Lipoprotein(a) and Risk of Coronary, Cerebrovascular, and Peripheral Artery Disease
The EPIC-Norfolk Prospective Population Study
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Objective—Although the association between circulating levels of lipoprotein(a) [Lp(a)] and risk of coronary artery disease (CAD) and stroke is well established, its role in risk of peripheral arterial disease (PAD) remains unclear. Here, we examine the association between Lp(a) levels and PAD in a large prospective cohort. To contextualize these findings, we also examined the association between Lp(a) levels and risk of stroke and CAD and studied the role of low-density lipoprotein as an effect modifier of Lp(a)-associated cardiovascular risk.

Methods and Results—Lp(a) levels were measured in apparently healthy participants in the European Prospective Investigation of Cancer (EPIC)-Norfolk cohort. Cox regression was used to quantify the association between Lp(a) levels and risk of PAD, stroke, and CAD outcomes. During 212,981 person-years at risk, a total of 2365 CAD, 284 ischemic stroke, and 596 PAD events occurred in 18,720 participants. Lp(a) was associated with PAD and CAD outcomes but not with ischemic stroke (hazard ratio per 2.7-fold increase in Lp(a) of 1.37, 95% CI 1.25–1.50, 1.13, 95% CI 1.04–1.22 and 0.91, 95% CI 0.79–1.03, respectively). Low-density lipoprotein cholesterol levels did not modify these associations.

Conclusion—Lp(a) levels were associated with future PAD and CAD events. The association between Lp(a) and cardiovascular disease was not modified by low-density lipoprotein cholesterol levels. (Arterioscler Thromb Vasc Biol. 2012;32:3058-3065.)

Key Words: atherosclerosis ■ lipoproteins ■ peripheral arterial disease ■ stroke ■ vascular biology

Lipoprotein(a) [Lp(a)] is a liver-synthesized lipoprotein that is composed of an apolipoprotein B100 molecule covalently bound to the glycoprotein apolipoprotein(a). Circulating levels of Lp(a) are associated with an increased risk of future coronary artery disease (CAD) and stroke.1 The mechanisms underlying these associations have not been fully elucidated, but Lp(a) is considered to play an active role in the process of vascular inflammation and atherothrombosis.2-4 Interestingly, Lp(a) levels are highly heritable and common variations in LPA, the gene encoding apolipoprotein(a), have been found to be associated with CAD risk,5-7 suggesting that Lp(a) may not only be a risk marker but also have a causal role in atherogenesis.

Peripheral arterial disease (PAD) commonly results from progressive narrowing of arteries in the lower extremities attributable to atherosclerosis. PAD affects 4% to 14%5-10 of the general population and its prevalence increases with age, affecting up to 15% to 30% of adults over the age of 70.10,11 It is commonly associated with functional impairment and can ultimately result in the need for vascular surgery or lower limb amputation. In addition, coexistent CAD and cerebrovascular disease are highly prevalent in patients with PAD, and the 1-year risk of cardiovascular events was 21% in a prospective cohort study.12 Aggressive medical treatment of atherosclerotic risk factors has been shown to significantly decrease morbidity and mortality associated with PAD.13 In this context, it is essential to identify factors contributing to the risk of PAD. Despite strong evidence for associations between Lp(a) and cardiovascular disease, the association between Lp(a) and PAD remains uncertain. Although some cross-sectional studies have suggested a positive association between Lp(a) levels and PAD risk,14-17 evidence from large-scale prospective studies is limited. Lp(a) levels were associated with incident PAD in the Edinburgh Artery Study18 and with PAD progression in individuals presenting for noninvasive lower limb arterial assessment.19 However, a large
prospective study of healthy women found only a marginally significant association.20 Another large-scale prospective study showed no association between Lp(a) levels and PAD.21 We therefore assessed the association between Lp(a) levels and future risk of PAD. To contextualize these findings, we examined associations between Lp(a) levels and risk of stroke and CAD. As some studies suggest that the association between Lp(a) and CAD may be modified by low-density lipoprotein cholesterol (LDL-C) levels,22–24 we also assessed the role of LDL-C in these associations.

Materials and Methods

Study Design

The European Prospective Investigation of Cancer (EPIC)-Norfolk is a prospective population study of 25,639 male and female inhabitants of Norfolk, United Kingdom, aged between 39 and 79 years. The design, recruitment, and characteristics of the EPIC-Norfolk study have been published previously.25 Briefly, EPIC-Norfolk is part of the 10-country collaborative EPIC study designed to investigate determinants of cancer. Additional data were obtained to enable assessment of determinants of other diseases such as CAD. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire with additional data collection performed by trained nurses at a clinic visit. The study cohort was similar to UK population samples in the Health Survey of England with regard to many characteristics including age, sex, anthropometry, blood pressure, and lipids, but with a lower proportion of smokers.

All individuals have been flagged for mortality at the UK Office of National Statistics, with vital status ascertained for the entire cohort. The death certificates were coded by trained nosologists according to the International Classification of Diseases 10th revision (ICD-10). In addition, hospitalized participants were identified by using their unique National Health Service number through data linkage with the East Norfolk Health Authority (ENCORE) database, which identifies all hospital contacts throughout England and Wales for residents of Norfolk. Participants were identified as having been hospitalized or having died because of a cardiovascular event if the corresponding ICD-10 code was recorded as the underlying cause of that hospitalization or mortality. For the purposes of this analysis, 3 separate outcome measures were considered: PAD events, stroke, and CAD-related events. For PAD, mortality and hospitalization coded as ICD-10 I70 to I73 were considered as events. CAD events were defined by ICD-10 codes I20 to I25. Stroke outcomes were subdivided into ischemic (I63), hemorrhagic (I60, I61, I62), and unclassified stroke (I64). Follow-up was censored on March 31st 2008, which was the date up to which complete data on mortality and hospitalizations were available. The study complies with the Declaration of Helsinki. The Norwich District Health Authority Ethics Committee approved the study and all participants gave written informed consent.

Laboratory Measurements

Nonfasting blood samples were collected from participants at baseline. Samples were stored frozen at −80°C for approximately 15 years before measurement. Lp(a) levels were measured with an immunoturbidimetric assay using polyclonal antibodies directed against epitopes in apolipoprotein(a) (Denka Seiken, Coventry, United Kingdom). This assay has been shown to be apolipoprotein(a) isoform independent. Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations were measured with the RA 1000 (Bayer Diagnostics, Basingstoke, United Kingdom) and apolipoprotein A-I and apolipoprotein B were measured by rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards.26 LDL-C levels were calculated using the Friedewald formula. C-reactive protein (CRP) levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and biotinylated monoclonal antibodies (CLB anti-CRP-2) as the detecting antibody. Results were related to a standard consisting of commercially available CRP (Behringwerke AG, Marburg, Germany). The lower detection limit was 0.1 mg/L.

Statistical Analysis

To examine Lp(a) as a continuous variable, Lp(a) was logarithmically transformed because the relationship between Lp(a) and CAD has been described to be log-linear in previous studies.1 Log-transformed Lp(a) was noted to be normally distributed with an SD of 0.99. Therefore, the results presented for 1 SD increase in Ln[Lp(a)] must be interpreted as equivalent to a 2.7-fold (e0.99) increase in Lp(a) levels. It has been recognized that adjustment for total cholesterol may attenuate the association between Lp(a) and CAD because total cholesterol includes cholesterol contained in the Lp(a) particle which may mediate some of the effects of Lp(a). To account for this, we used adjusted cholesterol = total cholesterol − Lp(a) cholesterol, where Lp(a) cholesterol (in mg/dL) = 0.15×Ln[Lp(a)] (in mg/dL)+1.24 as previously described.1,27

Sex-specific quartiles of Lp(a) were generated because the distribution of Ln[Lp(a)] was found to significantly vary between men and women. Baseline characteristics of study participants were compared within sex-specific quartiles of Lp(a) using ANOVA for comparison of continuous variables and χ2 test on 1 df for categorical variables. Triglycerides and CRP levels were logarithmically transformed to normalize data before the analysis.

Cox regression modeling was applied to assess the association between Lp(a) and outcome measures of PAD, stroke, and CAD. These associations were expressed using hazard ratios and corresponding 95% CI by sex-specific quartiles (using the lowest quartile as reference) and by standardized Ln[Lp(a)]. Statistical significance of associations was assessed by tests for trend, likelihood ratio tests between a model including and excluding sex-specific Lp(a) quartiles as ordered variables. The P value for this was derived on a χ2 distribution on 1 df. Nonlinearity was measured as the likelihood ratio tests between models including Lp(a) quartiles as linear variables and indicator variables. The P value for nonlinearity was derived from a χ2 distribution on 2 df. Each outcome was censored as the first occurring event of either hospitalization or mortality. Follow-up time was calculated up to either the first event or the last date of follow-up or the date of death. Hazard ratio estimates in the Cox model were adjusted according to 3 models. Model 1 adjusted for sex and age. Model 2 adjusted for age, sex, body mass index, total cholesterol adjusted for Lp(a) levels, HDL-C, and triglycerides. Model 3 adjusted for the variables in model 2 and in addition smoking, diabetes mellitus, baseline antihypertensive treatment, baseline lipid-lowering therapy, alcohol consumption, physical activity level, serum creatinine levels, fibrinogen levels, apolipoprotein A-I, apolipoprotein B, CRP levels, history of myocardial infarction at baseline, history of stroke at baseline, family history of myocardial infarction, family history of stroke, postmenopausal status, and use of hormone replacement therapy. Testing for statistical interaction was carried out in 2 groups of LDL-C split by median LDL-C (≤3.9 mmol/L vs >3.9 mmol/L). Likelihood ratio testing was carried out between models including and excluding an interaction term between Ln[Lp(a)] and the binary LDL-C variable, and results were assessed on a χ2 distribution with 1 df. In additional exploratory analyses, we also assessed interactions between Lp(a) and age, sex, sex, baseline diabetes mellitus, and serum creatinine levels in relation to risk of CAD. For assessment of statistical interaction, age and serum creatinine were included in the model as continuous variables, and sex and baseline diabetes mellitus were included as binary categorical variables.

The assumption of proportionality of hazard over time for the Cox regression was tested using the Schoenfeld residual and no violations were found. The assumption of linearity was tested using restricted cubic splines with the number of knots set to 3 and no nonlinearity was observed. Cox regression models were fitted as time varying covariates in the model. The significance of addition
of a time varying covariate for these exposure variables was assessed by a likelihood ratio test between models including and excluding the time varying covariate. All analyses were carried out in Stata version 11. \( P < 0.05 \) was considered significant.

**Results**

Lp(a) levels were available for 18,720 participants. The mean age at entry of participants was 59 years (SD 9), and 55% of participants were women. During 212,981 person-years at risk, a total of 2,365 CAD events, 653 strokes (284 ischemic, 122 hemorrhagic, and 247 unclassified), and 596 PAD events were reported. Baseline characteristics of the cohort are represented in Table 1. Baseline characteristics were compared between sex-specific quartiles of Lp(a) because the distribution of \( \ln[Lp(a)] \) was found to vary significantly between men and women (Table 1), with women more likely to have higher Lp(a) levels (\( P < 0.0001 \)). Age, body mass index, total cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein A-I, apolipoprotein B, systolic blood pressure, CRP, and creatinine levels were associated with Lp(a) levels, as were postmenopausal status and hormone replacement therapy use (Table 2).

### Association Between Lp(a) Levels and Cardiovascular Outcomes

#### Association With PAD

Lp(a) was associated with PAD-related hospitalization and mortality; the fully adjusted hazard ratio for PAD for a 2.7-fold increase in Lp(a) (equating to a 1 SD increase in \( \ln[Lp(a)] \)) was 1.37 (95% CI 1.25–1.50; Table 3, Model 3). Adjustment for known confounders did not attenuate the magnitude of the association observed. The magnitude of the fully adjusted association observed remained the same even when total cholesterol or LDL-C unadjusted for the Lp(a) component was included as a covariate in Model 3. This association was further examined by plotting the adjusted hazard ratios for PAD for sex-specific quartiles of Lp(a) (Figure 1). The association appeared curvilinear, and the test for nonlinearity was statistically significant (\( P = 0.0002 \); Table 3). There was no statistical interaction between Lp(a) and LDL-C with regard to risk of PAD (\( P = 0.60 \)). The association between Lp(a) and PAD did not show statistically significant deviation from proportionality of hazards over time (\( P = 0.07 \)).

#### Table 1. Baseline Characteristics in the EPIC-Norfolk Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>18,720</td>
<td>8,381</td>
<td>10,339</td>
<td></td>
</tr>
<tr>
<td>Mean Lp(a) level, mg/dL</td>
<td>21.1(23.6)</td>
<td>20.4(22.5)</td>
<td>21.8(24.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lp(a) range, mg/dL</td>
<td>0.1–175.0</td>
<td>0.1–166.4</td>
<td>0.5–175.0</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>59.2(9.2)</td>
<td>59.5(9.1)</td>
<td>58.9(9.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2(3.8)</td>
<td>26.4(3.2)</td>
<td>26.1(4.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>406(2.2)</td>
<td>261(3.1)</td>
<td>145(1.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2,100(11.3)</td>
<td>989(11.9)</td>
<td>1,111(10.9)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>7,797(42.0)</td>
<td>4,525(54.4)</td>
<td>3,272(32.0)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>8,668(46.7)</td>
<td>2,810(33.8)</td>
<td>5,858(57.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.2(1.2)</td>
<td>6.0(1.1)</td>
<td>6.3(1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.0(1.0)</td>
<td>3.9(1.0)</td>
<td>4.0(1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.42(0.43)</td>
<td>1.24(0.33)</td>
<td>1.57(0.44)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.80(1.08)</td>
<td>2.03(1.21)</td>
<td>1.61(0.93)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apolipoprotein A-I, g/L</td>
<td>1.55(0.33)</td>
<td>1.43(0.28)</td>
<td>1.64(0.31)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apolipoprotein B, g/L</td>
<td>0.97(0.24)</td>
<td>0.97(0.23)</td>
<td>0.98(0.26)</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>135(18)</td>
<td>137(17)</td>
<td>133(19)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82(11)</td>
<td>84(11)</td>
<td>81(11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>3.1(6.3)</td>
<td>3.0(5.9)</td>
<td>3.1(6.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>Creatinine, ( \mu )mol/L</td>
<td>86.8(21.3)</td>
<td>94.9(19.9)</td>
<td>80.3(20.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>599(3.2)</td>
<td>474(5.7)</td>
<td>125(1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of stroke</td>
<td>262(1.4)</td>
<td>154(1.8)</td>
<td>108(1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Postmenopausal status</td>
<td>8,056(43)</td>
<td>NA</td>
<td>8,056(78)</td>
<td></td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2,149(11.5)</td>
<td>NA</td>
<td>2,149(20.8)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>1,177(6.3)</td>
<td>NA</td>
<td>1,177(11.4)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>7,005(37.4)</td>
<td>NA</td>
<td>7,005(67.8)</td>
<td></td>
</tr>
</tbody>
</table>

All estimates presented are unadjusted. For continuous variables, means are presented with standard deviation between parentheses. For categorical variables, frequencies are presented with percentage between parentheses. Lp(a) indicates lipoprotein(a); LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*Total cholesterol unadjusted for the Lp(a) component was used for these comparisons.*
The association between Lp(a) and ischemic, hemorrhagic, and unclassified stroke outcomes was not statistically significant when fully adjusted for potential confounders (Table 3, Model 3). Using LDL-C or total cholesterol, uncorrected for the Lp(a) component for adjustment did not alter the magnitude of association. Risk of ischemic, hemorrhagic, and unclassified strokes was not modified significantly by LDL-C levels ($P=0.91, 0.76, \text{ and } 0.51,$ respectively). There was no significant deviation from the assumption of proportionality of hazards over time ($P=0.67$).

### Association With CAD
Lp(a) levels were found to be associated with CAD hospitalization and mortality (Table 3, Model 3), with a fully adjusted hazard ratio of 1.13 (95% CI 1.04–1.22) per 1 SD increase in Ln[Lp(a)]. Again, adjustment with total cholesterol or LDL-C without correction for the Lp(a) component did not alter the strength of association. We further examined the shape of the association between Lp(a) and CAD by plotting the adjusted hazard ratios for within sex-specific Lp(a) quartiles (Figure 2). The association appeared curvilinear and the test for nonlinearity was statistically significant in the sex- and age-adjusted model ($P=0.009$) but not in the fully adjusted model ($P=0.17$). The hazard function was found to deviate significantly from the assumption of proportionality over time for the association between standardized Ln[Lp(a)] and CAD ($P=0.005$ for Schoenfeld residuals). Addition of time varying covariates to the fully adjusted model was found to be statistically significant ($P=0.01$ for likelihood ratio tests on 1 df) with the hazard ratio for a 2.7-fold increase in Lp(a) declining 0.92-fold per Ln(year) of analysis time. The hazard ratio function for the Lp(a)-CAD association over 15 years of analysis time is depicted in Figure 3. Exclusion of the first 5 years of analysis time attenuated the association between standardized Ln[Lp(a)] and CAD. The adjusted hazard ratio for a 2.7-fold increase in Lp(a) was 1.10 (95% CI 1.01–1.20) with the first 5 years of analysis time excluded, compared with 1.23 (95% CI 1.03–1.46) during the first 5 years of analysis. The hazard ratio did not significantly deviate from the proportionality assumption beyond this 5-year period ($P=0.82$). There was no evidence of statistical interaction with LDL-C levels ($P=0.84$ for likelihood ratio test on 1 df). Additional exploratory analyses did not show statistically significant interaction between Lp(a) and age ($P=0.87$), sex ($P=0.89$), diabetes mellitus at baseline ($P=0.14$), or serum creatinine (0.79) for risk of CAD.

### Table 2. Baseline Characteristics Within Sex-Specific Quartiles of Lp(a)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Lp(a) level, mg/dL</td>
<td>4.1(1.1)</td>
<td>8.5(1.5)</td>
<td>17.3(4.3)</td>
<td>54.7(24.9)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Lp(a) range, mg/dL</td>
<td>0.1–6.2</td>
<td>5.9–11.7</td>
<td>11.2–27.9</td>
<td>26.6–175.0</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Female sex</td>
<td>2588 (55%)</td>
<td>2585 (55%)</td>
<td>2582 (55%)</td>
<td>2584 (55%)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.9(9.2)</td>
<td>58.3(9.2)</td>
<td>59.6(9.1)</td>
<td>59.0(9.1)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4(4.0)</td>
<td>26.1(3.8)</td>
<td>26.3(3.7)</td>
<td>26.1(3.7)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>104(2.2)</td>
<td>104(2.2)</td>
<td>100(2.1)</td>
<td>98(2.1)</td>
<td>0.97</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>2486(53.4)</td>
<td>2493(53.7)</td>
<td>2486(53.7)</td>
<td>2432(52.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Never</td>
<td>2166(46.6)</td>
<td>2148(46.3)</td>
<td>2145(46.3)</td>
<td>2209(47.6)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L*</td>
<td>6.0(1.2)</td>
<td>6.1(1.1)</td>
<td>6.3(1.2)</td>
<td>6.4(1.2)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.7(1.0)</td>
<td>3.8(1.0)</td>
<td>4.1(1.0)</td>
<td>4.2(1.0)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4(0.43)</td>
<td>1.42(0.42)</td>
<td>1.40(0.40)</td>
<td>1.44(0.45)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.89(1.26)</td>
<td>1.77 (1.03)</td>
<td>1.77(1.01)</td>
<td>1.76(1.03)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Apolipoprotein A-I, g/L</td>
<td>1.53(0.36)</td>
<td>1.55(0.33)</td>
<td>1.55(0.32)</td>
<td>1.57(0.31)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Apolipoprotein B, g/L</td>
<td>0.92(0.25)</td>
<td>0.95(0.24)</td>
<td>1.00(0.24)</td>
<td>1.01(0.24)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134.6(18.0)</td>
<td>134.6(18.2)</td>
<td>136.2(18.6)</td>
<td>134.8(18.3)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82.2(11.3)</td>
<td>82.0(11.1)</td>
<td>82.7(11.2)</td>
<td>82.1(11.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>2.9(6.6)</td>
<td>2.8(5.4)</td>
<td>3.3(6.1)</td>
<td>3.2(6.9)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>84.6(20.2)</td>
<td>86.9(18.6)</td>
<td>88.5(25.8)</td>
<td>87.3(19.8)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>121(2.6)</td>
<td>150(3.2)</td>
<td>137(2.9)</td>
<td>191(4.1)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>History of stroke</td>
<td>60(1.3)</td>
<td>60(1.3)</td>
<td>65(1.4)</td>
<td>77(1.7)</td>
<td>0.39</td>
</tr>
<tr>
<td>Postmenopausal status</td>
<td>1939(75.1)</td>
<td>1986(76.9)</td>
<td>2082(80.7)</td>
<td>2049(79.3)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>918(35.5)</td>
<td>838(32.4)</td>
<td>745(28.9)</td>
<td>825(31.9)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Never</td>
<td>1665(64.5)</td>
<td>1746(67.6)</td>
<td>1835(71.1)</td>
<td>1759(68.1)</td>
<td></td>
</tr>
</tbody>
</table>

All estimates presented within sex-specific quartiles of Lp(a) are unadjusted. For continuous variables, means are presented with standard deviation between parentheses. For categorical variables, frequencies are presented with percentage between parentheses. Lp(a) indicates lipoprotein(a); LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*Total cholesterol unadjusted for the Lp(a) component was used for these comparisons.
In this large-scale prospective population study of 18,720 participants, we found that Lp(a) levels were associated with future risk of PAD-related hospitalizations and death, suggesting that Lp(a) is a risk factor for a broad spectrum of cardiovascular diseases. We also confirmed the positive association between Lp(a) and CAD risk.

The physiological role of Lp(a) remains undefined, but it is postulated to influence tissue healing, innate immunity, and infection. Its role in the pathophysiology of atherosclerosis may relate to its capacity to transport proinflammatory oxidized phospholipids. 28,29 Lp(a) is now regarded as an independent and highly heritable risk factor that may be causal in affecting cardiovascular risk. 5,6 It is estimated that in the United States, elevated Lp(a) levels (≥25 mg/dL) are present in ≈30% of whites and 60% to 70% of blacks, which corresponds to ≈100,000,000 Americans. 30 Supporting evidence for the atherogenicity of Lp(a) comes from Mendelian randomization studies, 5,6 which show that lifelong exposure to altered Lp(a) levels because of LPA gene variants, where Lp(a) levels were inversely related to the number of genetically determined apo(a) K4-2 repeats, results in altered cardiovascular risk. 5 In addition to its atherogenicity through the cholesterol component, apo(a) itself has been shown to

### Table 3. Cardiovascular Outcomes by Sex-Specific Lp(a) Quartiles and Per 1 Standard Deviation Increase in Ln[Lp(a)]

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model</th>
<th>n</th>
<th>Events</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>1 SD Ln[Lp(a)]</th>
<th>(P^{\text{ Trend*}})</th>
<th>Nonlinearity (P^{\text{†}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAD</td>
<td>1</td>
<td>18716</td>
<td>596</td>
<td>1.01(0.79–1.31)</td>
<td>1.04(0.81–1.34)</td>
<td>1.94(1.55–2.43)</td>
<td>1.32(1.22–1.43)</td>
<td>&lt;0.00001</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17867</td>
<td>544</td>
<td>1.07(0.82–1.41)</td>
<td>1.06(0.81–1.38)</td>
<td>2.09(1.64–2.65)</td>
<td>1.36(1.25–1.48)</td>
<td>&lt;0.00001</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15930</td>
<td>484</td>
<td>1.02(0.76–1.37)</td>
<td>1.09(0.82–1.45)</td>
<td>2.06(1.59–2.67)</td>
<td>1.37(1.25–1.50)</td>
<td>&lt;0.00001</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Stroke ischemic</td>
<td>1</td>
<td>18719</td>
<td>284</td>
<td>0.82(0.59–1.14)</td>
<td>0.78(0.56–1.08)</td>
<td>0.85(0.61–1.17)</td>
<td>0.96(0.85–1.08)</td>
<td>0.30</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17870</td>
<td>272</td>
<td>0.81(0.58–1.14)</td>
<td>0.80(0.57–1.11)</td>
<td>0.80(0.57–1.12)</td>
<td>0.94(0.83–1.07)</td>
<td>0.20</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15932</td>
<td>244</td>
<td>0.85(0.59–1.21)</td>
<td>0.81(0.57–1.16)</td>
<td>0.73(0.51–1.05)</td>
<td>0.91(0.79–1.03)</td>
<td>0.10</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Stroke hemorrhagic</td>
<td>1</td>
<td>18719</td>
<td>122</td>
<td>0.93(0.55–1.58)</td>
<td>0.98(0.59–1.63)</td>
<td>1.20(0.73–1.97)</td>
<td>1.09(0.91–1.30)</td>
<td>0.43</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17870</td>
<td>118</td>
<td>0.91(0.53–1.57)</td>
<td>0.96(0.57–1.62)</td>
<td>1.20(0.72–1.98)</td>
<td>1.08(0.90–1.30)</td>
<td>0.45</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15932</td>
<td>99</td>
<td>1.06(0.59–1.91)</td>
<td>0.94(0.52–1.70)</td>
<td>1.25(0.71–2.20)</td>
<td>1.07(0.88–1.32)</td>
<td>0.53</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Stroke unclassified</td>
<td>1</td>
<td>18719</td>
<td>247</td>
<td>0.67(0.46–0.96)</td>
<td>0.73(0.52–1.02)</td>
<td>0.83(0.60–1.17)</td>
<td>0.84(0.83–1.07)</td>
<td>0.38</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17870</td>
<td>234</td>
<td>0.72(0.50–1.04)</td>
<td>0.77(0.54–1.10)</td>
<td>0.80(0.59–1.20)</td>
<td>0.94(0.82–1.07)</td>
<td>0.43</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15932</td>
<td>207</td>
<td>0.74(0.50–1.11)</td>
<td>0.85(0.58–1.25)</td>
<td>0.90(0.61–1.32)</td>
<td>0.96(0.83–1.11)</td>
<td>0.78</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>1</td>
<td>18715</td>
<td>2365</td>
<td>1.07(0.95–1.21)</td>
<td>1.12(0.99–1.26)</td>
<td>1.49(1.33–1.67)</td>
<td>1.17(1.13–1.22)</td>
<td>&lt;0.0001</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17866</td>
<td>2202</td>
<td>1.09(0.96–1.24)</td>
<td>1.11(0.98–1.26)</td>
<td>1.49(1.32–1.68)</td>
<td>1.17(1.13–1.23)</td>
<td>&lt;0.0001</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15930</td>
<td>1984</td>
<td>1.01(0.88–1.16)</td>
<td>1.11(0.97–1.27)</td>
<td>1.33(1.17–1.52)</td>
<td>1.13(1.04–1.22)</td>
<td>&lt;0.0001</td>
<td>0.174</td>
<td></td>
</tr>
</tbody>
</table>

Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, body mass index, total cholesterol adjusted for Lp(a) levels, HDL cholesterol, and triglycerides. Model 3 was adjusted for all covariates in Model 2 in addition to smoking, alcohol consumption, baseline antihypertensive therapy, baseline lipid-lowering therapy, diabetes mellitus, physical activity level, serum creatinine levels, fibrinogen levels, apolipoprotein A-I, apolipoprotein B, CRP levels, history of myocardial infarction at baseline, history of stroke at baseline, family history of myocardial infarction, family history of stroke, postmenopausal status, and use of hormone replacement therapy. Lp(a) indicates lipoprotein(a); PAD, peripheral artery disease; CAD, coronary artery disease.

\*P trend: \(P\) value for the likelihood ratio test for trend.

†P nonlinearity is the \(P\) value for the likelihood ratio test for nonlinearity.

**Discussion**

In this large-scale prospective population study of 18,720 participants, we found that Lp(a) levels were associated with future risk of PAD-related hospitalizations and death, suggesting that Lp(a) is a risk factor for a broad spectrum of cardiovascular diseases. We also confirmed the positive association between Lp(a) and CAD risk.

The physiological role of Lp(a) remains undefined, but it is postulated to influence tissue healing, innate immunity, and infection. Its role in the pathophysiology of atherosclerosis may relate to its capacity to transport proinflammatory oxidized phospholipids. 28,29 Lp(a) is now regarded as an independent and highly heritable risk factor that may be causal in affecting cardiovascular risk. 5,6 It is estimated that in the United States, elevated Lp(a) levels (≥25 mg/dL) are present in ≈30% of whites and 60% to 70% of blacks, which corresponds to ≈100,000,000 Americans. 30 Supporting evidence for the atherogenicity of Lp(a) comes from Mendelian randomization studies, 5,6 which show that lifelong exposure to altered Lp(a) levels because of LPA gene variants, where Lp(a) levels were inversely related to the number of genetically determined apo(a) K4-2 repeats, results in altered cardiovascular risk. 5 In addition to its atherogenicity through the cholesterol component, apo(a) itself has been shown to

![Adjusted Hazard Ratios for PAD by Sex-Specific Lp(a) Quartile](image1.png)

**Figure 1.** Nonlinearity of the association between lipoprotein(a) [Lp(a)] and risk of peripheral artery disease. Adjusted hazard ratios and corresponding 95% CI by sex-specific quartiles of Lp(a).

![Adjusted Hazard Ratios for CAD by Sex-Specific Lp(a) Quartile](image2.png)

**Figure 2.** Nonlinearity of the association between lipoprotein(a) [Lp(a)] and risk of coronary artery disease. Adjusted hazard ratios and corresponding 95% CI by sex-specific quartiles of Lp(a).
exert proatherogenic effects,\textsuperscript{31} which includes its ability to increase endothelial cell permeability and enhance expression of adhesion molecules leading to monocyte recruitment and retention. Moreover, apo(a) has been shown to promote smooth muscle cell proliferation, carry proinflammatory and proatherogenic oxidized phospholipids,\textsuperscript{28} and promote macrophage foam cell formation, apoptosis, and plaque vulnerability.\textsuperscript{3}

The role of inflammatory processes in atherosclerotic diseases has been examined extensively, and most studies have focused primarily on CAD and cerebrovascular disease. Studies examining the role of inflammatory and atherogenic biomarkers in PAD have been limited.\textsuperscript{32} PAD is highly prevalent, affecting an estimated 27 million adults in North America and Europe.\textsuperscript{33} It is well recognized that patients with PAD have greater functional impairment and a higher risk of fatal and nonfatal cardiovascular events compared with patients with symptomatic atherosclerosis in other vascular beds.\textsuperscript{34} Early treatment of modifiable risk factors can substantially reduce the morbidity associated with disease. This underlines the importance of delineating risk factors associated with PAD.\textsuperscript{35}

The association between Lp(a) levels and PAD risk has been investigated in only a small number of prospective studies and was not addressed in the Emerging Risk Factors Collaboration (ERFC) meta-analysis. The observed association between Lp(a) and PAD risk is consistent with data from the Edinburgh Artery Study, which included 208 cases of incident PAD.\textsuperscript{18} Two other prospective studies did not observe such an association, which may have been attributable to the relatively small number of PAD cases in those studies.\textsuperscript{20,21}

The observed association between Lp(a) levels and CAD risk is consistent with various previous studies and also with findings of the ERFC meta-analysis.\textsuperscript{1} In this study, the Lp(a)-associated hazard ratio for CAD was observed to reduce over time. This nonproportionality of hazard has not been reported previously for this association. Several explanations could be advanced for this observation: (1) Reverse association bias attributable to subclinical disease in participants at baseline, causing a stronger association to be observed in the first few years of follow-up; (2) Changes in levels of other variables over time, such as lipid levels and use of statins, because this would not have been accounted for in adjustment for confounding; (3) Variability in Lp(a) levels over time leading to duration-dependent regression-dilution, with stronger associations being observed closer to the time of baseline measurement; and (4) Survivor bias attributable to relatively healthy individuals surviving over longer periods of time. The median follow-up time of this study was 16 years. Few prospective studies have analyzed such a large sample of individuals over such a prolonged period of time,\textsuperscript{3} which may explain why this has not been previously observed. The significance of this finding is unclear and will require further exploration in cohorts with long durations of follow-up. If this observation is substantiated in other prospective cohort studies with long term follow-up, this may have implications for clinical risk prediction.

We did not observe a statistically significant association between Lp(a) levels and stroke subtypes. These results are inconsistent with results of the pooled analysis in ERFC, which reported a significant association with ischemic stroke with a total of 1903 events in the final analysis.\textsuperscript{3} By contrast, our findings are similar to results from the Heart Protection Study, which did not show a significant association between LPA genetic variants and ischemic stroke in 1326 cases of prevalent stroke and 507 cases of incident stroke.\textsuperscript{36} These differences may be attributed to several factors, including the small number of incident ischemic stroke events in the EPIC-Norfolk cohort as compared with the ERFC,\textsuperscript{1} misclassification of the pathogenesis of stroke events attributable to inadequate diagnostic information, and differences in exposure assessment attributable to the use of genetic markers as a proxy for Lp(a) in the Heart Protection Study.

Our results for associations between Lp(a) and cardiovascular outcomes are consistent with the Heart Protection Study, which observed statistically significant associations between genetic variants in the LPA region and CAD, PAD, but not with ischemic stroke.\textsuperscript{36} Lp(a) levels were not directly reported in this study, so the magnitude of association observed cannot be directly compared. However, the estimates for association between Lp(a) and CAD were of lower magnitude than those reported with LPA genetic variants in the PROCARDIS study.\textsuperscript{6} Several explanations could be advanced for this, including overadjustment for confounders and intermediates, lack of adjustment for regression-dilution, and lower effect sizes attributable to less than lifelong exposure to Lp(a), as compared with genetically elevated Lp(a). In addition, PROCARDIS was a study enriched for individuals with early CAD, and the associations with Lp(a) observed here may not reflect those seen in the general population.

This study also assessed modification of Lp(a)-associated cardiovascular risk by LDL-C levels. Several studies have reported that the association between Lp(a) and CAD risk is stronger in patients with concomitant high plasma LDL-C levels.\textsuperscript{20-21} Although the ERFC meta-analysis did not perform subgroup analyses by LDL-C levels, subgroup analyses by non–HDL-C levels suggested that the risk estimates were somewhat stronger among people with higher non–HDL-C levels.\textsuperscript{1} However, the test for statistical interaction in the ERFC meta-analysis was not statistically significant.\textsuperscript{1} In EPIC-Norfolk we did not observe any evidence for such an
interaction, which might be because the risk of Lp(a)-related CAD is independent of LDL-C levels or because we did not have the statistical power to detect an interaction in this study.

When interpreting the results of our study, several aspects need to be taken into account. An important strength of this study is the large number of cardiovascular outcomes, and PAD outcomes in particular. Also, Lp(a) measurements in this study were performed with an apo(a) isoform independent assay, as recommended by the National Heart, Lung, and Blood Institute.37 An important limitation is the fact that outcome definitions were derived from clinical practice and were not independently adjudicated. The end point stroke is therefore likely to comprise a heterogeneous group of underlying diseases such as atherosclerotic carotid artery disease as well as thromboembolic events attributable to atrial fibrillation and other cardiac sources of embolism. This might have resulted in an underestimation of the effect of Lp(a) on stroke attributable to atherosclerosis. Second, the combined group of PAD diseases was diagnosed on the basis of ICD-10 codes I70-I73, which may have included several cases of nonatherosclerotic diseases like Raynaud phenomenon and vasculitis. Although we expect the number of such diagnoses to be low compared with atherothrombotic PAD, we cannot exclude the possibility that this may have diluted the Lp(a)-PAD association. Third, this study may not have had adequate statistical power to detect interactions between LDL-C levels and Lp(a)-associated cardiovascular risk. Fourth, whereas total cholesterol was corrected for its Lp(a) fraction before adjustment in all associations, other lipid fractions could not be adjusted for their Lp(a) components, because the proportional contribution of these to Lp(a) is unknown. This may have attenuated observed associations with cardiovascular outcomes. Fifth, because repeat measurements of Lp(a) were unavailable for individuals in this cohort, we could not correct for within-individual variation in these, which may have led to attenuation of observed estimates. Finally, no changes in lipid-lowering therapy were recorded during follow-up. This may be particularly relevant for statins, because these may actually increase Lp(a) levels.38 The initiation of such medication could have altered Lp(a) levels and the inherent cardiovascular risk.

In summary, we provide new evidence of the association of Lp(a) with PAD outcomes and confirmed an independent and positive association between Lp(a) levels and CAD risk. The shape of the Lp(a)-PAD association was curvilinear. We did not find any evidence for an interaction between Lp(a) levels and LDL-C levels in determining risk of CAD, stroke, or PAD. Our data suggest that Lp(a) is an independent risk factor for PAD and CAD risk and support future efforts to identify effective and safe Lp(a)-lowering therapies in an effort to lower cardiovascular risk.39,40

Acknowledgments
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Disclosures
None.

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Lipoprotein(a) and Risk of Coronary, Cerebrovascular, and Peripheral Artery Disease: The EPIC-Norfolk Prospective Population Study

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