van Miert, Barbara A. Hutten, John J.P. Kastelein, Jan Albert Kuivenhoven, Kay-Tee Khaw, S. Matthijs Boekholdt

Background—Lipoprotein lipase (LPL) is associated with coronary artery disease (CAD) risk, but prospective population data are lacking. This is mainly because of the need for cumbersome heparin injections, which are necessary for LPL measurements. Recent retrospective studies, however, indicate that LPL concentration can be reliably measured in serum that enabled evaluation of the prospective association between LPL and future CAD.

Methods and Results—LPL concentration was determined in serum samples of men and women in the EPIC-Norfolk population cohort who developed fatal or nonfatal CAD during 7 years of follow-up. For each case (n/H11005/H11006), 2 controls, matched for age, sex, and enrollment time, were identified. Serum LPL concentration was lower in cases compared with controls (median and interquartile range: 61 [43–85] versus 66 [46–92] ng/mL; \( P < 0.0001 \)). Those in the highest LPL concentration quartile had a 34% lower risk for future CAD compared with those in the lowest quartile (odds ratio [OR] 0.66; confidence interval [CI], 0.53 to 0.83; \( P < 0.0001 \)). This effect remained significant after adjustment for blood pressure, diabetes, smoking, body mass index, and low-density lipoprotein (LDL) cholesterol (OR, 0.77; CI, 0.60–0.99; \( P = 0.02 \)). As expected from LPL biology, additional adjustments for either high-density lipoprotein cholesterol (HDL-C) or triglyceride (TG) levels rendered loss of statistical significance. Of interest, serum LPL concentration was positively linear correlated with HDL and LDL size.

Conclusions—Reduced levels of serum LPL are associated with an increased risk for future CAD. The data suggest that high LPL concentrations may be atheroprotective through decreasing TG levels and increasing HDL-C levels. (Arterioscler Thromb Vasc Biol. 2006;26:637-642.)

Key Words: preheparin lipoprotein lipase cardiovascular diseases lipoproteins lipids cholesterol

Lipoprotein lipase (LPL) hydrolyzes plasma triglycerides (TG) that are packaged in chylomicrons and very-low-density lipoproteins (VLDLs). This catalytic activity results in the formation of cholesterol-rich lipoprotein remnants and generates constituents for the anti-atherogenic high-density lipoprotein (HDL) pool.\(^1\) LPL also enhances the hepatic clearance of atherogenic lipoprotein remnants via the low-density lipoprotein (LDL) receptor.\(^2\) The crucial role of LPL in lipid metabolism is illustrated by genetic LPL deficiency, a rare disorder characterized by severe hypertriglyceridemia and low HDL cholesterol (HDL-C) levels.\(^3\)

Over the past 25 years, the relation between LPL and coronary artery disease (CAD) has been addressed using various approaches. Patients with genetic LPL deficiency have been studied in detail, and although there are indications that these patients have premature atherosclerosis,\(^4\) there are also reports indicating that a complete lack of LPL, and thus a lack of formation of atherogenic lipoprotein remnants, does not underlie increased CAD risk.\(^5\) In the families in which LPL-deficient probands were identified, the heterozygotes were shown to be at increased risk for atherosclerosis.\(^6\) Nevertheless, reliable data on CAD risk are unavailable because only small groups of affected individuals were studied. The use of (multiple) variants at the LPL gene locus has provided more insight. In fact, numerous genetic association studies, and studies on frequent functional variants such as LPL D9N and N291S, have shown that loss of LPL function is associated with CAD.\(^7\)\(^-\)\(^10\) In addition, the majority of animal studies clearly indicate that LPL protects against (diet-induced) atherosclerosis.\(^11\)\(^,\)\(^12\) Biochemical assessment of...
LPL function has also frequently been used to assess the role of LPL in atherogenesis. However, these studies are hampered by the need to administer heparin intravenously to release sufficient LPL from the endothelium to measure reliably LPL activity and LPL concentration. Because heparinization is time-consuming, not standardized, and induces bleeding risk, most investigators have only studied limited numbers of diseased and/or nondiseased individuals. The bulk of these studies have indicated that (post-heparin) LPL activity is decreased in hypertriglyceridemic subjects and other patients at increased risk for CAD.\textsuperscript{13–19} Recently, the availability of a highly sensitive enzyme-linked immunosorbent assay, which can measure accurately freely circulating LPL concentration in nonheparinized serum has provided a tool to more easily assess the relationship between LPL and CAD. Olivecrona and colleagues were the first who studied how preheparin LPL (from now on referred to as serum LPL) relates to plasma lipoproteins and post-heparin LPL.\textsuperscript{20} It was recognized that the majority of serum LPL is catalytically inactive\textsuperscript{21} and likely represents a mere catabolic product of catalytically active LPL that is bound to the endothelium. Also, it was demonstrated that serum LPL concentration is not associated with post-heparin LPL concentration or LPL activity. This was not unexpected because LPL levels are controlled by many factors, including differential transcriptional regulation in adipose and skeletal muscle tissue, post-translational modification and translocation over the endothelium, retro-endocytosis, binding to heparan sulfate-containing proteoglycans, lipoproteins, and receptors, and hepatic clearance.\textsuperscript{22–25} Despite this, serum LPL concentration was strongly positively related with HDL-C and negatively with VLDL-TG, although the latter relation was weak. Japanese investigators, using a commercially available LPL ELISA, have recently confirmed that serum LPL is not associated with post-heparin LPL concentration and LPL activity.\textsuperscript{26,27} but at the same time their data suggest that serum LPL concentration reflects whole-body LPL production or the systemic potential to hydrolyze plasma TG. In agreement with the studies of Tornval and Vilella, they showed that serum LPL is strongly correlated with HDL-C and inversely related to plasma levels of TG, whereas no correlations with total cholesterol and low-density lipoprotein cholesterol (LDL-C) were found.\textsuperscript{27,28} Two cross-sectional analyses have shown that men with acute myocardial infarction have lower serum LPL concentration compared with healthy controls.\textsuperscript{29} In addition, serum LPL is reported to be inversely related with the extent of coronary atherosclerosis.\textsuperscript{30} However, prospective data in humans showing that LPL is atheroprotective are lacking.

Based on these data, we hypothesized that in apparently healthy individuals, low concentrations of serum LPL are associated with an increased risk for future CAD. We tested this hypothesis in a large prospective nested case-control study.

**Methods**

We performed a nested case-control study among participants in the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort study, a population of 25,663 men and women between ages 45 and 79. EPIC-Norfolk is part of the 10-country collaborative EPIC study designed to investigate determinants of cancer.\textsuperscript{31} From the outset, additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases. Recruitment of participants was performed by mail from age–sex registers of general practices. The recruitment rate was relatively low as addressed by Day et al in one of the first study reports.\textsuperscript{32} At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire, which included questions about cigarette smoking habit and past medical history, and attended a clinic visit where additional data collection was undertaken by trained nurses using standardized protocols as previously described.\textsuperscript{33} This included anthropometry, blood pressure measurements, and a nonfasting blood sample. Body mass index (BMI) was estimated as weight in kg divided by height in meters squared. All individuals have been flagged for mortality at the UK Office of National Statistics, with vital status ascertained for the entire cohort. Death certificates for all decedents were coded by trained nosologists according to the International Classification of Diseases (ICD)\textsuperscript{9th} revision. Death was considered caused by CAD if the underlying cause was coded as ICD 410 to 414. In addition, participants admitted to hospital were identified using their unique National Health Service number by data linkage with ENCORE (East Norfolk Health Authority database), which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified having CAD during follow-up if they had a hospital admission and/or died with CAD as underlying cause. We report results with follow-up to January 2003, an average of 6–7 years. The study was approved by the Norwich District Health Authority Ethics Committee and all participants gave signed informed consent.

**Participants**

We have previously described similar designed nested case-control studies of the EPIC-Norfolk cohort.\textsuperscript{32–34} All individuals who reported a history of heart attack or stroke at the baseline clinic visit were excluded. Cases were individuals in whom a fatal or nonfatal CAD developed during follow-up until November 2003. Controls were study participants who remained free of any cardiovascular disease during follow-up. We matched 2 controls to each case by sex, age (within 5 years), and time of enrollment (within 3 months).

**Biochemical Analysis**

Levels of total cholesterol, HDL-C, and TG in nonfasted serum samples were measured with the RA 100 (Bayer Diagnostics, Basingstoke, UK), and LDL-C levels were calculated using the Friedewald formula.\textsuperscript{35} LDL size and HDL size were assessed by proton nuclear magnetic resonance spectroscopy as described previously.\textsuperscript{36} Serum LPL concentrations were measured using a commercially available sandwich enzyme-linked immunosorbent assay (Dainippon Pharmaceutical Co, Ltd, Japan). Pooled plasma from healthy volunteers (n = 200) was used as a control in each individual LPL assay and the interassay variance was found 8.2%. Samples were analyzed in random order to avoid systemic bias. Researchers and laboratory personnel were blinded to identifiable information, and could identify samples by number only.

**Statistical Analysis**

Baseline characteristics were compared between cases and controls using a mixed effect model for continuous variables or conditional logistic regression for categorical variables, which takes into account the matching for sex, age, and enrollment time. Because TG and serum LPL levels had a skewed distribution, values were log-transformed before being used in the statistical analyses as continuous variables. In the Tables, we show untransformed medians and corresponding interquartile ranges. Serum LPL levels were categorized in quartiles based on the distribution in the controls. Mean levels of traditional cardiovascular risk factors were calculated per LPL quartile. Linear associations between LPL quartiles and traditional risk factors were calculated using linear regression for contin-
TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 1980)</th>
<th>Cases (n = 1006)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>63 (1243)</td>
<td>63 (637)</td>
<td>Matched</td>
</tr>
<tr>
<td>Age, y</td>
<td>65.1 ± 7.8</td>
<td>65.2 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>Diabetes % (n)</td>
<td>1.9 (37)</td>
<td>6.7 (67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>8 (164)</td>
<td>16 (155)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>51 (1006)</td>
<td>52 (514)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>40 (788)</td>
<td>33 (327)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4 ± 3.5</td>
<td>27.3 ± 3.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139.1 ± 17.9</td>
<td>143.9 ± 18.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83.7 ± 11.1</td>
<td>85.8 ± 11.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.3 ± 1.2</td>
<td>6.5 ± 1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.1 ± 1.0</td>
<td>4.3 ± 1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.37 ± 0.40</td>
<td>1.27 ± 0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7 (1.2–2.3)</td>
<td>1.9 (1.4–2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum LPL concentration, ng/mL</td>
<td>66 (46–92)</td>
<td>61 (43–85)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD, percentage (n), or median (interquartile range).

BMI indicates body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LPL, lipoprotein lipase.

TABLE 2. Distribution of CAD Risk Factors by Serum LPL Quartiles

<table>
<thead>
<tr>
<th>LPL Quartile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P*</th>
<th>R</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL range, ng/mL</td>
<td>&lt;46</td>
<td>47–65</td>
<td>66–91</td>
<td>&gt;92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case/control</td>
<td>315/495</td>
<td>259/495</td>
<td>215/495</td>
<td>217/495</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>13 (98)</td>
<td>11 (88)</td>
<td>9 (70)</td>
<td>11 (84)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous</td>
<td>56 (439)</td>
<td>49 (388)</td>
<td>52 (403)</td>
<td>49 (387)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>32 (249)</td>
<td>39 (309)</td>
<td>40 (310)</td>
<td>40 (316)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>27.4 ± 3.5</td>
<td>26.7 ± 3.5</td>
<td>26.3 ± 3.6</td>
<td>26.1 ± 3.9</td>
<td>&lt;0.0001</td>
<td>−0.141</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6.7(54)</td>
<td>3.0 (24)</td>
<td>2.6 (21)</td>
<td>1.6 (13)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.3 ± 1.1</td>
<td>6.3 ± 1.2</td>
<td>6.4 ± 1.3</td>
<td>6.4 ± 1.2</td>
<td>0.945</td>
<td>0.058</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.1 ± 1.0</td>
<td>4.1 ± 1.0</td>
<td>4.2 ± 1.1</td>
<td>4.2 ± 1.1</td>
<td>0.441</td>
<td>0.034</td>
<td>0.072</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.17 ± 0.32</td>
<td>1.27 ± 0.35</td>
<td>1.40 ± 0.37</td>
<td>1.53 ± 0.43</td>
<td>&lt;0.0001</td>
<td>0.349</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.4 ± 1.3</td>
<td>2.1 ± 1.1</td>
<td>1.8 ± 1.3</td>
<td>1.6 ± 0.8</td>
<td>&lt;0.0001</td>
<td>−0.246</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL size, nm</td>
<td>20.7 ± 0.6</td>
<td>20.9 ± 0.6</td>
<td>21.1 ± 0.6</td>
<td>21.2 ± 0.5</td>
<td>&lt;0.0001</td>
<td>0.301</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL size, nm</td>
<td>8.7 ± 0.4</td>
<td>8.8 ± 0.4</td>
<td>9.0 ± 0.5</td>
<td>9.1 ± 0.5</td>
<td>&lt;0.0001</td>
<td>0.331</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* indicates linearity between serum LPL quartiles and risk factor levels. R indicates Pearson’s correlation between log-transformed serum LPL levels and risk factor levels. † indicates the corresponding P value.
CI, 0.60 to 0.99; model 2) for the comparison of extreme quartiles (P for linearity = 0.02). Additional adjustment for either TG (model 3) or HDL-C (model 4) levels, 2 parameters that are intrinsically correlated with LPL, rendered loss of statistical significance (P for linearity 0.17 and 0.16, respectively). This suggest that LPL mediates is protective effects through these parameters. This is in accordance with LPL biology in that LPL is the sole enzyme responsible for the clearance of plasma triglycerides and also provides constituents that contribute the pool of HDL.

**Discussion**

**Lipoprotein Lipase and Coronary Artery Disease**

This prospective study shows that levels of serum LPL are inversely related to future CAD in apparently healthy men and women. In agreement with previous studies, we observed that this parameter is strongly associated with LPL, rendering loss of statistical significance (P for linearity 0.17 and 0.16, respectively). This suggest that LPL mediates is protective effects through these parameters. This is in accordance with LPL biology in that LPL is the sole enzyme responsible for the clearance of plasma triglycerides and also provides constituents that contribute the pool of HDL.

**Lipoprotein Lipase Biology**

In trying to understand how low levels of LPL in the circulation are associated with increased cardiovascular risk, we refer to the idea of Tornval et al that this parameter may represent a catabolic product of biologically active LPL. There are several lines of evidence in support of the hypothesis that this parameter somehow reflects total LPL body production. First, peroxisome proliferated activated receptor alpha and gamma agonists that are known to increase LPL gene expression increase serum LPL concentration. Second, insulin concentrations that control LPL gene expression levels also affect serum LPL concentration. A recent study furthermore shows that variation at the LPL gene locus also affects serum LPL concentration. Specifically, it was reported that carriers of a common LPL gene variant (LPLS447X) have increased levels of serum LPL, whereas others have shown that this mutation protects against CAD. Thus, serum LPL concentration may be a marker for the amount of systemically available (catalytically) active LPL, when taken into notice that LPL is the sole lipolytic enzyme that is responsible for the breakdown of plasma triglycerides. Serum LPL mass may, however, also have a direct atheroprotective role in mediating the clearance of atherogenic lipoproteins remnants. These assumptions need confirmation in mechanistic studies into triglyceride catabolism.

**Considerations**

Several aspects of the current study warrant attention. First, CAD events were ascertained through death certification and hospital admission data, which are likely to lead both to under ascertainment and to misclassification of cases. However, previous validation studies in our cohort indicate high specificity of such case ascertainment. Second, the recruitment rate for the EPIC Norfolk study was relatively low, but the study population is representative of the general British population for all classical risk factors except for a low smoking rate. Third, serum levels of LPL and other lipid-related variables were determined in a single nonfasting sample that was obtained at a nonuniform time of the day. Diurnal variation, variation over time, and differences in the time span because the last meal could have affected these variables. The latter is especially true for TG levels. We underline, however, that in the Western World, people live...
under constant postprandial conditions. Therefore, studies into the associations between lipids, lipoproteins, and CAD risk are, in our opinion, best performed under nonfasting conditions. Random measurement error in both case ascertainment and time variations would lead to an underestimation of any relationships between risk factors and CAD risk. The extent of measurement error, however, is unlikely to differ from those for other risk factors or from other prospective studies.

Conclusions
We show that apparently healthy men and women with reduced levels of serum LPL have an increased risk for future CAD. The data suggest that high LPL concentrations may be atheroprotective through associations with decreased TG levels and increased HDL-C levels.

Acknowledgments
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References
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