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/H110051006), 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. Olivecrona and colleagues were the first who studied 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. It was recognized that the majority of serum LPL is catalytically inactive and likely represents a mere catabolic product of post-translational modification and translocation over the endothelium, retro-endocytosis, binding to heparan sulfate-containing proteoglycans, lipoproteins, and receptors, and hepatic clearance. Despite this, serum LPL concentration was strongly positively related with HDL-C and negatively with VLDL-TG, although the latter relation was weak. 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. We have previously described similar designed nested case-control studies of the EPIC-Norfolk cohort. 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. LDL size and HDL size were assessed by proton nuclear magnetic resonance spectroscopy as described previously. 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 continu-
Results

Baseline Characteristics
We identified 1006 individuals in whom CAD developed during a, on average, 6-year follow-up. A total of 974 cases could each be matched to 2 controls. For the remaining 32 cases, we could identify only 1 control per case. Thus, the control group consisted of 1980 people. At baseline, cases were more likely to have diabetes and be smokers compared with controls (Table 1). Furthermore, BMI, systolic blood pressure, diastolic blood pressure, and plasma levels of total cholesterol (TC), LDL-C, and TG were significantly higher in cases compared with controls. In contrast, HDL-C levels were significantly lower in cases compared with controls. Serum LPL concentration in serum was significantly lower in cases compared with controls: 61 (43–85) ng/mL versus 66 (46–92) ng/mL (P<0.0001).

Serum LPL and Other CAD Risk Factors
Linear negative associations with serum LPL concentration quartiles were observed for BMI and the number of subjects with diabetes and TG (P for linearity was <0.0001; Table 2). For HDL-C, HDL size, and LDL size, we identified a linear positive association with serum LPL quartiles (P for linearity <0.0001 for all 3 parameters). Similar significant linear associations between serum LPL quartiles and LDL-C or TC were not observed.

Serum LPL Relation to CAD
The risk of future CAD decreased with increasing LPL quartiles such that people in the highest quartile had an OR of 0.66 (95% CI, 0.53 to 0.83), compared with those in the lowest quartile (P for linearity <0.0001; model 1, Table 3). Looking for pathways through which LPL might offer the observed protection, we used multivariate analyses. After adjustment for systolic blood pressure, diabetes, BMI, LDL-C, and smoking, a significant association between LPL quartiles and risk for CAD remained present (OR, 0.77; 95%
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 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 diabetes, HDL-C, and TG, but not with TC and LDL-C levels. To obtain insight in the pathways through which LPL concentration can offer atheroprotection, we performed multivariate analyses. Corrections for systolic blood pressure, diabetes, smoking, BMI, and LDL-C levels did not strongly affect the relationship between serum LPL concentration and CAD. Further correction of HDL-C and TG levels, however, rendered loss of statistical significance indicating that the relationship of serum LPL concentration with CAD is largely explained by these factors. This result agrees with the fact that LPL is at the start of a (catalytic) cascade that culminates in the breakdown of plasma triglycerides thereby releasing apolipoproteins, phospholipids and other constituents from chylomicrons and VLDL to the HDL pool. Because others recently provided evidence for a positive association between serum LPL concentration and LDL size, an important player in atherogenesis, we examined whether LDL size as measured by nuclear magnetic resonance differed between individuals in the 4 concentration quartiles. The data confirmed a strong positive correlation between serum LPL concentration and LDL size. In addition, we also found a positive correlation with HDL size, a parameter that is associated with decreased CAD risk. The latter observations support the association of a high serum LPL concentration with a more beneficial lipid profile.

**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 lipoprotein 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

EPIC-Norfolk is supported by program grants from the Medical Research Council UK and Cancer Research UK, and with additional support from the European Union, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and the Wellcome Trust. Parts of the lipid measurements described in this article were funded by an educational grant from the Future Forum, The Hague, The Netherlands. We thank the participants, general practitioners, and staff in EPIC-Norfolk.

References

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 and S. Matthijs Boekholdt

*Arterioscler Thromb Vasc Biol*. 2006;26:637-642; originally published online December 22, 2005;
doi: 10.1161/01.ATV.0000201038.47949.56

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/3/637

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints:** Information about reprints can be found online at:
http://www.lww.com/reprints

**Subscriptions:** Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/