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Lipoprotein-Associated Phospholipase A2, High-Sensitivity C-Reactive Protein, and Risk for Incident Coronary Heart Disease in Middle-Aged Men and Women in the Atherosclerosis Risk in Communities (ARIC) Study

Christie M. Ballantyne, MD; Ron C. Hoogeveen, PhD; Heejung Bang, PhD; Josef Coresh, MD, PhD; Aaron R. Folsom, MD, MPH; Gerardo Heiss, MD, PhD; A. Richey Sharrett, MD, DrPH

Background—Measuring C-reactive protein (CRP) has been recommended to identify patients at high risk for coronary heart disease (CHD) with low LDL cholesterol (LDL-C). Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a proinflammatory enzyme associated primarily with LDL.

Methods and Results—In a prospective, case cohort study in 12,819 apparently healthy middle-aged men and women in the Atherosclerosis Risk in Communities study, the relation between Lp-PLA2, CRP, traditional risk factors, and risk for CHD events over a period of ≈6 years was examined in a proportional hazards model, stratified by LDL-C. Lp-PLA2 and CRP levels were higher in the 608 cases than the 740 noncases. Both Lp-PLA2 and CRP were associated with incident CHD after adjustment for age, sex, and race with a hazard ratio of 1.78 for the highest tertile of Lp-PLA2 and 2.53 for the highest category of CRP versus the lowest categories. Lp-PLA2 correlated positively with LDL-C (r = 0.36) and negatively with HDL-C (r = −0.33) but not with CRP (r = −0.05). In a model adjusted for traditional risk factors including LDL-C, the association of Lp-PLA2 with CHD was attenuated and not statistically significant. For individuals with LDL-C below the median (130 mg/dL), Lp-PLA2 and CRP were both significantly and independently associated with CHD in fully adjusted models. For individuals with LDL-C <130 mg/dL, those with both Lp-PLA2 and CRP levels in the highest tertile were at the greatest risk for a CHD event.

Conclusions—Lp-PLA2 and CRP may be complementary in identifying individuals at high CHD risk who have low LDL-C. (Circulation. 2004;109:837-842.)

Key Words: coronary disease | epidemiology | inflammation | risk factors

Although screening for elevated LDL cholesterol (LDL-C) remains a major component of national guidelines for the prevention of coronary heart disease (CHD), LDL-C level is insufficient to identify individuals who would develop CHD, because many CHD events occur in individuals without elevated LDL-C, indicating the influence of other risk factors. Inflammation plays an important role in both atherosclerosis and atherothrombotic events, and several biomarkers of inflammation, including high-sensitivity C-reactive protein (hs-CRP), interleukin-6, and soluble intercellular adhesion molecule-1, have been associated with increased risk for CHD events. hs-CRP measurement has been recommended for some patients to refine risk assessment because hs-CRP levels have been shown to provide additional predictive information beyond traditional risk factors such as LDL-C. Increased hs-CRP levels may also be useful to identify patients with low LDL-C who are at increased CHD risk and may benefit from statin therapy.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme that can hydrolyze oxidized phospholipids to generate lysophosphatidylcholine and oxidized fatty acids, which have proinflammatory properties. However, hydrolysis of platelet-activating factor and other phospholipids by Lp-PLA2 could also reduce inflammation, and it is not clear whether Lp-PLA2 is proinflammatory or anti-inflammatory in humans.
Lp-PLA₂ is associated primarily with LDL, and enzyme activity is increased in small, dense LDL.9

In a case–cohort analysis of the West of Scotland Coronary Prevention Study (WOSCOPS), high baseline Lp-PLA₂ levels were associated with increased risk for CHD events, even after adjustment for traditional risk factors and hs-CRP.10 In a case–control analysis from the Women’s Health Study, baseline Lp-PLA₂ levels were higher in women with subsequent cardiovascular events but were not associated with increased CHD risk after adjustment for traditional risk factors and hs-CRP.11

The purpose of this study was to examine whether levels of Lp-PLA₂ and hs-CRP in middle-aged American men and women were associated with increased risk for incident CHD in the Atherosclerosis Risk in Communities (ARIC) study.

Methods

Study Population
The ARIC design, objectives, sampling strategies, and examination techniques have been described previously.12 ARIC is a large, biracial cohort study of 15,792 adults 45 to 64 years old. A baseline examination was conducted in 1987 to 1989, with 3 more examinations through 1999.

Participants were followed up for incident CHD, defined by combinations of chest pain, ECG changes, cardiac enzyme levels, and surgical revascularization. Potential CHD events were reviewed by 2 members of the ARIC Morbidity and Mortality Classification Committee, and any differences between reviewers were adjudicated by the committee chairperson.

Study Design
Because plasma samples from the first visit were depleted, Lp-PLA₂ and hs-CRP were measured in duplicate in plasma from visit 2 (1990–1992) in individuals who subsequently developed a CHD event (cases) and in a cohort random sample (CRS). Of the 14,560 participants with visit 2 data, 1,272 were excluded because of CHD or missing CHD information before visit 2, 376 for transient ischemic attack or stroke, and 93 who belonged to an underrepresented minority group. The potential full cohort consisted of 12,819 participants with visit 2 data. Body mass index (BMI) was derived from measured height and weight. Hypertension was defined by the committee chairperson.

Statistical Analyses
For the primary analysis, all variables were categorized, with cutpoints taken from the National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines for cholesterol19 and the Joint National Committee VI guidelines for blood pressure.20 Some categories were combined to maintain sufficient numbers of events per cell. Tertiles of the major study covariate, Lp-PLA₂, were used. For hs-CRP, both tertiles and American Heart Association (AHA)/Centers for Disease Control and Prevention (CDC) cutpoints were examined. Covariates also were treated as continuous to examine the potential (non)linear trend in ancillary analyses. The primary null hypothesis was that Lp-PLA₂ is not predictive of CHD events, over and above a set of traditional risk factors included in the model, with special attention to the interrelationship of Lp-PLA₂, LDL-C, and hs-CRP. The association was tested at the 0.05 level using a Wald test for the 2-sided alternative.

Crude and adjusted (for demographic factors: race, sex, age) means or proportions of baseline variables were examined in cases versus noncases using ANCOVA and logistic regression.21 Beyond basic demographic variables, variables for risk factors recommended by ATP III19 for CHD risk assessment were considered as potential confounders: LDL-C, HDL cholesterol (HDL-C), total cholesterol, diabetes, smoking, and hypertension. Weighted Pearson and Spearman’s rank correlation coefficients were computed between variables among subjects in the CRS. In all analyses, weighting schemes based on sampling proportions were used so that the resulting inferences were pertinent to the entire cohort.

For the association between Lp-PLA₂ level and incident CHD, the Cox proportional hazards model was used to investigate the independent and joint prognostic effects, accounting for the fact that all incident CHD cases are included but only a stratified random sample of the full cohort. The statistical method and computer software used were developed for case–cohort design within a framework of proportional hazard regression, with an appropriate modification to take into account the stratified nature of the CRS and robust variance estimation.22,23 The results were summarized as hazard ratios (HRs) with 95% CIs. Tests for various potential interactions and (non)linearity were conducted as a secondary analysis, and subgroup analyses were considered to confirm findings. For the overall association of Lp-PLA₂ and outcome, a χ² test statistic was calculated. All other overall associations were tested similarly. SAS version 8 was used for all statistical analyses, except that SUDAAN version 8.0.0 was used to compute probability values for weighted correlation coefficients.

Results
Of the 608 CHD events, 41.6% were nonfatal myocardial infarctions, 9.5% were silent myocardial infarctions, 39.0% were revascularization procedures, and 9.9% were fatal events, with mean time to event 4.1 years. Baseline charac-
**TABLE 1. Weighted-Adjusted Means or Prevalences of Risk Factors at Baseline (Visit 2)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=608)</th>
<th>Noncases (n=740)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>58.5</td>
<td>56.7</td>
<td>...</td>
</tr>
<tr>
<td>Female, %*</td>
<td>32.2</td>
<td>58.9</td>
<td>...</td>
</tr>
<tr>
<td>African American, %*</td>
<td>22.9</td>
<td>25.0</td>
<td>...</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>28.2</td>
<td>19.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.7</td>
<td>28.1</td>
<td>0.014</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>27.8</td>
<td>15.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (history), %</td>
<td>51.0</td>
<td>32.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>127.5</td>
<td>121.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73.1</td>
<td>72.6</td>
<td>0.350</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>219.7</td>
<td>207.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>144.8</td>
<td>124.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45.6</td>
<td>51.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL‡</td>
<td>145.1</td>
<td>131.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp-PLA₂, µg/L</td>
<td>404.0</td>
<td>373.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>4.05</td>
<td>3.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Unadjusted mean or proportion; all others are age-, race-, and sex-adjusted mean or proportion.

†Median LDL-C was 145 mg/dL for cases and 129 mg/dL for noncases.

**TABLE 2. Weighted Correlation Between Lp-PLA₂ and Other Risk Factors**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Pearson Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.01</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-0.05</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.13</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

NS indicates nonsignificant (P≥0.05)

**TABLE 3. CHD HRs (95% CI) by hs-CRP Categories Defined by AHA/CDC§**

<table>
<thead>
<tr>
<th>hs-CRP Categories*</th>
<th>Average Risk (1.0–3.0 mg/L)</th>
<th>High Risk (&gt;3.0 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 †</td>
<td>1.61 (1.21–2.16)</td>
<td>2.53 (1.88–3.40)</td>
</tr>
<tr>
<td>Model 2 †</td>
<td>1.31 (0.96–1.80)</td>
<td>1.72 (1.24–2.39)</td>
</tr>
<tr>
<td>Model 2,‡ LDL-C &lt;130 mg/dL</td>
<td>1.18 (0.71–1.96)</td>
<td>1.76 (1.02–3.03)</td>
</tr>
</tbody>
</table>

*Low risk (<1 mg/L) is reference; AARC tertiles were <1.01, 1.01–2.82, and >2.82 mg/L.
†Adjusted for age, sex, and race.
‡Adjusted for age, sex, race, smoking status, systolic blood pressure, LDL-C, HDL-C, and diabetes.
§n=204 cases and 369 noncases.

339 µg/L in women, 366 µg/L in individuals <55 years old, and 384 µg/L in individuals ≥55 years old, 388 µg/L in whites, and 333 µg/L in African Americans. 362 µg/L in diabetics and 376 µg/L in nondiabetics, and 404 µg/L in current smokers and 366 µg/L in nonsmokers.

Because hs-CRP tertiles in ARIC (<1.01, 1.01 to 2.82, and >2.82 mg/L) were similar to the cutpoints defined in the AHA/CDC guidelines (<1 mg/L, 1 to 3 mg/L, >3 mg/L), the AHA/CDC cutpoints were used to facilitate comparison across studies. In a Cox proportional hazards model adjusted for age, sex, and race, high hs-CRP as defined by the AHA/CDC cutpoint of >3.0 mg/L was associated with a significant increase in risk (2.53 HR, 95% CI 1.88 to 3.40) (Table 3). Further adjustment for smoking, hypertension, diabetes, LDL-C, and HDL-C attenuated risk associated with high hs-CRP, but risk remained significantly elevated (1.72 HR, 95% CI 1.24 to 2.39). For individuals with LDL-C <130 mg/dL, approximately the median LDL-C in this population, high hs-CRP was associated with increased CHD risk (1.76 HR, 95% CI 1.02 to 3.03). The use of tertiles for hs-CRP and the addition of BMI to the model did not significantly alter the findings (data not shown).

Lp-PLA₂ levels in the highest tertile (>422 µg/L) were associated with increased CHD risk (1.78 HR, 95% CI 1.33 to 2.38) in a model adjusted for age, sex, and race (Table 4). In a Cox proportional hazards model also adjusted for traditional risk factors, including LDL-C and HDL-C, the relative risk...
associated with the upper tertile of Lp-PLA2 was attenuated and no longer significant. Tests for potential interaction with variables used in the model showed no interaction with race or sex but a significant 3-way interaction of Lp-PLA2 with BMI in the model did not alter these findings. To address the impact of the 3-way effect modification, we added the interaction of Lp-PLA2 and hs-CRP in the model, which was significant predictor after adjustment for traditional risk factors including LDL-C and HDL-C, but further analysis motivated by the significant interactions among Lp-PLA2, hs-CRP, and LDL-C indicated that the association remained significant, independent of other traditional risk factors but modifiable by hs-CRP in individuals with low LDL-C (<130 mg/dL). We did not find any significant, meaningful nonlinearity of Lp-PLA2 using polynomial and spline regression, but complex nonlinearity remains a possibility. Moreover, the significant 3-way interaction (P=0.02 in a model with categorical variables and P=0.001 in a model with continuous variables) needs more statistical investigation in future research in this and other populations. The differing results among these studies may be attributed to the markedly different populations. WOSCOPS enrolled middle-aged hypercholesterolemic men in Scotland, with LDL-C entry criterion within a narrow range of 174 to 232 mg/dL, high prevalence of other risk factors, and 5-year event rate of 7.9% in the placebo group (1.6%/yr). In addition, half the patients in WOSCOPS were assigned to pravastatin therapy, which lowered LDL-C by 26% on average. The Women’s Health Study examined middle-aged American women who were mostly professionals and had a lower event rate (1.4% over 6.2 years, or 0.2%/yr),

Discussion
In the ARIC study, Lp-PLA2 and hs-CRP levels were higher in middle-aged American men and women who subsequently developed CHD events than in those who remained free of CHD.

Two previous studies have examined Lp-PLA2 and hs-CRP levels in cases with incident CHD and controls, and both studies found positive associations of Lp-PLA2 and hs-CRP with CHD risk. In ARIC, both Lp-PLA2 and hs-CRP were associated with incident CHD after adjustment for age, sex, and race, with 1.78 HR (95% CI 1.33 to 2.38) for the highest Lp-PLA2 tertile (≥422 µg/L) and 2.53 HR (95% CI 1.88 to 3.40) for hs-CRP >3.0 mg/L. The HRs for high Lp-PLA2 in this study are similar to those reported in WOSCOPS and the Women’s Health Study in the unadjusted models. Although all 3 studies found that Lp-PLA2 levels were increased in individuals with subsequent CHD events, results varied among the studies when statistical models examined whether Lp-PLA2 level had additional predictivity beyond traditional risk factors. In WOSCOPS, Lp-PLA2 remained a significant predictor after adjustment for traditional risk factors and inflammatory factors, including hs-CRP. However, in the Women’s Health Study, the predictivity of Lp-PLA2 was no longer statistically significant after adjustment for cardiovascular risk factors. In the ARIC study, the relation between Lp-PLA2 and CHD risk was attenuated after adjustment for traditional risk factors including LDL-C and HDL-C.
intensify therapy. There is a consensus that individuals with CHD risk >20% need preventive intervention and therefore CRP measurement will not influence therapy. Screening very-low-risk populations is not recommended and not thought to be cost-effective, although some groups have considered intermediate risk to include 6% to 20%.27

The ATP III guidelines recommend that calculated 10-year risk for a CHD event be used to determine which patients should receive lipid-lowering therapy and the target level for LDL-C. Two large placebo-controlled randomized clinical trials of statins in high-risk patients have shown that patients benefited from therapy regardless of baseline LDL-C.28,29 In the current guidelines, for individuals with LDL-C <130 mg/dL, drug therapy is not recommended in primary prevention and is considered optional for individuals with CHD or equivalent (ie, 10-year risk estimate >20%).30 If high-risk patients with LDL-C <130 mg/dL receive significant benefit from lipid-lowering drug therapy, as suggested by recent clinical trials, future guidelines may focus more on novel ways to assess CHD risk in low–LDL-C patients to determine who should be selected. This is an important population for CHD prevention, because approximately one third of all events (204 cases in ARIC) occurred in persons with LDL-C <130 mg/dL.

A post hoc analysis of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) suggested that measuring hs-CRP may be useful for targeting statin therapy in primary prevention of acute coronary events.37 AFCAPS/TexCAPS enrolled middle-aged men and women with average LDL-C and low HDL-C levels, and for individuals with LDL-C below the median (149.1 mg/dL), only those with elevated hs-CRP had reduced risk with lovastatin therapy.

The results from ARIC support the rationale that Lp-PLA2 and hs-CRP may be useful to identify patients at increased CHD risk who have low LDL-C (<130 mg/dL) and are not targeted for drug therapy by the current guidelines. ARIC had only a single measurement of Lp-PLA2 and hs-CRP; associations might have been stronger with multiple measurements of these biomarkers. In addition, the risk for Lp-PLA2 may have been underestimated because of a lower reliability coefficient for the Lp-PLA2 manual ELISA than for the hs-CRP automated immunoturbidimetric assay (0.76 versus 0.95). A large prospective randomized trial will test the hypothesis that hs-CRP measurement can identify high-risk patients with LDL-C <130 mg/dL and no clinical evidence of CHD who may benefit from statin therapy.30

Although our results support the use of novel blood tests to identify high-risk patients who may benefit from primary prevention, they are also consistent with a putative causal role for both Lp-PLA2 and hs-CRP in atherogenesis and CHD events. Lp-PLA2 is bound primarily with LDL, with an increased concentration in small, dense LDL.9 Small LDL has enhanced penetration into the vessel wall31 and enhanced susceptibility to oxidation.32 Lp-PLA2 is the enzyme responsible for the hydrolysis of oxidized phospholipids and the generation of lysophosphatidylcholine, which can lead to increased expression of adhesion molecules. Thus, increased Lp-PLA2 in LDL may enhance the atherogenicity of LDL by increasing vascular inflammation. High levels of hs-CRP, which is an acute-phase reactant, may also provoke vascular inflammation.33 and hs-CRP may preferentially bind to oxidized LDL.34 Individuals with low LDL-C but high Lp-PLA2 and hs-CRP may therefore have much greater atherogenicity from LDL than would be expected by the absolute level of LDL-C. Statins lower LDL-C level and LDL particle number, reduce hs-CRP level,7 and reduce Lp-PLA2 activity.35 However, many patients continue to have elevated hs-CRP and Lp-PLA2 even on statin therapy (Chris J. Packard, DSc, personal communication, 2003). Other therapies, such as weight loss and high-dose aspirin, also reduce hs-CRP levels. Fibrates have been shown to reduce Lp-PLA2 activity,36 and a novel agent that inhibits Lp-PLA2 is currently in phase II development.37 Therefore, in addition to potentially identifying high-risk but currently untreated patients who may benefit from therapies such as statins to reduce CHD events, measurement of Lp-PLA2 and hs-CRP may be useful to identify cohorts of patients for clinical trials to determine whether inhibition of Lp-PLA2 or reduction/inhibition of hs-CRP reduces CHD events. In summary, both Lp-PLA2 and hs-CRP may be complementary in identifying middle-aged individuals with high CHD risk but low LDL-C.

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