Association of Lipoprotein-Associated Phospholipase A$_2$ Mass and Activity With Calcified Coronary Plaque in Young Adults

The CARDIA Study

Carlos Iribarren, Myron D. Gross, Jeanne A. Darbinian, David R. Jacobs Jr, Stephen Sidney, Catherine M. Loria

Objective—To examine the association of lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) mass and activity with calcified coronary plaque in young adults.

Methods and Results—Nested case-control study among CARDIA participants at the year 15 examination (2000 to 2001, 33 to 45 years old). Cases ($n=266$) were those with and controls ($n=266$) those without evidence of calcified coronary plaque by computed tomography matched 1:1 on sex and race. Lp-PLA$_2$ mass and activity were significantly higher in cases ($296\pm101$ ng/mL and $36.4\pm12.3$ nmol/mL per minute) than in controls ($267\pm80$ ng/mL and $32.9\pm11.8$ nmol/mL per minute). In age-adjusted conditional logistic regression, the odds ratio (OR) of calcified coronary plaque per 1 standard deviation (SD) increment was 1.40 (95% CI, 1.17 to 1.67) and 1.39 (95% CI, 1.14 to 1.70) for Lp-PLA$_2$ mass and activity, respectively. After adjusting for multiple covariates including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and C-reactive protein, a statistically significant association remained for Lp-PLA$_2$ mass (OR, 1.28; 95% CI, 1.03 to 1.60) but not for activity (OR, 1.09; 95% CI, 0.84 to 1.42). No evidence was found for interaction between Lp-PLA$_2$ mass or activity with LDL-C as predictors of calcified coronary plaque.

Conclusion—An independent association of Lp-PLA$_2$ mass with calcified coronary plaque existed in young adults. Therefore, Lp-PLA$_2$ mass may be a useful marker of subclinical cardiovascular risk. (Arterioscler Thromb Vasc Biol. 2005;25:216-221.)

Key Words: phospholipases ■ coronary calcification ■ young adults ■ atherosclerosis ■ risk factors
first quartile and after adjusting for clinical and metabolic factors.17

The aims of this investigation were to describe the demographic, lifestyle, and biochemical correlates of Lp-PLA2 mass and activity in a sample of white and black young adults and to characterize the association of Lp-PLA2 mass and activity with the presence of calcified coronary plaque ascertained by cardiac computed tomography (CT).

Methods
The Coronary Artery Risk Development in Young Adults (CARDIA) study is an ongoing investigation of heart disease risk factors and subclinical CAD among black and white men and women aged 18 through 30 years at study inception in 1985 to 1986. More details of study design, recruitment, and procedures have been published elsewhere.18,19 Participants were recruited in 4 field centers: Birmingham, Ala; Chicago, Ill; Minneapolis, Minn; and Oakland, Calif. A nested case-control study design was used with data collected at the CARDIA Year 15 examination (June 2000 to October 2001), in which the participants were between the ages of 33 and 45. Cases (n=266) were all study participants who had evidence of calcified coronary plaque (ie, coronary calcium score >0), and controls were members with a calcium score of zero individually matched to cases based on sex and race. Of the original 282 cases, 16 were excluded in the final analysis for missing data on covariates of interest.

Age, race/ethnicity, and educational attainment were ascertained by self-report. Alcohol and tobacco use were ascertained by self-report and by interviewer-administered questionnaire if the participant reported ever drinking or smoking.20 Blood samples were drawn after an overnight fast. The protocols for lipid determination can be found elsewhere.21–23 Anthropometric measures (weight, height, waist and hip girths) were performed according to the CARDIA study protocol.24 Body mass index was calculated as weight/height2. C-reactive protein (CRP) was measured with an ultrasensitive enzyme-linked immunosorbent assay based on purified protein and polyclonal anti-CRP antibodies (Calbiochem).25 Hypertension was defined as self-report of hypertension and/or systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of antihypertensive medication. Diabetes was defined as self-report of diabetes mellitus and/or fasting blood glucose ≥126 mg/dL and/or use of antidiabetic medication. Hormone replacement therapy and use of cholesterol lowering medication were ascertained by self-report. The institutional review boards of the field centers approved the study, and all participants gave informed written consent.

Measurement of Lp-PLA2 Mass and Activity
Lp-PLA2 mass was measured using a 2-site enzyme-linked immunosorbent assay (diaDexus Inc, South San Francisco, Calif) on serum samples stored at −70°C for an average of 18 months.27 The highest standard in the assay is 1000 ng/mL and the lowest limit of detection is 1 ng/mL. The interassay coefficient of variation was 20%. The correlation between split Lp-PLA2 mass measurements in 119 randomly selected samples was 0.91.

Lp-PLA2 activity was measured at the Molecular Epidemiology and Biomarker Research Laboratory (University of Minnesota, Minneapolis) using a method described by Stafforini et al28 with slight modifications. We used Sep-Pak Plus C18 cartridges from Waters instead of Kaker Chemical Co, and our units are reported as nmol/mL per minute instead of μmol/mL per hour. The substrate for this analysis was obtained from Avanti Polar Lipids part number 840009. The interassay coefficient of variation was 9%, and in 294 split sample pairs the correlation was 0.76.

CT Scanning Protocol
Two scans were obtained on each participant, 1 to 2 minutes apart. Participants were scanned in supine position (without getting off the table between scans) over a hydroxyapatite phantom to adjust for scanner and participant differences in CT attenuation numbers (ie, Hounsfield units) across field centers. The Chicago and Oakland field centers used Imatron C-150 scanners, the Birmingham field center used a GE Lightspeed Qxi scanner, and the Minneapolis field center used a Siemens VZ scanner. An experienced and trained CT image analyst (blinded to norcan data and to the order of images) identified the courses of the coronary arteries using specially developed image-processing software and calculated an Agaston score modified to account for slice width and adjusted to standardized CT attentuations values based on the calibration phantom, which was imaged with each participant.29 Additional details of the phantom calibration and CT protocol can be found in our previous publications.30,31 In the analysis, we used the mean of the 2 readings. There were 2921 (96.5%) concordant positive (score >0) or concordant negative (score =0) scans and 107 (3.5%) discordant scans (ie, one zero scan and one nonzero scan). Of the discordant scans, 9 (8%), 33 (31%), 4 (4%), 56 (%), and 5 (5%) were adjudicated to definitely negative, probably negative, cannot tell, probably positive, and definitely positive, respectively. For the first 3 categories, the adjudicated score was set to zero; for the last 2 categories, the adjudicated score was set to the score of the positive scan. A second expert reader who was not from the Reading Center trained in the software usage reviewed 100 discordant scan pairs using the same adjudication process. The agreement was 91%.

Statistical Analysis
Univariate case-control differences were ascertained using the t test for normally distributed continuous variables, the Wilcoxon rank-sum test for continuous variables that were not normally distributed (alcohol intake, triglycerides and CRP), and the χ2 test for categorical variables. To assess bivariate associations of Lp-PLA2 mass, height and activity with other covariates, we computed means and SD (or proportions) of the covariates according to sex-/race-specific tertiles of Lp-PLA2 mass and activity, respectively. Tests for linear trend were estimated using the significance of Pearson correlation coefficients for continuous variables and the Cochran–Armitage test for 2 by n categorical variables. Heterogeneity of means across groups was assessed with analysis of variance (ANOVA). Conditional logistic regression was then applied to quantify the relative odds of calcified coronary plaque (ie, calcium score >0 versus calcium score =0) associated with 1 (sex- and race-specific) SD linear increment in Lp-PLA2 mass and activity, respectively. We also implemented conditional logistic models with Lp-PLA2 mass and activity entered as [sex- and race-specific] tertiles 2, 3 with tertile 1 as the reference group. Both the SD and the tertile cutoffs were estimated among controls. Because of the high correlation between mass and activity, we performed separate models for mass and activity. Three sequential models were fitted: model 1, adjusting for age only (sex and race are adjusted for by design); model 2, adjusting for (in addition to age) education attainment, alcohol consumption, cigarette smoking, body mass index, waist circumference, diabetes and hypertension; model 3, further adjusting for LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides; and model 4, further adjusting for CRP. We also considered a variation of model 3 that included only LDL-C.

Because of previous epidemiological evidence indicating a stronger association of Lp-PLA2 mass among individuals with low LDL-C, we tested the significance (in fully adjusted models) of interactions of Lp-PLA2 mass and activity with LDL-C as predictors of calcified coronary plaque. Because none of the interactions was statistically significant (P for interaction between Lp-PLA2 mass and LDL-C=0.52; P for interaction between Lp-PLA2 activity and LDL-C=0.68), we did not perform stratified analyses by LDL-C level. The SAS software, version 8 (SAS Institute), was used in all statistical analyses.

To examine whether there is a quantitative relationship between Lp-PLA2 mass and activity and coronary artery calcium (not just presence or absence of calcified coronary plaque), we used an alternative analytical approach consisting of linear regression models with log (calcium score +1) as the dependent variable, Lp-PLA2 mass and activity with the presence of calcified coronary plaque ascertained by cardiac computed tomography (CT).
Results

Compared with controls, cases tended to be slightly older (by 1.5 years) and had lower educational attainment (Table 1). Cases were significantly more likely to be current smokers, to have a history of hypertension, and to be using cholesterol-lowering medication. Although diabetes was twice as common in cases as in controls, this difference did not reach statistical significance. Hormone replacement therapy was very rare in our sample (<3%) and did not differ by case-control status. Waist circumference, LDL-C, and triglycerides were significantly higher, whereas HDL-C was significantly lower in cases compared with controls. Both Lp-PLA₂ mass and activity were significantly higher in cases than in controls. However, no statistically significant differences were found for alcohol intake, body mass index, and CRP level between cases and controls. Among the cases, the average (±SD) calcium score was 85 (± 272); approximately half of the cases had a calcium score <20 and 32% had a calcium score of ≥50. In descriptive analysis combining cases and controls (Figure 1), Lp-PLA₂ mass was highest in white men, followed by white women, black men, and black women (P ANOVA <0.0001). In turn, Lp-PLA₂ activity was highest in white men, followed by black men, white women, and black women (P ANOVA <0.0001).

Means of waist circumference, LDL-C, and triglycerides increased and HDL-C decreased linearly with the levels of both Lp-PLA₂ mass and activity (Table 2). There were also significant positive linear trends of age and educational attainment across levels of Lp-PLA₂ activity. There were no discernible trends with all the other covariates. The Pearson linear correlation between Lp-PLA₂ mass and activity was 0.61; Lp-PLA₂ mass correlated 0.39 with LDL-C and −0.25 with HDL-C; Lp-PLA₂ activity correlated 0.52 with LDL-C and −0.33 with HDL-C. The Spearman rank-order correlation with triglycerides was 0.11 for mass and 0.33 for activity.

The proportion of cases with calcified coronary plaque increased in a stepwise fashion with tertiles of both Lp-PLA₂ mass and activity in both men and women (Figure 2).

In age-adjusted conditional logistic regression (model 1), Lp-PLA₂ mass and activity were significantly associated with calcified coronary plaque (odds ratio [OR] per 1 standard deviation [SD] increment = 1.40; 95% CI, 1.17 to 1.67 for mass; OR, 1.39; 95% CI, 1.14 to 1.70 for activity) (Table 3). Age-adjusted OR for the upper tertile, relative to the lowest tertile, were 2.15 (95% CI, 1.36 to 3.42) for mass and 2.40 (95% CI, 1.52 to 3.81) for activity. After adjusting for age, educational attainment, smoking status, alcohol consumption, body mass index, waist circumference, diabetes, and hypertension (model 2), the risk relations did not change appreciably. Further adjustment for LDL-C, HDL-C, and triglycerides (model 3) resulted in attenuation of the strength of the associations but, whereas Lp-PLA₂ mass remained significantly related, Lp-PLA₂ activity was no longer significantly related to calcified coronary plaque. In a variation of model 3 with entry of LDL-C alone (in addition to model 2 covariates), the OR associated with 1 SD increment of Lp-PLA₂ mass was 1.29 (95% CI, 1.04 to 1.60), and the OR associated with 1 SD increment of Lp-PLA₂ activity was 1.18 (95% CI, 0.91 to 1.52). Further adjustment for CRP (Model 4) did not change the results.

In the linear model of log (calcium score +1), the slope estimates associated with 1 SD increment (standard error; P) were as follows (first value is for Lp-PLA₂ mass, second is for Lp-PLA₂ activity): 0.25 (0.07; 0.0006) and 0.26 (0.08; 0.002) after adjustment for age, sex, and race (the inclusion of sex and race in model 1 is necessary because the linear model is not matched); 0.22 (0.07; 0.003) and 0.22 (0.08; 0.007) in model 2; 0.15 (0.08; 0.06) and 0.11 (0.10; 0.27) in model 3; and 0.15 (0.08; 0.05) and 0.11 (0.09; 0.28) in model 4.

## Table 1. Characteristics of Cases and Controls: The CARDIA Study, Year 15 Examination (2000 to 2001)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=266)</th>
<th>Controls (n=266)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42.1±3.0</td>
<td>40.5±3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex/race groups (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White men</td>
<td>131 (49)</td>
<td>131 (49)</td>
<td>1.00</td>
</tr>
<tr>
<td>Black men</td>
<td>58 (22)</td>
<td>58 (22)</td>
<td></td>
</tr>
<tr>
<td>White women</td>
<td>44 (17)</td>
<td>44 (17)</td>
<td></td>
</tr>
<tr>
<td>Black women</td>
<td>33 (12)</td>
<td>33 (12)</td>
<td></td>
</tr>
<tr>
<td>Educational attainment, y</td>
<td>14.3±2.7</td>
<td>15.0±2.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Cigarette smoking status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>128 (48)</td>
<td>159 (60)</td>
<td>0.001</td>
</tr>
<tr>
<td>Current</td>
<td>85 (32)</td>
<td>48 (18)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>192 (72)</td>
<td>229 (86)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>74 (28)</td>
<td>37 (14)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol-lowering agents (%)</td>
<td>245 (92)</td>
<td>255 (96)</td>
<td>0.07</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (8)</td>
<td>11 (4)</td>
<td></td>
</tr>
<tr>
<td>Hormone replacement therapy (%)</td>
<td>246 (93)</td>
<td>257 (99)</td>
<td>0.04</td>
</tr>
<tr>
<td>No</td>
<td>20 (8)</td>
<td>9 (3)</td>
<td></td>
</tr>
<tr>
<td>Mean alcohol intake, mL/day†</td>
<td>6.3±5.0</td>
<td>5.2±4.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.9±5.9</td>
<td>28.1±5.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>93.7±13.7</td>
<td>90.6±12.5</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>124±38</td>
<td>112±32</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>46±14</td>
<td>49±15</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides, mg/dL†</td>
<td>107±2</td>
<td>93±2</td>
<td>0.0003</td>
</tr>
<tr>
<td>C-reactive protein, mg/L†</td>
<td>1.37±2</td>
<td>1.26±2</td>
<td>0.12</td>
</tr>
<tr>
<td>Lp-PLA₂ mass, mg/mL</td>
<td>296±101</td>
<td>267±80</td>
<td>0.0004</td>
</tr>
<tr>
<td>Lp-PLA₂ activity, nmol/mL/min</td>
<td>36.4±12.3</td>
<td>32.9±11.8</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Entries are means±SD or percents.

*Alcohol intake for individuals who self-reported any alcohol consumption during past year.

†Geometric mean and standard deviation.
Discussion

This study documents, for the first time to our knowledge in young adults, an independent association of Lp-PLA2 mass with presence of calcified coronary plaque and amount of coronary calcium and thus supports the notion that Lp-PLA2 may be relevant to atherogenesis. In this regard, recent evidence indicates that Lp-PLA2 has great affinity for small, dense LDL particles, known to be more susceptible to oxidative modification and to be atherogenic,33 and that Lp-PLA2 byproducts may be involved in apoptosis of macrophages and smooth muscle cells, a process that can lead to atherosclerosis progression.34

Our findings confirm and extend earlier results of the West of Scotland Coronary Prevention Study, in which Lp-PLA2 mass was a strong, independent predictor of coronary events.5 After adjustment for age, systolic blood pressure, plasma triglycerides, LDL-C, HDL-C, fibrinogen, white cell count, and CRP, an increase of 1 SD in Lp-PLA2 mass was associated, in Scottish men, with 1.18 increased hazard of coronary events (95% CI, 1.05 to 1.33). Our estimate of the risk relation with calcified coronary plaque, using a slightly different group of covariates, was 1.28 (95% CI, 1.03 to 1.60). Furthermore, in the current analysis, the OR associated with the highest tertile of Lp-PLA2 mass (2.2) was of similar magnitude to the multivariate OR observed for diabetes (2.6), hypertension (2.2), or current smoking (2.2).

An association was also found between Lp-PLA2 activity and calcified coronary plaque, but this relationship dropped below the threshold for statistical significance after adjustment for lipids, particularly LDL-C. We believe that the reason behind this attenuation is the stronger correlation between enzymatic activity and LDL-C ($r = 0.52$) than between enzymatic mass with LDL-C ($r = 0.39$). It could be argued that adjustment for LDL-C may be inappropriate in this case because Lp-PLA2 may be in the causal pathway connecting LDL oxidation with atherosclerosis, creating statistical colinearity.

Lp-PLA2 mass and activity differed significantly by sex–race groups, being highest in white men and lowest in black women. In agreement with the data from Packard et al,5 Lp-PLA2 mass and CRP were uncorrelated, suggesting that Lp-PLA2 and CRP represent different metabolic pathways.

The calcification of atheroma occurs early in plaque development as part of the inflammatory pathophysiologic cascade of CAD, and is actively regulated like bone mineralization.35

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<table>
<thead>
<tr>
<th>Tertiles of Lp-PLA2 Mass</th>
<th>Tertiles of Lp-PLA2 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
<td>Middle</td>
</tr>
<tr>
<td>Age, y</td>
<td>41.1 41.5 41.3</td>
</tr>
<tr>
<td>Educational attainment, y</td>
<td>14.9 15.0 14.8</td>
</tr>
<tr>
<td>Alcohol intake, mL/day*</td>
<td>11.0 8.0 7.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.13 28.64 28.68</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>91.11 92.13 93.18</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>108.1 115.11 131.45</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>51.12 46.26 45.42</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>10.6 14.4 22.1</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL*</td>
<td>86.9 88.9 87.9</td>
</tr>
<tr>
<td>Lp-PLA2 mass, ng/mL</td>
<td>200.3 268.52 376.08</td>
</tr>
<tr>
<td>Lp-PLA2 activity, nmol/mL/min</td>
<td>29.24 33.91 40.85</td>
</tr>
<tr>
<td>Ever smoking, %</td>
<td>34 29 37</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>6 7 6</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>20 21 21</td>
</tr>
<tr>
<td>Cholesterol-lowering agents, %</td>
<td>6 6 5</td>
</tr>
<tr>
<td>Hormone replacement therapy, %</td>
<td>2 4 2</td>
</tr>
</tbody>
</table>

*Geometric means.
Although its value in cardiovascular risk stratification is not definitely established, coronary artery calcium may be an independent predictor of angiographic CAD and of coronary events.\textsuperscript{37–39}

In our study, CRP was not associated with presence of calcified coronary plaque, a finding that is in agreement with 2 recent studies using coronary calcium scores as the outcome.\textsuperscript{40–42} It has been argued that CRP may measure the degree of plaque destabilization and ongoing ulceration or thrombosis rather than atherosclerotic mass.\textsuperscript{43}

The strengths of our study include being a population-based, bi-ethnic sample, the lack of interference of treatments (only 29 participants were using cholesterol agents and 4 women were using hormone replacement therapy), and the availability of multiple established cardiac risk factors. The main limitations are the observational and cross-sectional nature of the data (precluding causal inference) and the fact that we have not accounted for the CARDIA cohort for known genetic polymorphisms in the PAF-AH gene. A G94–100 formation in exon 9, shown to be present in 4% of Japanese men, has been shown to be associated with reduced Lp-PLA\textsubscript{2} activity and with increased risk of CAD.\textsuperscript{44} Further research is needed to reconcile these findings in Japanese men with the findings reported here that increased mass and activity are associated with calcified coronary plaque.

In conclusion, Lp-PLA\textsubscript{2} mass (but not its activity) was independently associated with calcified coronary plaque among young adults. Additional studies are warranted to elucidate the contributions of Lp-PLA\textsubscript{2} bioproducts on the risk of atherothrombosis and the value of selective inhibitors of Lp-PLA\textsubscript{2} activity in combating atherosclerosis.

Acknowledgments

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References


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