Lipoprotein(a) Is Associated Differentially With Carotid Stenosis, Occlusion, and Total Plaque Area

Jonathan H. Klein, Robert A. Hegele, Daniel G. Hackam, Marlys L. Koschinsky, Murray W. Huff, J. David Spence

Background—Lipoprotein(a) [Lp(a)] is a putative risk factor for myocardial infarction and stroke and is related to thrombosis and impaired fibrinolysis. We studied relationships of Lp(a) with carotid stenosis, occlusion, and total plaque area, distinct phenotypes of atherosclerosis that may be differentially affected by cardiovascular risk factors.

Methods and Results—Multivariable linear regression analysis was used to study relationships of Lp(a) to phenotypes of carotid atherosclerosis among 876 consecutive patients from an atherosclerosis prevention clinic with complete data for all variables used in the model. Occlusion of an internal carotid artery was present in 22 (2.5%) patients (one with bilateral occlusions). Risk factors predicted carotid plaque area, stenosis, and occlusion differently. Lp(a) was a significant independent predictor of baseline stenosis (P<0.0001) but not of plaque area (P=0.13); in logistic regression, Lp(a) significantly predicted occlusion (P=0.001). Patients with occlusion had significantly higher levels of Lp(a): 0.27±0.25 g/L versus 0.17±0.18 g/L, without occlusion; P=0.007.

Conclusion—Lp(a) was a significant independent predictor of carotid stenosis and occlusion, but not of carotid plaque area, supporting the hypothesis that the effect of Lp(a) on atherogenesis and cardiovascular risk is largely related to thrombosis and impaired fibrinolysis. Stenosis and occlusion may not be attributable to plaque progression, but to plaque rupture and thrombosis; this relationship may also apply to other arterial beds. (Arterioscler Thromb Vasc Biol. 2008;28:1851-1856)

Key Words: lipoprotein(a) ■ carotid ■ atherosclerosis ■ stenosis ■ plaque

Previous research suggests that plasma lipoprotein(a) [Lp(a)] is a strong and independent risk factor for myocardial infarction and ischemic stroke. Cardiovascular events are extremely complex, involving not only the underlying problem of plaque initiation and progression, but also plaque rupture, thrombosis, impaired fibrinolysis, and other processes. As atherosclerotic plaque develops, arteries enlarge (compensatory enlargement) to maintain luminal size and optimize shear rates. Spence and Hegele have suggested that ultrasound phenotypes reflect different processes in the progression of atherosclerosis from intimal thickening, through plaque growth, to plaque rupture, stenosis, and occlusion. Total plaque area is probably related to endothelial dysfunction, hypertension, lipids, oxidative stress, and smooth muscle proliferation. Stenosis and occlusion, on the other hand, are probably more dependent on factors predisposing to plaque rupture, such as inflammation, weakening of matrix, and possibly infection, and to thrombosis and impaired thrombolysis. It is possible that MRI phenotypes, such as lipid core and other aspects of plaque composition, may reflect still other influences on atherosclerosis such as effects of lipid lowering therapy, propensity for macrophages to imbibes lipids, and other biological processes including those affecting calcification (which may affect yet another imaging phenotype of atherosclerosis: coronary calcium assessed by computerized tomography).

Lp(a) contains a biochemical moiety similar in structure to low-density lipoprotein and several domains similar to plasminogen, a proenzyme related to fibrinolysis. Combining a proatherogenic factor with an antifibrinolytic factor makes Lp(a) an interesting candidate for a link between plaque and stenosis and a likely risk factor for thrombotic events, including atherosclerotic occlusion.

We hypothesized, based on preliminary observations in a smaller sample, that Lp(a) may be differentially related to carotid plaque and stenosis via thrombosis and impaired thrombolysis, and that occlusion, being the ultimate consequence of plaque rupture and thrombosis, may be particularly related to Lp(a).

In this study we analyze the role of Lp(a) in carotid total plaque area (TPA), stenosis, and occlusion.
Methods

Setting
The sample comprised consecutive patients referred to JDS in the Atherosclerosis Prevention Clinic, Premature Atherosclerosis Clinic, and the Stroke Prevention Clinic of University Hospital, London, Ontario, Canada. Each patient had measurement of both carotid stenosis and total carotid plaque area as well as a complete data profile for plasma Lp(a) and all the other variables used in the multiple regression model: age, sex, total cholesterol, triglycerides, HDL cholesterol, systolic blood pressure, smoking (pack-years), diabetes, and treatment of lipids and cholesterol. Lp(a) was measured using a monoclonal antibody-based sandwich ELISA as previously described. An Advanced Technology Laboratories (ATL) Mark 9 was used before 2000 and an ATL HDI 5000 thereafter (Phillips). The technologists scanned along the length of the right and left common, internal and external carotid arteries between the angle of the jaw and the clavicle. They then determined the largest extent of each plaque present and traced the outline of each plaque in a longitudinal view with a cursor. The machine’s microprocessor computed the plaque area for each plaque; summing the individual plaque areas yielded the total plaque area. Intraobserver and interobserver reliability (intraclass correlation) were \( \kappa = 0.94 \) and \( \kappa = 0.85 \), respectively. For branches that were occluded, the entire cross-sectional area of the branch was regarded as occupied by plaque.

Total carotid stenosis was defined as the sum of the percent stenosis in the right and left internal carotid arteries; the upper limit of total stenosis (for a patient with bilateral carotid occlusion) was thus 200%. Stenosis was measured by Doppler peak frequency shift before 2003 and Doppler peak velocity after 2003, and was calibrated angiographically from 100 angiograms (200 arteries) measured in the North American Symptomatic Carotid Endarterectomy Trial. Carotid occlusion was defined by absence of flow on Doppler ultrasound with color flow.

Key Variables
In a prespecified protocol, we evaluated the determinants of total carotid plaque area (the sum of the cross-sectional areas of all plaques seen in the internal, external and common carotid arteries on both sides), total carotid stenosis (the sum of the percent stenosis of both internal carotid arteries), and occlusion. The variables used in the analyses had previously been shown in multivariable analysis to maximally predict baseline plaque area and stenosis. Age, sex, and smoking status were self reported and corroborated by clinic records. Baseline blood pressure was defined as the highest systolic reading in either arm from either of the patient’s first 2 clinic appointments. Variables representing therapy for hyperlipidemia and hypertension were included to account for lowering of baseline blood pressure and cholesterol levels by treatment.

Biochemical marker values were taken using blood samples obtained after a 12-hour fast. The means of duplicate determinations of plasma levels of Lp(a) were obtained in the labs of Dr Hegele (approximately a third of the samples) using a commercial ELISA (Terumo Inc). The anti-Lp(a) antibody (iD1) used in our studies has been demonstrated to be highly specific to epitopes on Lp(a) and does not cross-react with plasminogen. In the laboratory of Dr Huff (approximately a third of the samples) was measured using a Macra Lp(a) Kit obtained from Trinity Biotech. The intraassay coefficients of variation was 5.6%.

In the laboratory of Dr Koschinsky (approximately a third of the samples) the plasma Lp(a) method used a monoclonal antibody-based sandwich ELISA as described by Wong et al. The results from this assay were comparable to the Macra Lp(a) assay, which was also used in her laboratory. The other biochemical variables were assayed in the University Hospital laboratory using routine methods.

Ultrasound Methods
Total carotid plaque area was measured by 2 registered vascular ultrasound technologists using a high-resolution ultrasound scanner as previously described. An Advanced Technology Laboratories (ATL) Mark 9 was used before 2000 and an ATL HDI 5000 thereafter (Phillips). The technologists scanned along the length of the right and left common, internal and external carotid arteries between the angle of the jaw and the clavicle. They then determined the largest extent of each plaque present and traced the outline of each plaque in a longitudinal view with a cursor. The machine’s microprocessor computed the plaque area for each plaque; summing the individual plaque areas yielded the total plaque area. Intraobserver and interobserver reliability (intraclass correlation) were \( \kappa = 0.94 \) and \( \kappa = 0.85 \), respectively. For branches that were occluded, the entire cross-sectional area of the branch was regarded as occupied by plaque.

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Statistical Analysis
Statistical analysis was performed using SPSS version 16.0 for Windows. Baseline plaque area was transformed using a cube root transformation to normalize its distribution (Figure 1). Total stenosis was untransformed, as the distribution approximated normality (Figure 1). Linear multivariable regression was used for analyses of normalized plaque area and stenosis, and logistic regression with backward elimination of variables at \( P = 0.10 \) according to likelihood ratio was used for occlusion. A 2-tailed probability value of 0.05 was considered significant. A variable called “era” was used as a surrogate for the laboratory in which the Lp(a) analyses were performed, as we did not record which laboratory performed each
assay, but the assays were performed in different laboratories at different times: first in Toronto in the laboratory of Dr Hegele, then in Kingston, Ontario in the laboratory of Dr Koschinsky, and finally in London, Ontario in the laboratory of Dr Huff.

Results

All 876 consecutive patients seen in the clinic between 1990 and 2007 for whom we had complete data for all the variables required for the multivariable regression analysis were included in the analyses. Slightly less than half the population was female, with a similar proportion receiving antihypertensive therapy (Table 1). Forty percent were former smokers, and 12% continued to smoke. The distribution of total plaque area and its cube root transformation (Exponent 1/3, used to normalize the distribution for multivariate linear regression) and total stenosis are shown in Figure 1; 22 (2.5%) patients had occlusion of at least 1 internal carotid artery; only 1 had occlusion of both.

Determinants of Baseline Total Carotid Plaque Area, Stenosis, and Occlusion

Table 2 shows the multivariable linear regression model for total plaque area; Table 3 shows the multivariable linear regression model for total stenosis. The risk factors included in the analysis were based on our previous studies, and as we previously reported, explained a much higher proportion of plaque area than of stenosis; the $R^2$ for plaque area was 0.49, versus 0.14 for stenosis. Lp(a) did not predict plaque area ($P=0.13$), but was a significant independent predictor of stenosis ($P<0.0001$). In logistic regression, Lp(a) significantly predicted occlusion ($P=0.001$). Figure 2 shows the relationship between quintiles of Lp(a) with plaque area and stenosis. There was a significant relationship for stenosis ($P=0.022$), but not for plaque area ($P=0.225$; ANOVA).

Age, sex, systolic blood pressure, pack-years of smoking, total cholesterol, diabetes, and lipid therapy were significant independent predictors of plaque area, as they had been in previous studies. The regression model showed that plasma Lp(a) was significantly predictive of baseline stenosis ($P<0.001$) as were age, sex, and pack years of smoking.

The risk factors we investigated were related differently to plaque area and stenosis. Importantly, plasma Lp(a) concentr-
tration was a significant independent predictor of carotid stenosis and occlusion, but not of total plaque area. Age, significant for both traits, played a much greater role in predicting plaque area, accounting for 49% of the explained variance while explaining only 23% of the variance for stenosis. Baseline systolic blood pressure and diabetes were significant for plaque area, but not for stenosis. Female sex, significant for both traits and explaining about 15% of the variance of each, was directly predictive of stenosis but inversely related to plaque area, in agreement with a previous report.\(^1\)

Plots of residual scores against the variables used in the regression models showed no obvious nonlinearities; for age and smoking pack-years linearity appears reasonable. One outlier for Lp(a) was identified by these plots, but when the regression analyses were performed with that case excluded, the results were not materially different.

Even though the number of patients with occlusion was small, Lp(a) was a significant independent predictor of occlusion in multivariable regression (*P*=0.001); none of the traditional risk factors predicted occlusion (Table 4). Age was much less predictive of occlusion than of plaque area or stenosis. Patients with occlusion had significantly higher levels of Lp(a): 0.27±0.25 g/L versus 0.17±0.18 g/L without occlusion; *P*=0.007.

During the 3 eras in which Lp(a) was measured in the 3 different laboratories, the age of the clinic population increased because a higher proportion were referred for stroke or TIA, and the pattern of treatment changed toward more intensive therapy of with lipid-lowering drugs. Table 5 shows the key variables used in the regression models, in the 3 eras (approximately 1990 to 1998, 1998 to 2003, and 2003 to 2008) in which the Lp(a) was measured in the 3 laboratories. Tests for interaction between Lp(a) and era were performed in SAS. There was no significant interaction: For the cubed root transformation of plaque *F*(2,886)=0.67, *P*=0.510; for total stenosis *F*(2,886)=2.45, *P*=0.087.

### Discussion

Lipoprotein(a), a putative biochemical risk factor for atherosclerotic events, is chemically similar to thrombotic factors.

### Table 4. Logistic Regression Model for Baseline Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th><em>P</em> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per decade)</td>
<td>1.48</td>
<td>0.98, 2.23</td>
<td>0.062</td>
</tr>
<tr>
<td>Female</td>
<td>2.38</td>
<td>0.92, 6.17</td>
<td>0.075</td>
</tr>
<tr>
<td>On lipid therapy</td>
<td>0.26</td>
<td>0.10, 0.67</td>
<td>0.005</td>
</tr>
<tr>
<td>Era</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Era 1 (Hegele)</td>
<td>0.27</td>
<td>0.07, 1.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Era 2 (Koschinsky)</td>
<td>0.47</td>
<td>0.16, 1.4</td>
<td>0.18</td>
</tr>
<tr>
<td>Lp(a) (per SD)</td>
<td>1.7</td>
<td>1.23, 2.34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Variables were excluded (at *P*>0.10) by backward elimination based on likelihood ratios; the final step (Step 6) is shown (n=876; *P*<0.0001; Chi-Square).

Variables entered on Step 1: Age by decade, sex, systolic pressure, cholesterol, smoking (pack-years), on lipid therapy, on therapy for hypertension, Era, Lp(a) per standard deviation.

### Table 5. Key Variables in the Three Eras in Which Lp(a) Was Measured in the Three Laboratories

<table>
<thead>
<tr>
<th>Era</th>
<th>Age</th>
<th>95% CI</th>
<th><em>P</em> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52.48</td>
<td>11.176</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>50.50</td>
<td>12.122</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>57.46</td>
<td>11.800</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>139.28</td>
<td>18.150</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>145.35</td>
<td>19.882</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>146.86</td>
<td>19.445</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.3567</td>
<td>0.88649</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>5.1715</td>
<td>1.02190</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.8386</td>
<td>1.19335</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.8283</td>
<td>1.10794</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>1.8855</td>
<td>1.24577</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.7151</td>
<td>1.02191</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>1.1577</td>
<td>0.38710</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>1.2669</td>
<td>0.46789</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.3965</td>
<td>0.41268</td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.18540</td>
<td>0.200756</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>0.14052</td>
<td>0.168208</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.18454</td>
<td>0.159902</td>
<td></td>
</tr>
</tbody>
</table>

Era 1, Hegele lab; 2, Koschinsky lab; 3, Huff lab.
This similarity may explain our observation that Lp(a) is significantly related to stenosis and occlusion, which in most instances are the consequence of plaque rupture and thrombosis. Stenosis can be regarded as evidence that a plaque event, such as rupture and thrombosis, has previously occurred. Our results showed that Lp(a) was predictive of baseline stenosis and occlusion, but not of baseline plaque area. This supports the concept that thrombotic factors may play a more important role in development of stenosis than of plaque. Patients with carotid stenosis are at high risk of stroke and myocardial infarction, and therefore warrant treatment of traditional risk factors to prevent future plaque events.

Our results support the possibility that treatments to lower Lp(a) would be worthy of consideration. Niacin has been shown to reduce Lp(a), along with other risk factors such as triglycerides and LDL cholesterol, and to raise levels of HDL cholesterol. New formulations have significantly reduced adverse effects, such as flushing attributable to vasodilation, which may encourage the use of niacin in cardiovascular therapy. Ethanol-extracted soy protein may represent another possible therapy to lower Lp(a).

The type of lipid-lowering therapy in use in our patients seems unlikely to have affected our results: at baseline most patients (76.7%) were taking statins; some (16.9%) were also taking fibrates; few (6%) were taking niacin.

An important potential weakness of this study is that the Lp(a) measurements were performed in 3 different laboratories, in 3 different eras: the first group were performed in the laboratory of Dr Hegele, the second group in the laboratory of Dr Koschinsky, and the third group in the laboratory of Dr Huff. We did not save as a variable the laboratory in which the levels were performed, so we could not analyze whether the laboratory per se had a significant effect in the models. The variable called “era”, which was used as a surrogate for these 3 laboratories, was significantly related to plaque area, but not to stenosis or occlusion. Patients in the final era were significantly older, with significantly higher blood pressures, but significantly lower levels of cholesterol, related undoubtedly to more intensive therapy in that era.

Traditional risk factors, such as sex, diabetes, and systolic blood pressure, predicted plaque, stenosis, and occlusion differently, further supporting our hypothesis that the 3 phenotypes are biologically distinct. At any age, men have more plaque, whereas women have more apparent stenosis as measured by blood velocity, probably reflecting a smaller average arterial diameter among women. That finding was reproduced in this study population. Whereas all the traditional risk factors and lipid therapy were independently significant predictors of plaque area, fewer variables predicted stenosis and only lipid-lowering therapy, sex, and Lp(a) significantly predicted occlusion. The proportion of explained variance for the stenosis model was about one third of that for the plaque area model. We suggest that this relationship may apply not only in the carotid arteries, but also in other arterial beds.

Conclusions
Carotid stenosis and total plaque area have different relationships with traditional risk factors, and with Lp(a). Carotid occlusion, probably attributable in large part to thrombosis and impaired fibrinolysis, bears a stronger relationship to Lp(a) than to other risk factors. It seems likely that the role of Lp(a) in atherogenesis is largely based on its effects on coagulation and fibrinolysis. Our findings suggest that therapy to lower Lp(a) might reduce the risk of cardiovascular events, even though it might not reduce the burden of atherosclerosis. Clinical trials of Lp(a) lowering should be considered to determine whether therapy for Lp(a) might reduce cardiovascular events. In such trials it may be important to account for confounding effects of antithrombotic therapy such as warfarin. Our results also suggest that imaging surrogates such as ultrasound measurement of intima-media thickness, 3D plaque volume or vessel wall volume, MRI vessel wall volume, or coronary calcification would not be appropriate for such studies, but that measurement of stenosis may be useful.

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Maria DiCicco RVT and Janine Desroches RVT performed the measurements of carotid plaque area and stenosis. Victoria Coates entered and edited much of the data used in these analyses.

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Disclosures
None.

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