Lipoprotein-Associated Phospholipase A$_2$ Is an Independent Marker for Coronary Endothelial Dysfunction in Humans


Objective—The purpose of the current study was to determine whether lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) is associated with coronary endothelial dysfunction and is a predictor of endothelial dysfunction in humans.

Methods and Results—Patients (172) with no significant coronary artery disease (<30% stenosis) undergoing assessment of coronary endothelial function were studied. Endothelial function was assessed by the change in coronary blood flow and coronary artery diameter in response to intracoronary acetylcholine. Plasma concentrations of Lp-PLA$_2$ were measured, and patients were divided into tertiles. Patients in tertiles 2 and 3 had a significantly lower change in coronary blood flow (63.8±73.2 and 32.0±71.7 versus 78.4±73.2%; P<0.001) and greater epicardial coronary artery vasoconstriction (−14.1±14.7 and −23.3±25.1 versus −9.5±15.2% mean diameter change; P<0.001) in response to acetylcholine. Patients with coronary endothelial dysfunction had significantly higher serum concentrations of Lp-PLA$_2$ than those with normal endothelial function (246.2±71.6 versus 209±56.7 ng/mL; P=0.001). The odds ratio for coronary endothelial dysfunction in patients with Lp-PLA$_2$ in the highest tertile was 3.3 (95% CI, 1.6 to 6.6).

Conclusions—Lp-PLA$_2$ is independently associated with coronary artery endothelial dysfunction and is a strong predictor of endothelial dysfunction in humans.

Key Words: lipoprotein-associated phospholipase A$_2$ ■ endothelial function ■ inflammatory markers

Coronary artery disease is the leading cause of morbidity and mortality in Western society and is a worldwide epidemic. The identification of patients at risk for coronary events and those in the early stages of atherosclerosis is essential for primary prevention. Predicting cardiac events, however, can be challenging, and current imaging techniques are limited in their ability to detect early atherosclerosis. Coronary endothelial dysfunction can be considered a marker for early atherosclerosis and has been shown to be associated with an increased risk of ischemic cardiac events and stroke. A systemic biomarker that is an independent predictor of coronary endothelial dysfunction would be valuable in identifying patients in the early stages of coronary atherosclerosis and those who are at risk for future cardiac events.

See page 5

Four population-based studies have shown that elevated lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) levels are associated with an increased risk of coronary heart disease and ischemic stroke. Lp-PLA$_2$ is a member of the phospholipase A$_2$ family of enzymes and is a 45.4-kDa protein produced by macrophages, T lymphocytes, and mast cells. Approximately 80% of Lp-PLA$_2$ circulates bound to low-density lipoprotein (LDL), whereas the other 20% is bound to high-density lipoprotein (HDL). Lp-PLA$_2$ hydrolyzes the sn2 ester bond in phospholipids of which the fatty acid moiety has been shortened or altered by oxidation to yield oxidized fatty acid and lysophosphatidylcholine. These metabolites have proinflammatory properties, and lysophosphatidylcholine has been shown to have adverse effects on endothelial function. Lp-PLA$_2$ could, therefore, play a direct role in the development of endothelial dysfunction and coronary artery disease. In addition, it may also serve as a useful biomarker for predicting coronary endothelial dysfunction.

The current study was performed to test the hypothesis that Lp-PLA$_2$ is associated with coronary endothelial dysfunction and is an independent predictor of endothelial dysfunction.

Methods

The study was approved by the Institutional Review Board at the Mayo Clinic. Written informed consent was obtained from patients ≥18 years of age undergoing coronary angiography and coronary endothelial function assessment for the assessment of chest pain. Because epicardial coronary artery disease, coronary bridging, and cardiomyopathy can alter coronary blood flow measurements, patients with a ≥30% epicardial coronary artery stenosis, coronary artery bridging in any segment of the left anterior descending
coronary artery, and an ejection fraction <40% were excluded. Patient characteristics and past medical history were obtained from a review of medical records. Blood samples for routine clinical laboratories (complete blood count, serum electrolytes, lipid profile, and C-reactive protein) were drawn in the fasting state 48 hours before the procedure. Blood samples for Lp-PLA₂ were drawn at the time of coronary angiography once it was determined that the patient had none of the exclusion criteria and could be included in the study. Systemic hemodynamics were invasively measured at the time of cardiac catheterization. Patients were instructed to not take any vasoactive medications within 48 hours of coronary angiography to prevent interference with endothelial function assessment.

Assessment of Coronary Endothelial Function

After diagnostic angiography and the exclusion of significant obstructive coronary artery disease, endothelium-dependent coronary vasoactivity was assessed as described previously. A 6- or 7-French guiding catheter was placed in the left main, and a Doppler guide wire (Flowire, Volcano Inc) within a coronary-infusion catheter (Ultrafuse, SciMed Life System) was positioned into the midportion of the left anterior descending coronary artery. Intracoronary acetylcholine was used to assess endothelial function. The direct response to acetylcholine is smooth muscle cell contraction and vasoconstriction. However, in patients with normal endothelial function, this effect is counterbalanced by vasodilatation because of the release of nitric oxide from the endothelium. Intracoronary acetylcholine at increasing concentrations of 10⁻⁶, 10⁻⁷, and 10⁻⁸ M was infused to obtain effective coronary concentrations of 10⁻⁵, 10⁻⁶, and 10⁻⁷ M, respectively. Each dose of acetylcholine was infused for 3 minutes through the infusion catheter into the left anterior descending coronary artery to assess endothelium-dependent vasoactivity.

To assess coronary endothelial function, coronary artery diameter and coronary blood flow were measured and calculated after each infusion of acetylcholine. The coronary artery diameter was measured by an independent investigator in the segment 5 mm distal to the tip of the Doppler wire. Measurements were made using an online quantitative coronary angiography program (Medis Corporation). The systematic and random errors of the quantitative coronary angiography system have been validated previously, and the system has been shown to have small, nonsignificant systematic, and random errors. The reproducibility of the measurements from the current laboratory is ±2%. Coronary blood flow (CBF) was determined as \( \pi \times (\text{coronary artery diameter}/2)^2 \times \text{average peak velocity (APV)/2} \). The velocity signals are instantaneously obtained from the Doppler wire by an online fast Fourier transform. Two previous studies have validated the velocity signals obtained by this method, and an analysis of data from the current laboratory demonstrated that the variation in repeated measurements is ±3%.

According to previous studies correlating the response to intracoronary acetylcholine and clinical outcomes, “microvascular endothelial dysfunction” was defined as an increase in CBF ≤50%, and “epicardial endothelial dysfunction” was defined as a change in epicardial coronary artery diameter less than or equal to −20% in response to the maximum dose of acetylcarnine. In addition, a prior study by Zeiher et al demonstrated that an increase in CBF <50% in response to acetylcholine is associated with exercise-induced ischemia in the setting of normal epicardial coronary arteries. Endothelial dysfunction was defined as having microvascular or epicardial endothelial dysfunction.

Lp-PLA₂ Measurement

Lp-PLA₂ levels were measured in plasma aliquots that were obtained at the time of coronary angiography and stored at −70°C using an enzyme-linked immunosorbent assay (PLAC test, diaDexus, Inc). Samples were incubated in microtiter plate wells with an immobilized monoclonal antibody (2C10) against Lp-PLA₂. A secondary monoclonal antibody (4B4) labeled with horseradish peroxidase was used to identify the enzyme, and recombiant Lp-PLA₂ was used as the standard reference. The range of detection was 50 to 1000 ng/mL, and the intraassay coefficients of variation were 7.8% at 276 ng/mL, 6.1% at 257 ng/mL, and 13.5% at 105 ng/mL. The 2C10 monoclonal antibody against Lp-PLA₂ has been shown to have no cross-reactivity with other A₂ phospholipases. All of the assays were performed by a single investigator, who was blinded to the clinical characteristics and results of endothelial function assessment.

Statistical Analysis

Patients were divided into 3 groups defined by the tertiles of the Lp-PLA₂ distribution. Continuous variables with little-to-mild skew were presented as mean±SD, and skewed measures were presented as median and interquartile ranges (IQR). Discrete variables were summarized as frequencies and within-group percentages. A trend for group differences across the 3 groups was tested using linear contrasts in association with 1-way ANOVA for continuous variables (with log transformation of the skewed variables) and the Armitage test for trend for discrete variables.

The association between Lp-PLA₂ tertiles and endothelial function independent of other covariates was assessed using linear regression models. Linear contrast was used to test for trend in endothelial function across Lp-PLA₂ tertiles. Covariates were selected for model adjustment if they were significantly associated with Lp-PLA₂ at the 0.10 significance level. The correlation between continuous covariates and Lp-PLA₂ was measured with Spearman’s rank correlation coefficient. The associations between dichotomous variables and logged values of Lp-PLA₂ were tested with Student t tests. Age was not significantly associated with Lp-PLA₂ but was included for clinical relevance. The covariates included in the models were age, sex, body mass index, creatinine (log-transformed), total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides (log-transformed), and use of lipid lowering medication.

Results

Patient Characteristics

Patients (172) with Lp-PLA₂ concentrations ranging from 110 to 443 ng/mL were studied. Patients were divided into 3 groups based on the following tertiles of Lp-PLA₂: tertile 1 (110 to 181.4 ng/mL; n = 57), tertile 2 (181.48 to 239.6 ng/mL; n = 58), and tertile 3 (240 to 443 ng/mL; n = 57). Compared with patients in the lowest tertile (Table 1), there was a significant trend for a greater percentage of men in tertiles 2 and 3 (47% and 54% versus 14%; \( P = 0.001 \)) and a greater serum creatinine [1.0 (IQR, 0.9 to 1.1) and 1.1 (0.9 to 1.2) versus 1.0 (0.8 to 1.0) mg/dL; \( P = 0.013 \)]. Patients in tertiles 2 and 3 also had a significantly greater total cholesterol (181.3 ± 43.4 and 193.3 ± 37.1 versus 169.2 ± 36.0 mg/dL; \( P = 0.001 \)), higher LDL (106.1 ± 35.5 and 115.9 ± 29.5 versus 88.6 ± 27.7 mg/dL; \( P < 0.001 \)), lower HDL (48.7 ± 16.2 and 46.5 ± 15.5 versus 56.2 ± 15.6 mg/dL; \( P = 0.001 \)), and higher triglycerides [121.5 (IQR, 87 to 160) and 125.0 (78 to 205) versus 98 (60 to 147) mg/dL; \( P = 0.005 \)]. There was no significant difference among the 3 groups in terms of age, prevalence of hypertension and diabetes, smoking history, C-reactive protein, and use of cardiac medications (aspirin, β-blockade, angiotensin-converting enzyme inhibitors, and lipid-lowering agents).
Endothelial Function

Microvascular endothelial function as determined by the percentage change in CBF in response to the maximal dose of intracoronary acetylcholine is shown in Figure 1. Patients in tertiles 2 and 3 had a significantly lower percentage change in CBF than those in tertile 1 (63.8 ± 73.2 and 32.0 ± 71.7 versus 78.4 ± 73.2%; \( P = 0.001 \)). This difference remained significant (\( P = 0.008 \)) after adjusting for age, sex, body mass index, serum creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and the use of lipid-lowering medications.

Epicardial endothelial function as determined by the percentage change in coronary artery diameter in response to the maximal dose of acetylcholine is shown in Figure 2. Patients in tertiles 2 and 3 had a greater reduction in coronary artery diameter than those in the lowest tertile (14.1 ± 14.7 and 23.3 ± 25.1 versus 9.5 ± 15.2%; \( P = 0.001 \)). After adjusting for age, sex, body mass index, serum creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and use of lipid-lowering medications, the trend remained significant (\( P = 0.006 \)).

As shown in Figure 3, patients with coronary endothelial dysfunction had a significantly higher serum concentration of Lp-PLA₂ (209 ± 56.7 versus 246.2 ± 71.6; \( P = 0.001 \)). The odds ratio of coronary endothelial dysfunction (Table 2) for patients in the highest tertile of Lp-PLA₂ was 3.3 (95% CI, 1.6 to 6.6).

Endothelial-Independent Function

There was no difference in the change in coronary artery diameter in response to intracoronary nitroglycerin among the 3 groups. The CFR was also similar between the 3 groups (Table 1).

Analysis in Patients Not on Lipid-Lowering Agents

A subset analysis was performed in the 103 patients not taking lipid-lowering agents. Patients were divided into 3...
TABLE 2. Odds Ratio for Coronary Endothelial Dysfunction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp-PLA₂ &gt;240 ng/mL</td>
<td>3.3</td>
<td>1.6 to 6.6</td>
</tr>
<tr>
<td>Total cholesterol &gt;200 mg/dL</td>
<td>1.2</td>
<td>0.57 to 2.5</td>
</tr>
<tr>
<td>LDL &gt;160 mg/dL</td>
<td>1.2</td>
<td>0.30 to 4.9</td>
</tr>
<tr>
<td>HDL &lt;40 mg/dL</td>
<td>2.2</td>
<td>1.0 to 4.5</td>
</tr>
<tr>
<td>Triglycerides &gt;150 mg/dL</td>
<td>1.8</td>
<td>0.9 to 3.7</td>
</tr>
</tbody>
</table>

Discussion

The current study demonstrates that circulating Lp-PLA₂ levels are associated with coronary endothelial dysfunction independent of other cardiac risk factors and are elevated in patients with early atherosclerosis as detected by endothelial dysfunction. Furthermore, Lp-PLA₂ is an independent predictor of coronary endothelial dysfunction. These results support a role for Lp-PLA₂ as a biomarker for coronary endothelial dysfunction and early coronary atherosclerosis in humans.

Previous studies have shown that Lp-PLA₂ is associated with cardiac events and risk factors. In our study, there was an association between Lp-PLA₂ and serum lipid concentrations, which is consistent with the results of previous studies. There appears to be a direct relationship between Lp-PLA₂ and total cholesterol, LDL, and triglycerides and an inverse relationship with HDL. Because Lp-PLA₂ is bound to LDL, these relationships may be attributable to the close association between the two. Our study also showed that Lp-PLA₂ levels are higher in men than women, which is consistent with results from the Rotterdam study. The current study supports the lack of association between age and Lp-PLA₂, a finding that was also shown in the West of Scotland Coronary Prevention trial (WOSCOPS) and the Arteriosclerosis Risk in Communities study (ARIC), and Rotterdam studies. Finally, although Lp-PLA₂ is a marker of inflammation, it appears to be independent of C-reactive protein. This finding is supported by previous studies and underscores the unique and novel characteristics of Lp-PLA₂ as a marker of coronary endothelial dysfunction and early atherosclerosis.

In addition to cardiac events, Lp-PLA₂ has been associated with an increase in the risk of stroke. The results of the current study suggest that the mechanism may involve endothelial dysfunction, which has been shown to be a risk factor for stroke. Endothelial dysfunction promotes an inflammatory, proliferative, and procoagulatory milieu, which may contribute to thromboembolic stroke. In addition, vasospasm secondary to endothelial dysfunction may also result in ischemic damage to the walls of small blood vessels and subsequent intracerebral hemorrhage.

Despite the association with other cardiac risk factors, the current study found that Lp-PLA₂ is independently associated with coronary artery endothelial dysfunction and is a strong predictor of endothelial dysfunction in patients with no significant coronary artery disease. In addition, the effects of Lp-PLA₂ on endothelial function remain significant even after the analysis of patients who were not taking lipid-lowering agents. This suggests that Lp-PLA₂ may play a direct role in endothelial damage and the initiation of cardio-
nary atherosclerosis. The mechanism may involve the inflammatory process, which has been shown to be present in the early stages of coronary artery disease. The production and release of Lp-PLA₂ by lymphocytes and macrophages may become increased under inflammatory conditions. The metabolism of oxidized phospholipids by Lp-PLA₂ increases the circulating levels of oxidized free fatty acid and lysophosphatidylcholine. Lysophosphatidylcholine can then have adverse effects on endothelial function via increased oxidative stress, modulation of leukocyte chemotaxis, and inhibition of endothelial cell migration to sites of endothelial damage. Coronary endothelial dysfunction and the development of atherosclerosis may be the mechanism by which Lp-PLA₂ becomes increased under inflammatory conditions. The mechanism may involve the inflammatory process, which has been shown to be present in the early stages of coronary artery disease.18 The production and release of Lp-PLA₂ by lymphocytes and macrophages may become increased under inflammatory conditions. The metabolism of oxidized phospholipids by Lp-PLA₂ increases the circulating levels of oxidized free fatty acid and lysophosphatidylcholine. Lysophosphatidylcholine can then have adverse effects on endothelial function via increased oxidative stress, modulation of leukocyte chemotaxis, and inhibition of endothelial cell migration to sites of endothelial damage.

Coronary endothelial dysfunction and the development of atherosclerosis may be the mechanism by which Lp-PLA₂ results in increased cardiac events. Thus, Lp-PLA₂ may play a significant role in the pathophysiology and mechanism of coronary atherosclerosis.

A direct role in the initiation of coronary endothelial dysfunction and the development of early coronary artery disease would make Lp-PLA₂ an ideal and novel biomarker for identifying patients at risk for future cardiac events. Lp-PLA₂ could also be a potential therapeutic target. Although statins have been shown to have anti-inflammatory properties, a specific inhibitor to an inflammatory mediated process may provide additional therapeutic benefits in reducing cardiac events. Novel inhibitors to Lp-PLA₂ have been developed and are currently under investigation.

To our knowledge, this is the first study to demonstrate that Lp-PLA₂ is an independent predictor for coronary endothelial dysfunction. The strength of the current study is that coronary endothelial function was directly assessed with intracoronary acetylcholine, and coronary angiography was performed to exclude patients with preexisting coronary artery disease.

In conclusion, Lp-PLA₂ is a predictor of coronary endothelial dysfunction. This relationship is independent of other cardiac risk factors and supports the role of Lp-PLA₂ as a novel biomarker for early coronary artery disease and endothelial dysfunction.

Acknowledgments

We thank DiaDexus Inc for providing the Lp-PLA₂ assay.

References


### TABLE 3. Characteristics of Patients not on Lipid-Lowering Therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1 (n=34)</th>
<th>Tertile 2 (n=35)</th>
<th>Tertile 3 (n=34)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.1±13.9</td>
<td>45.1±11.7</td>
<td>47.8±10.8</td>
<td>0.83</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>7 (21)</td>
<td>16 (46)</td>
<td>18 (53)</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>15 (45)</td>
<td>8 (23)</td>
<td>16 (47)</td>
<td>0.88</td>
</tr>
<tr>
<td>Diabetes, no. (%)</td>
<td>0 (0)</td>
<td>2 (6)</td>
<td>4 (12)</td>
<td>0.038</td>
</tr>
<tr>
<td>Hyperlipidemia, no. (%)</td>
<td>11 (33)</td>
<td>15 (43)</td>
<td>16 (47)</td>
<td>0.25</td>
</tr>
<tr>
<td>History of smoking, no. (%)</td>
<td>7 (21)</td>
<td>13 (37)</td>
<td>16 (47)</td>
<td>0.022</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.3±6.0</td>
<td>28.2±6.0</td>
<td>29.7±6.9</td>
<td>0.012</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg), median (IQR)</td>
<td>93.5 (87.0 to 104.0)</td>
<td>94.0 (86.0 to 105.0)</td>
<td>93.0 (84.0 to 109.0)</td>
<td>0.093</td>
</tr>
<tr>
<td>Creatinine (mg/dL), median (IQR)</td>
<td>1.0 (0.8 to 1.0)</td>
<td>1.0 (0.9 to 1.1)</td>
<td>1.1 (0.9 to 1.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>186.9±43.0</td>
<td>181.5±42.5</td>
<td>200.0±34.3</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>103.8±33.8</td>
<td>110.7±35.2</td>
<td>119.4±28.2</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>58.4±18.3</td>
<td>46.0±14.1</td>
<td>47.5±15.9</td>
<td>0.007</td>
</tr>
<tr>
<td>Triglycerides (mg/dL), median (IQR)</td>
<td>89.0 (53 to 114)</td>
<td>122.0 (78 to 158)</td>
<td>132.0 (86 to 233)</td>
<td>0.005</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL), median (IQR)</td>
<td>0.2 (0.1 to 0.7)</td>
<td>0.2 (0.1 to 0.5)</td>
<td>0.3 (0.1 to 0.9)</td>
<td>0.30</td>
</tr>
<tr>
<td>Coronary flow reserve, median (IQR)</td>
<td>2.9 (2.6 to 3.1)</td>
<td>3.1 (2.6 to 3.5)</td>
<td>3.0 (2.7 to 3.6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aspirin, no. (%)</td>
<td>19 (56)</td>
<td>13 (37)</td>
<td>14 (41)</td>
<td>0.22</td>
</tr>
<tr>
<td>β blocker, no. (%)</td>
<td>11 (32)</td>
<td>7 (20)</td>
<td>7 (21)</td>
<td>0.26</td>
</tr>
<tr>
<td>ACE inhibitor, no. (%)</td>
<td>10 (29)</td>
<td>4 (11)</td>
<td>4 (12)</td>
<td>0.06</td>
</tr>
<tr>
<td>% change in coronary blood flow with acetylcholine</td>
<td>81.2±71.9</td>
<td>62.5±82.4</td>
<td>45.3±71.3</td>
<td>0.05</td>
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<tr>
<td>% change in coronary artery diameter with acetylcholine</td>
<td>−8.1±14.4</td>
<td>−15.3±16.0</td>
<td>−21.2±23.9</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Test for trend.
Yang et al Lp-PLA2 and Endothelial Function


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