Effect of Lp(a) on the Early Functional and Structural Changes of Atherosclerosis

Olli T. Raitakari, Mark R. Adams, David S. Celermajer

Abstract—Epidemiologic studies have shown a significant relationship between elevated plasma levels of Lp(a) and increased risk of cardiovascular events; however, the mechanisms by which elevated Lp(a) levels produce this increased risk are not known. To test the hypothesis that high Lp(a) levels might contribute to the development of subclinical atherosclerosis, we examined the influence of Lp(a) levels on early functional and structural atherosclerotic vascular changes. Flow-mediated (endothelium-dependent) and nitrate-mediated (smooth muscle–dependent) arterial dilations were measured by high-resolution ultrasound in 241 normal healthy subjects (aged 15 to 69 years; 116 men). In addition, carotid artery intima-media thickness was measured by ultrasound in 71 subjects. Plasma Lp(a) was measured using a 2-sided immunoradiometric assay (cohort median, 10 mg/dL; interquartile range, 3.9 to 24.4 mg/dL). In these subjects, there were no significant relationships between Lp(a) and arterial endothelial function, smooth muscle responses, or carotid wall thickness (P>0.25). By contrast, other lipid risk factors, such as LDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratio, were significantly correlated with abnormal arterial function and structure (P≤0.01). These data suggest that elevated Lp(a) levels do not confer cardiovascular risk by contributing to the early functional or structural changes of atherosclerosis. (Arterioscler Thromb Vasc Biol. 1999;19:990-995.)

Key Words: endothelium ■ preclinical atherosclerosis ■ ultrasound ■ carotid artery

Lp(a) was first described by Berg in 1963.1 It differs from LDL by the presence of a glycosylated protein of variable mass, termed apolipoprotein(a), which is linked by a covalent bond to apoB100 and has approximately 80% structural homology with plasminogen, a key protein of the coagulation cascade.2 Most3–7 but not all previous studies8–10 have shown that elevated Lp(a) is an independent risk factor for coronary heart disease. The exact mechanism by which Lp(a) confers cardiovascular risk is unknown; however, both proatherogenic and prothrombogenic effects have been hypothesized.2,11–13

The early stages of atherosclerosis are associated with changes in arterial function and structure that can now be studied noninvasively using high-resolution ultrasound. A key early event in atherosclerosis is endothelial dysfunction,14,15 which can be detected in systemic conduit arteries by measuring flow-mediated dilation.16 Subtle structural changes, such as thickening of the arterial intima-media complex, also occur early in the atherosclerotic disease process.17,18 Many conventional risk factors, such as smoking, hypercholesterolemia, hypertension, and diabetes, have recently been shown to be significantly associated with impaired arterial endothelial function19 and with increased arterial wall thickness,20–22 consistent with their accepted role in atherogenesis. Much less is known, however, about the effects of Lp(a) on these early markers of arterial disease in healthy asymptomatic subjects. The purpose of the present study was therefore to examine the effects of plasma Lp(a) levels on early functional and structural atherosclerotic vascular changes in a cohort of normal healthy subjects.

Methods

Subjects
We studied 241 healthy subjects aged 40±15 years, (range, 15 to 69 years; 116 men, 125 women). None of these subjects had any history or clinical signs of coronary atherosclerosis, diabetes mellitus, familial hypercholesterolemia, or homozgyous homocystinuria. All subjects were white. The subjects were recruited from hospital staff and volunteers from the community. None of the subjects was taking any regular cardioactive medications. There were 190 nonsmokers (79%), 40 current smokers (17%) and 11 ex-smokers (4%). Lifetime smoking dose was categorized on basis of self-reported lifetime total pack-years smoked: nonsmokers (0 pack-years), very light (0 to 4 pack-years), light (5 to 9 pack-years), moderate (10 to 19 pack-years), and heavy (≥20 pack-years). There were 82 postmenopausal women and 59 of them (47% of all women) were taking hormone-replacement therapy: 41 were taking a combination estrogen and progesterin and 18 were on estrogen only (after hysterectomy). All studies were approved by the local committees on ethical practice, and all subjects gave informed consent.

Lipoprotein Measurements
Fasting serum levels of Lp(a) were determined using commercially available solid-phase 2-site immunoradiometric assay kits (Mercodia Apo(a) RIA, Mercodia AB), which measures the apolipoprotein(a)
molecule. Fasting serum total cholesterol and triglyceride concentrations were measured using standard enzymatic methods (Boehringer Mannheim GmbH) with a fully automated analyzer (Hitachi 704 or 747; Hitachi Ltd). HDL cholesterol (HDL-C) was measured after precipitation with phosphotungstic-stain-magnesium. The LDL-C concentration was calculated using the Friedewald formula.\(^\text{23}\)

## Ultrasound Studies
Ultrasound study to examine brachial artery flow–mediated dilation was performed in all subjects, and carotid artery ultrasound was performed for measurement of mean common carotid artery intima-media thickness (IMT) in 71 participants (age, 42±13 years; range, 29 to 69 years), including 62 nonsmokers, 1 current smoker, and 8 ex-smokers. All studies were performed using an Acuson 128XP/10 mainframe (Acuson) with a 7.0-MHz linear array transducer.

### Arterial Physiology Testing
The ultrasound method for measuring endothelium-dependent and smooth muscle–dependent arterial dilation has been previously described.\(^\text{1-4,29}\) In brief, brachial artery diameter was measured from B-mode ultrasound images. In all studies, scans were obtained at rest, during reactive hyperemia, again at rest, and after sublingual isosorbide dinitrate spray 2.5 mg. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was taken continuously for 30 seconds before and 90 seconds after cuff deflation, including a repeat flow velocity recording for the first 15 seconds after the cuff was released. Thereafter, 10 to 15 minutes was allowed for vessel recovery, after which a further resting scan was taken. Sublingual nitroglycerin in standard antianginal doses (glyceryl trinitrate spray 400 μg or isosorbide dinitrate spray 2.5 mg) was then administered, and 3 to 4 minutes later the last scan was acquired.

### Carotid Artery Studies
All scans were performed by operators following a predetermined, standardized scanning protocol for the right and left carotid arteries, as described by Salonen and Salonen\(^\text{26}\) and Blankenhorn et al.\(^\text{27}\) using images of the far wall of the distal 10 mm of the common carotid arteries. Three scanning angles were used in each case; anterior oblique, lateral, and posterior oblique. The image was focused on the posterior (far) wall, and images were recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface, as described previously.\(^\text{26}\) All scans were recorded on super-VHS videotape for subsequent off-line analysis. Images were digitized using a video frame-grabber interfaced with a personal computer and analyzed with custom-made analysis software. Two end-diastolic frames were selected, digitized, and analyzed for mean IMT, and the average reading from these 2 frames was calculated, for both right and left carotid arteries. We have previously reported good intraobserver and interobserver repeatability values, and within subject reproducibility, using this method.\(^\text{28}\) The interobserver error for mean IMT was 0.035±0.03 mm (range, 0 to 1.17 mm; coefficient of variation [CV], 2.5%), and the intraobserver variability was 0.07±0.07 (range, 0 to 0.26 mm; CV, 6%).

### Statistical Analysis
Descriptive data are expressed as mean±SD, unless otherwise stated. Comparisons between groups were performed with independent samples t tests, nonparametric Mann-Whitney U tests, or χ² tests, as appropriate. Associations were examined by calculating univariate Spearman’s correlation coefficients. Because IMT measurements correlated linearly with age in this data set, partial correlation coefficients were also calculated between the measured variables and age-adjusted IMT. Multivariate linear regression models were used to study the independent determinants of arterial function and structure. The values for vascular parameters were normally distributed. However, because the distributions for Lp(a) and triglycerides were skewed, their values were log_{10}-transformed before regression analyses. Statistical significance was inferred at a P value ≤0.05. All statistical analyses were performed by using the Statistical Analysis System.\(^\text{29}\)

### Results
The study characteristics of the 241 healthy subjects are summarized in Table I. As expected, the distribution of Lp(a) values was skewed toward lower values. The median Lp(a) value was 10 mg/dL (interquartile range, 3.9 to 24.4 mg/dL, range, 1 to 125.4 mg/dL). Lp(a) correlated significantly with LDL-C concentration (r=0.19, P=0.003), but not with the other measured lipids, subject age (r=0.09, P=0.18), sex (r=−0.06, P=0.39), or smoking status (r=−0.11, P=0.10).

### Table I. Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>241</td>
</tr>
<tr>
<td>Males/Females</td>
<td>116/125</td>
</tr>
<tr>
<td>Age, y</td>
<td>40±15 (15–69)</td>
</tr>
<tr>
<td>Vessel size, mm</td>
<td>3.8±0.7 (2.5–5.6)</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>5.0±3.5 (range, –1.8–15.6)</td>
</tr>
<tr>
<td>Nitroglycerin-mediated dilation, %</td>
<td>16.7±5.9 (0.4–33.3)</td>
</tr>
<tr>
<td>Flow at rest, mL/min</td>
<td>65±53</td>
</tr>
<tr>
<td>Reactive hyperemia, %</td>
<td>608±289</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>18.3 (median, 10; interquartile range, 3.9–24.4)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.17±0.99 (range, 2.80–7.80)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.13±0.94 (range 1.05–5.89)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.43±0.44 (0.72–3.51)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.23±0.64 (0.30–4.10)</td>
</tr>
<tr>
<td>LDL-C/HDLC ratio</td>
<td>2.46±1.08 (0.42–6.28)</td>
</tr>
<tr>
<td>Pack-years in smokers</td>
<td>12±12 (0.04–60)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±15 (90–180)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78±10 (55–108)</td>
</tr>
</tbody>
</table>

Data are mean±SD, range in parentheses unless otherwise indicated.
Lp(a) and Early Atherosclerosis

TABLE 2. Univariate Correlation Coefficients for Lp(a), Other Risk Variables, and Measures of Arterial Function and Structure

<table>
<thead>
<tr>
<th></th>
<th>FMD (n=241)</th>
<th>NMD (n=241)</th>
<th>IMT (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>0.02</td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.08</td>
<td>−0.16*</td>
<td>0.35**</td>
</tr>
<tr>
<td>LDL-C</td>
<td>−0.14**</td>
<td>−0.22***</td>
<td>0.33**</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.23***</td>
<td>0.21***</td>
<td>−0.15</td>
</tr>
<tr>
<td>LDL/C/HDL-C ratio</td>
<td>−0.29***</td>
<td>−0.28***</td>
<td>0.34**</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.17**</td>
<td>−0.23***</td>
<td>0.27*</td>
</tr>
<tr>
<td>Age</td>
<td>−0.08</td>
<td>−0.03</td>
<td>0.33**</td>
</tr>
<tr>
<td>Smoking‡</td>
<td>−0.10</td>
<td>−0.02</td>
<td>0.14</td>
</tr>
</tbody>
</table>

FMD, indicates flow-mediated endothelium-dependent dilation; IMT, intima-media thickness; NMD, nitrate-mediated smooth muscle–dependent dilation. Negative correlations with FMD or NMD indicate an association with arterial dysfunction, whereas positive correlations with IMT indicate an association with arterial wall thickening.

* P<0.05.
** P<0.01.
*** P<0.001.
‡ Smoking is coded as a categorical variable (nonsmoker, very light, light, moderate, heavy) on the basis of the history of pack-years smoked (see Methods). On multivariate analysis, cigarette smoking was associated with impaired FMD (P=0.015, see Table 3).

Lp(a) and Arterial Function

The univariate associations between lipid variables, age, smoking status, and vascular reactivity data are shown in Table 2. Lp(a) concentration showed no significant association with flow-mediated dilation (Figure 1) or nitrate-mediated dilation in the entire cohort, or in either sex (data not shown). Both flow-mediated and nitrate-mediated dilation were significantly and inversely related to LDL-C, LDL-C/HDL-C ratio, and triglycerides and directly to HDL-C concentration. In a multivariate regression model, the independent determinants of flow-mediated dilation included LDL-C/HDL-C ratio (P=0.017), smoking (P=0.015), and vessel size (P<0.001) (Table 3). Independent determinants of nitrate-mediated dilation included LDL-C/HDL-C ratio (P<0.001), sex (P<0.001), and vessel size (P<0.001).

To examine the influence of extremely high Lp(a) levels on vascular reactivity, we compared subjects in the highest Lp(a) quintile (Lp(a)=30 mg/dL, n=47) with those in the lowest (Lp(a)≤3 mg/dL, n=46). The level of 30 mg/dL represents the atherogenic threshold of plasma Lp(a) levels in most studies.4–7 Subjects with high Lp(a) levels had higher LDL-C concentrations (P=0.012), but otherwise the 2 groups had similar characteristics and showed no differences in the values for either flow-mediated or nitrate-mediated dilation (P>0.7).

Lp(a) and Arterial Structure

The associations between IMT and the measured risk variables are also shown in Table 2. Lp(a) concentrations showed no significant association with IMT in either group (Figure 2). IMT was significantly correlated with total cholesterol, LDL-C, LDL-C/HDL-C ratio, age, and triglycerides. Adjustment for age did not change the overall correlations or significances. For example, the partial correlation adjusted for age between Lp(a) and IMT remained nonsignificant (r=0.00, P=0.99). Furthermore, when the correlation analyses were stratified by age (using the median value of 37 years as the cut-point), the overall correlations and significances for IMT remained essentially the same as those shown in Table 2. In a multivariate regression model, IMT correlated significantly and directly with age (P<0.001) and LDL-C/HDL-C ratio (P=0.03).

Discussion

Lp(a) levels in the highest population quintile (greater than ~30 mg/dL) appear to be associated with approximately 2 to 3 mm of wall thickening on common carotid imaging. This finding is consistent with prior human and animal studies.4 The results are notable because Lp(a) is a far less studied risk factor relative to other lipids. The data from this study indicate that Lp(a) could be a novel therapeutic target for slowing the progression of atherosclerosis.

Figure 1. Scatterplot of Lp(a) values and flow-mediated dilation (FMD) in healthy subjects (n=241), showing no significant correlation.

Figure 2. Scatterplot of Lp(a) values and common-carotid IMT (n=71), showing no significant correlation.
3 times higher relative risk for cardiovascular events, compared with levels <30 mg/dL. This finding has been observed in men and women, and in several different regions of the world. Nevertheless, the mechanism of risk conferred by Lp(a), and its interaction with other traditional atherogenic factors, remains obscure. In this relatively large study of arterial function and structure, we have shown that elevated Lp(a) has no significant association with endothelial dysfunction, impaired smooth muscle responses, or arterial wall thickening. By contrast, in the same population, other lipid risk factors such as elevated LDL-C or LDL-C/HDL-C ratio were significantly associated with preclinical evidence of arterial damage in accordance with previous studies. The best single correlate for early functional and structural atherosclerotic changes in this study was the LDL-C/HDL-C ratio, which has previously been found to be superior to measurement of serum LDL-C alone, as a predictor of coronary heart disease risk in epidemiologic studies. Significant associations between risk factors and impaired nitrate-mediated vasodilation observed here suggest that early changes in the vessel wall during atherogenesis may not be limited to the endothelium, and that the reduction in vasodilation to exogenous sources of nitric oxide may be partly mediated by changes in vascular smooth muscle responsiveness.

Previous results regarding the relationship between Lp(a) and early functional and structural changes of atherosclerosis have been inconsistent. In smaller studies, elevated Lp(a) concentration has been shown to be related to peripheral endothelial dysfunction in children with familial hypercholesterolemia who have markedly elevated LDL-C levels, but not in normcholesterolemic control children, healthy adolescents, or normal young adults. In patients with angiographically normal or minimally diseased coronary arteries, elevated plasma Lp(a) levels have been linked to impaired coronary vasomotion induced by acetylcholine infusion; however, coronary vasodilatory response to dipyridamole has not been significantly related to plasma Lp(a) levels in healthy young subjects. The results concerning the relationship between high Lp(a) levels and early arterial structural changes are also inconsistent. Elevated serum Lp(a) concentrations have been associated with subclinical carotid atherosclerosis in patients with non-insulin-dependent diabetes mellitus in some studies, but not all. Furthermore, high Lp(a) has been shown to be a risk factor for increased IMT in patients with severe hypercholesterolemia, but not in normcholesterolemic subjects. Lavrencic et al found no association between IMT and Lp(a) concentration in a pooled cohort of young patients with familial hypercholesterolemia and controls. By contrast, the large data set from the Atherosclerosis Risk in Communities study has shown that Lp(a) concentration is weakly but significantly correlated with increased IMT values, both in white and African American men and women.

**Mechanisms**

Owing to its structural homology with LDL and plasminogen, Lp(a) has both atherogenic and thrombogenic potential. It is not yet known, however, whether Lp(a) has a role in the early phases (initiation, development) or late phases (thrombosis) of occlusive arterial disease, or whether the associated cardiovascular risk is mediated by some other mechanism. Several mechanisms have been proposed to explain the association between Lp(a) and atherosclerosis. In vitro, Lp(a) migrates to the vessel wall binds to macrophages, may be internalized in these cells after oxidative modification, and subsequently may be found in atherosclerotic plaques. Lp(a) has also been shown to promote the proliferation of smooth muscle cells and to enhance the expression of intracellular adhesion molecule-1 in cultured human umbilical vein endothelial cells. There are fewer in vivo studies of the potentially proatherogenic effects of Lp(a), however, and our current data support the suggestion that elevated Lp(a) levels might confer risk by potential effects on thrombogenesis, rather than by promoting early atherogenic events.

**Methodology**

Recently, developments in ultrasound have provided methods for the noninvasive study of the functional and structural changes that occur in arteries in early atherosclerosis in vivo, and therefore offer an opportunity to assess the relative importance of different vascular risk factors in the preclinical stages of atherosclerosis. In this study, we have used a recently described and validated test of arterial endothelial function that reflects mainly the endothelium-dependent release of nitric oxide in response to a physical stimulus (shear stress).

Previous in vitro and in vivo data have implicated arterial endothelial dysfunction as a key early event in atherosclerosis, preceding plaque formation and clinical events. Our current observations about Lp(a) therefore suggest that this factor does not influence endothelial function in otherwise healthy subjects. Endothelial function tested by the currently described method in the brachial artery correlates well with coronary endothelial function and with the angiographically determined extent of coronary atherosclerosis. Endothelium-independent, smooth-muscle-dependent dilation was studied by measuring the arterial dilator response to sublingual nitrates, which produce vasorelaxation by the cGMP pathway. Early structural changes were studied by measuring the IMT of the common carotid artery. This measurement also correlates significantly with traditional vascular risk factors and the severity and extent of coronary, carotid, and femoral atherosclerotic plaques, and also predicts the likelihood of future cardiovascular events in at-risk population groups. Both these surrogate measures of early atherosclerosis may be measured accurately and reproducibly in human subjects.

**Limitations**

The present study examined the relationships between Lp(a) and arterial reactivity and early atherosclerosis cross-sectionally. A more ideal approach would be prospective study of subjects before and after therapeutic interventions aimed at altering serum Lp(a) levels. Because Lp(a) levels are mainly determined genetically and there is no effective means to reduce Lp(a) levels without simultaneously affecting the levels of other lipoproteins, such an interventional study would be difficult to perform. We have studied only those volunteers approached and willing to consent to studies on the effects of risk factors on arterial physiology, and therefore some selection bias may be present. Nevertheless,
the asymptomatic subjects studied noninvasively presented with a wide range of ages, cholesterol and blood pressure levels, and smoking histories, which include the average population values. The number of subjects with data on arterial structure was limited, and this subgroup included very few smokers. Nevertheless, significant associations were present in this group between IMT and lipid risk factors other than Lp(a).

Conclusions

In summary, we did not find any influence of Lp(a) levels on either the functional or structural vascular changes associated with early stages of atherosclerosis in asymptomatic subjects. These data suggest that elevated Lp(a) levels do not confer cardiovascular risk by promoting early atherogenesis in vivo.

Acknowledgments

This study was financially supported by the Academy of Finland (O.T.R.) and by the Medical Foundation of Sydney University, Australia (D.S.C.).

References


Effect of Lp(a) on the Early Functional and Structural Changes of Atherosclerosis
Olli T. Raitakari, Mark R. Adams and David S. Celemajer

doi: 10.1161/01.ATV.19.4.990
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 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/19/4/990

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/