Epidemiology

Relationship of Oxidized Phospholipids on Apolipoprotein B-100 Particles to Race/Ethnicity, Apolipoprotein(a) Isoform Size, and Cardiovascular Risk Factors

Results From the Dallas Heart Study

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Background—Elevated levels of oxidized phospholipids (OxPLs) on apolipoprotein B-100 particles (OxPL/apoB) are associated with cardiovascular disease and predict new cardiovascular events. Elevated lipoprotein (a) [Lp(a)] levels are a risk factor for cardiovascular disease in whites and also in blacks if they carry small apolipoprotein(a) [apo(a)] isoforms. The relationship of OxPL/apoB levels to race/ethnicity, cardiovascular risk factors, and apo(a) isoforms is not established.

Methods and Results—OxPL/apoB levels were measured in 3481 subjects (1831 black, 1047 white, and 603 Hispanic subjects) in the Dallas Heart Study and correlated with age, sex, cardiovascular risk factors, and Lp(a) and apo(a) isoforms. Significant differences in OxPL/apoB levels were noted among racial/ethnic subgroups, with blacks having the highest levels compared with whites and Hispanics (P<0.001 for each comparison). OxPL/apoB levels generally did not correlate with age, sex, or risk factors. In the overall cohort, OxPL/apoB levels strongly correlated with Lp(a) (r=0.85, P<0.001), with the shape of the relationship demonstrating a “reverse L” shape for log-transformed values. The highest correlation was present in blacks, followed by whites and Hispanics; was dependent on apo(a) isoform size; and became progressively weaker with larger isoforms. The size of the major apo(a) isoform (number of kringle type IV repeats) was negatively associated with OxPL/apoB (r=−0.49, P<0.001) and Lp(a) (r=−0.61, P<0.001) regardless of racial/ethnic group. After adjustment for apo(a) isoform size, the relationship between OxPL/apoB and Lp(a) remained significant (r=0.67, P<0.001).

Conclusions—OxPL/apoB levels vary according to race/ethnicity, are largely independent of cardiovascular risk factors, and are inversely associated with apo(a) isoform size. The association of OxPL with small apo(a) isoforms, in which a similar relationship is present among all racial/ethnic subgroups despite differences in Lp(a) levels, may be a key determinant of cardiovascular risk. (Circulation. 2009;119:1711-1719.)

Key Words: atherosclerosis ■ lipoproteins ■ lipoprotein(a) ■ phospholipids ■ risk factors

Oxidized lipids play a central role in mediating a variety of immune, proinflammatory, and plaque-destabilizing processes that further amplify the inflammatory response.1 Plasma levels of specific oxidized phospholipids (OxPLs) on apolipoprotein B-100 (apoB) particles (OxPL/apoB) can be measured with the murine monoclonal antibody E06. OxPL/apoB levels are elevated in patients with coronary, carotid, and femoral artery disease2,3; with acute coronary syndromes4; and after percutaneous coronary intervention.5 Interestingly, in human plasma, E06-detectable OxPLs are preferentially carried by lipoprotein(a) [Lp(a)] compared with other apoB-100 particles.2-8

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We recently showed in the Bruneck population, which is entirely white, that OxPL/apoB and Lp(a) levels were strongly and significantly associated with the presence, extent, and interim development of carotid and femoral atherosclerosis from 1995 to 2000.3 OxPL/apoB and Lp(a) also predicted new cardiovascular events over a 10-year period independently of other risk factors and provided additional prognostic information within each Framingham Risk Score tertile.9

The Dallas Heart Study is a unique epidemiological survey of middle-aged asymptomatic subjects of different racial/ethnic groups (≈50% black, 30% white, and 20% Hispanic; 56%, female, 44% male) with the purpose of evaluating traditional cardiovascular risk factors, biomarkers, and non-invasive measures of atherosclerosis.10 The relationship of Lp(a) and apolipoprotein isoform sizes to coronary calcium in this cohort was previously described.11 The purpose of the
present study was to determine whether racial/ethnic differences exist in OxPL/apoB levels and to evaluate their relationship to cardiovascular risk factors.

Methods

Study Subjects

The Dallas Heart Study subjects were previously described in detail.\textsuperscript{10,11} The Dallas Heart Study is a multiethnic, probability-based sample of the Dallas County population in which blacks were systematically oversampled so that the final sample was 50% black. In this study, 3481 blood samples were available from 3 racial/ethnic groups at the baseline time point.

Determination of OxPL/apoB Levels

The content of OxPL/apoB was measured as previously described in detail by chemiluminescent ELISA using the murine monoclonal antibody E06, which binds to the phosphocholine head group of oxidized but not native phospholipids.\textsuperscript{2,3,6,12} As described, equal numbers of apoB-100 particles are captured from each plasma sample; thus, the content of OxPL is normalized for apoB-100 in each subject. Thus, by design, the OxPL/apoB measurement is independent of apoB-100 (and low-density lipoprotein [LDL] cholesterol) levels. The OxPL/apoB values are expressed as relative light units (RLUs) reflecting the amount of E06 bound to OxPL on apoB particles captured on microtiter well plates with antibody MB47. It is to be emphasized that the OxPL/apoB measure represents only those OxPLs recognized by E06 (ie, E06 immunoreactivity) and does not represent all OxPLs present on apoB particles. In particular, E06 does not recognize lysophosphocholine.\textsuperscript{12}

Determination of Lp(a) Levels and Apolipoprotein(a) Isootypes

Measurement of Lp(a) levels in nanomoles per liter was performed with a well-validated assay that is independent of apolipoprotein(a) [apo(a)] isoform size.\textsuperscript{11} If expressed as milligrams per deciliter, the values would be \( \approx 2.5 \) fold lower than in nanomoles per liter, although larger differences would be present at either extreme of apo(a) size. Apo(a) isoforms were measured as previously described.\textsuperscript{11} The analyses in this study were based on size of the major apo(a) isoform visualized on agarose gel electrophoresis, which is directly proportional to the number of kringle IV repeats.\textsuperscript{13} In this study, the major apo(a) isoform was associated with the smaller of the 2 alleles in 87% of subjects.

determination of Laboratory Variables

Total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol, triglycerides, high-sensitivity C-reactive protein, and lipoprotein-associated phospholipase A2 (Lp-PLA2) mass and activity were measured as previously described.\textsuperscript{14,15} Carbohydrate, lathosterol, sitosterol, homocysteine, monocyte chemoattractant protein-1, interleukin-18, peptidoglycan recognition protein-1, brain natriuretic peptide, troponin, and liver fat (by magnetic resonance imaging) were measured as previously described.\textsuperscript{15–21}

Statistical Analysis

Six groups were formed from 3 racial/ethnic groups and both sexes. Omnibus tests were performed among these groups on baseline demographic and laboratory variables using \( \chi^2 \) tests for nominal variables. For continuous variables, 1-way ANOVA or Kruskal-Wallis tests were used, depending on whether the variable was normally distributed. Significance tests for OxPL/apoB and \( \text{Lp(a)} \) values were computed on log-transformed variables using 3 \( \times \) 2 (racial/ethnic group \( \times \) sex) ANOVA models. Posthoc Tukey tests among racial/ethnic groups and between sexes within racial/ethnic groups were used for comparisons. Other tests of OxPL/apoB used nonparametric Kruskal-Wallis or Mann-Whitney \( U \) tests. Correlations were computed using Spearman’s rank-order method to avoid distributional assumptions. Partial correlation was conducted with log-transformed values of OxPL/apoB and \( \text{Lp(a)} \). Some analyses were stratified on the basis of kringle IV repeats grouped in tertiles.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Demographic and Laboratory Variables

Table 1 shows the baseline demographic and laboratory variables in male and female black, white, and Hispanic subjects. Significant baseline differences among groups were noted in all variables.

Absolute OxPL/apoB and \( \text{Lp(a)} \) Levels in Different Racial/Ethnic Groups

The data for these analyses were evaluated according to sex and race/ethnicity in a \( 2 \times 3 \) ANOVA design. Significant differences were found in log-transformed OxPL/apoB levels among the racial/ethnic subgroups, with blacks having the

**Table 1. Baseline Demographic and Laboratory Variables of the Study Participants**

<table>
<thead>
<tr>
<th></th>
<th>Black Women (n=1058)</th>
<th>Black Men (n=773)</th>
<th>White Women (n=546)</th>
<th>White Men (n=501)</th>
<th>Hispanic Women (n=351)</th>
<th>Hispanic Men (n=252)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>44.4±10.2</td>
<td>44.8±10.3</td>
<td>45.2±10.4</td>
<td>44.2±9.5</td>
<td>40.0±8.9</td>
<td>40.0±9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>32.0±8.0</td>
<td>28.4±6.4</td>
<td>28.6±7.5</td>
<td>28.2±5.2</td>
<td>30.1±6.9</td>
<td>28.7±4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>28.8</td>
<td>39.6</td>
<td>28.1</td>
<td>27.5</td>
<td>12.3</td>
<td>31.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>41.9</td>
<td>41.0</td>
<td>19.7</td>
<td>20.6</td>
<td>15.4</td>
<td>14.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>14.1</td>
<td>14.4</td>
<td>7.0</td>
<td>6.4</td>
<td>11.7</td>
<td>12.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>129.5±19.1</td>
<td>132.0±18.3</td>
<td>120.7±15.1</td>
<td>127.4±13.2</td>
<td>118.7±16.4</td>
<td>123.9±13.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.3±9.9</td>
<td>79.4±10.2</td>
<td>75.9±8.6</td>
<td>78.8±9.0</td>
<td>74.6±9.0</td>
<td>75.8±8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laboratory values, mg/dL</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>LDL-C</td>
<td>105.5±36.1</td>
<td>103.9±37.4</td>
<td>105.2±33.6</td>
<td>111.6±35.0</td>
<td>103.2±31.6</td>
<td>112.5±34.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>53.9±14.5</td>
<td>49.9±16.0</td>
<td>54.2±16.5</td>
<td>42.0±10.2</td>
<td>48.3±11.5</td>
<td>42.1±9.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Triglycerides</td>
<td>97.4±75.5</td>
<td>120.8±115.4</td>
<td>122.5±86.6</td>
<td>156.9±123.3</td>
<td>139.8±129.6</td>
<td>166.8±129.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>103.4±46.2</td>
<td>107.9±53.1</td>
<td>96.8±33.7</td>
<td>98.7±33.9</td>
<td>107.5±48.7</td>
<td>104.1±34.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, LDL cholesterol; and HDL-C, high-density lipoprotein cholesterol. n=3481.
highest levels, followed by whites and then by Hispanics ($P<0.001$ for each comparison; Figure 1A). Differences between men and women in OxPL/apoB within each subgroup were not significant except that black women had higher OxPL/apoB and Lp(a) values than black men ($P=0.002$). Lp(a) levels were higher in black ($P=0.005$) and Hispanic ($P=0.015$) women compared with their male counterparts, but levels were similar among white women and men ($P=0.42$; Figure 1B). The racial/ethnic differences remained significant when these groups were stratified by decade age groups (data not shown).

Frequency distribution graphs of log-transformed OxPL/apoB (Figure 2A) and Lp(a) (Figure 2B) levels among the racial/ethnic groups showed a positive skewness for Hispanics and whites; less positive skewness was noted in blacks.

**Distribution of apo(a) Isoforms, OxPL/apoB, and Lp(a) Levels in the Entire Cohort**

Figure 3A displays the distribution of apo(a) isoforms in the 3 racial/ethnic groups. In blacks, a gaussian distribution is present, whereas a bimodal distribution is more evident in whites and Hispanics. In the entire cohort with all 3 racial/ethnic groups combined, when plotted against the number of kringle IV repeats, OxPL/apoB (Figure 3B) and Lp(a) (Figure 3C) levels were highest in the subjects with small apo(a) isoforms and lowest in the subjects with large apo(a) isoforms. A larger variability was found in OxPL/apoB values in small apo(a) isoforms; a larger variability in Lp(a) values was present in large apo(a) isoforms.

**Relationship Between OxPL/apoB, Lp(a), and apo(a) Isoforms**

In the overall cohort, OxPL/apoB and Lp(a) levels were highly correlated (Spearman’s $r=0.85$, $P<0.001$). When plotted on a linear scale, the positive relationship of OxPL/
apoB to Lp(a) is noted in the entire cohort (Figure 4A). When plotted on a logarithmic scale (Figure 4B), evidence was found of a “reverse L” shape, with a weaker correlation between OxPL/apoB and Lp(a) \( (r = -0.14, P < 0.001) \) at Lp(a) levels \( 0 < 30 \) nmol/L and then a stronger correlation \( (r = 0.83, P < 0.001) \) for Lp(a) levels \( \geq 30 \) nmol/L. Furthermore, when OxPL/apoB was evaluated versus Lp(a) according to tertiles of apo(a) isoform size [number of kringle IV repeats of the major apo(a) isoform, 12 to 20, 21 to 26, and 27 to 41 repeats], a reverse L relationship was noted in apo(a) isoforms with 12 to 20 and 21 to 26 kringle IV repeats but was less evident in those with 27 to 41 repeats (Figure 4C).

Figure 5 displays the relationship between OxPL/apoB and Lp(a) with apo(a) isoform number in different racial/ethnicity groups. It can be appreciated that an inverse relationship is present between OxPL/apoB (Figure 5A) and Lp(a) (Figure 5B) and the number of kringle IV repeats in blacks \( (r = -0.614, P < 0.001) \), whites \( (r = -0.567, P < 0.001) \), and Hispanics \( (r = -0.570, P < 0.001) \). However, within each isoform class, blacks have higher OxPL/apoB and Lp(a) levels than whites and Hispanics.

Table 2 displays the correlations between OxPL/apoB, Lp(a), and apo(a) isoforms in the overall cohort and the 3 racial/ethnicity groups. The correlation between OxPL/apoB and Lp(a) was strongest in blacks, followed by whites and then Hispanics. When the data were analyzed in groups based on the size of the major apo(a) isoform, the racial/ethnic differences were less marked, with a negative correlation between the major apo(a) isoform and OxPL/apoB in all racial/ethnic subgroups, ranging from \( r = -0.50 \) in black women to \( r = -0.34 \) in Hispanic men.

In further analysis of data according to apo(a) tertiles, it is evident that a strong correlation \( (r = 0.85 \text{ to } 0.79) \) is noted between OxPL/apoB and Lp(a) in the group with 12 to 20 kringle IV repeats regardless of race/ethnicity. A more modest correlation is noted in all racial/ethnic subgroups in OxPL/apoB and 21 to 26 kringle IV repeats \( (r = 0.84 \text{ to } 0.43) \), with the strongest correlations in blacks and Hispanics. However, weak to no correlations were noted in all racial/ethnic subgroups with OxPL/apoB and 27 to 41 kringle IV repeats (Table 2).

In the overall cohort, after adjustment for apo(a) isoform size, the relationship between OxPL/apoB and Lp(a) remained significant \( (r = 0.67, P < 0.001) \). In addition, the partial correlation adjusted for the sum of the 2 kringle isoforms was 0.686 \( (P < 0.001) \). For black, white, and Hispanic subjects, the correlations were 0.729, 0.612, and 0.540, respectively, controlling for the sum of the 2 isoforms, and 0.714, 0.582, and 0.510, respectively, controlling for both major and minor isoforms \( (all P < 0.001) \). Adjusting for the socioeconomic status variables of income and education level did not affect any of the above relationships (data not shown).

**Relationship of OxPL/apoB to Clinical and Laboratory Variables**

In the overall cohort, correlations of OxPL/apoB with blood pressure were very weak \( (r = 0.078 \text{ for systolic pressure, } r = 0.089 \text{ diastolic pressure, } P < 0.001 \text{ for both}) \) and not significant with body mass index \( (r = 0.019, P = 0.27) \). OxPL/apoB levels were higher in subjects with versus without hypertension (median, 4910 versus 3755 RLU; \( P < 0.001 \)) but not in those with versus without diabetes mellitus (median, 4198 versus 4008 RLU; \( P = 0.135 \)). Very weak relationships were found between OxPL/apoB blood glucose levels (Spearman \( r = -0.051, P = 0.003 \)).

The relationship of OxPL/apoB and Lp(a) to a variety of laboratory variables is shown in Table 3. Although many of
these correlations were statistically significant, most were quite weak. Of interest, a negative association was present of both OxPL/apoB and Lp(a) with triglyceride levels and liver fat content measured with magnetic resonance imaging. Both OxPL/apoB and Lp(a) correlated very weakly with highsensitivity C-reactive protein. Weak correlations were also found between brain natriuretic peptide and OxPL/apoB and Lp(a). No relationship was found between OxPL/apoB or Lp(a) with monocyte chemoattractant protein-1, peptidoglycan recognition protein-1, or troponin levels. Lp-PLA2 mass correlated inversely but weakly with OxPL/apoB and Lp(a), as did Lp-PLA2 activity. No major differences were noted among the racial/ethnic groups in these associations.

**Discussion**

This large epidemiological study of apparently healthy young to middle-aged individuals demonstrates that significant racial/ethnic differences exist in OxPL/apoB levels, with black subjects displaying the highest levels, followed by whites and then by Hispanics. OxPL/apoB levels were largely independent of most clinical and laboratory variables. However, OxPL/apoB levels were distributed in a manner consistent with genetically determined Lp(a) levels and furthermore were positively correlated with small apo(a) isoforms. Interestingly, when the data were analyzed according to the size of the major apo(a) isoform, the racial/ethnic differences in OxPL/apoB were less marked. In fact, in subjects of all racial/ethnic subgroups with isoform sizes ≥30 kringle IV repeats, no significant relationship was noted between OxPL/apoB and Lp(a). These data suggest that OxPL/apoB levels may reflect a key component of the atherogenicity of elevated Lp(a) levels, particularly in the presence of small apo(a) isoforms.

**Distribution of OxPL/apoB Levels in Different Racial/Ethnic Groups**

Previous studies documenting the relationship between OxPL/apoB and Lp(a) were performed primarily in white populations (MAYO, Bruneck, and the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering [MIRACL] studies). The Dallas Heart Study documents that this relationship also exists in blacks and Hispanics but is quantitatively different in these groups compared with whites. For example, the highest correlation of OxPL/apoB with Lp(a) was present in blacks and the weakest was present in Hispanics compared with whites. High Lp(a) levels are inherited as a dominant quantitative trait, and most subjects (≈80%) express 2 distinct apo(a) alleles, although subjects with 1 allele or null alleles have been described. Elevated Lp(a) levels can be found in a large proportion of individuals in most racial/ethnicity groups, with the prevalence being lowest in whites and Asians. The median Lp(a) levels in black subjects and in Asian Indians from southern locations are 2- to 4-fold higher compared with whites, and up to 68% of blacks have Lp(a) levels >30 mg/dL, whereas levels above this threshold are present in ≈25% of whites. In whites, elevated Lp(a) levels are generally associated with small apo(a) isoforms in >80% of subjects. In blacks, elevated Lp(a) levels are distributed over a broader range of apo(a) isoform sizes, and plasma Lp(a) levels are higher in blacks within the same apo(a) isoform sizes as documented in this study. In particular, differences in Lp(a) levels...
between blacks and whites are prominent in the range of 20 to 25 kringle IV repeats, but the underlying reason why blacks have higher Lp(a) levels than whites for a similar isoform size is not understood.\textsuperscript{28} The present data are consistent with the interpretation that OxPL/apoB levels also are genetically determined in most subjects in a manner parallel to Lp(a) and highly reflect apo(a) isoform size.

### Relationship of OxPL/apoB and Lp(a) Levels

The OxPL/apoB assay was initially designed as a method to quantify minimally oxidized phospholipids on apoB particles as a measure of “oxidized LDL.” Unexpectedly, in clinical studies done to date, a significant correlation has been found between OxPL/apoB and Lp(a), in the range of $R=0.80$ to $0.90.\textsuperscript{2–7}$ This finding was confirmed in the present study, but a more extended analysis of this study provides further, novel insights into this relationship. For example, on a linear scale, the relationship of OxPL/apoB to Lp(a) appears positive and roughly linear, but on a logarithmic scale, a reverse L shape was noted, with a flat relationship up to Lp(a) levels of $\approx 30$ nmol/L and then a log-linear relationship at Lp(a) levels $>30$ nmol/L. Part of this association may be related to the different distribution of Lp(a) levels in this study, in which black patients had highest levels compared with whites and Hispanics, thus potentially creating an artificial relationship.

![Figure 5. Bar graph displaying the OxPL/apoB (A) and Lp(a) (B) levels according to tertiles of apo(a) isoform size in blacks, whites, and Hispanics.](image)

### Table 3. Correlations of OxPL/apoB and Lp(a) With Laboratory Variables

<table>
<thead>
<tr>
<th></th>
<th>OxPL/ApoB</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R$</td>
<td>$P$</td>
</tr>
<tr>
<td>TC</td>
<td>0.024</td>
<td>0.07</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.045</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.150</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>TG</td>
<td>$-0.156$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Liver fat by MRI</td>
<td>$-0.136$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.085</td>
<td>0.001</td>
</tr>
<tr>
<td>Lathosterol</td>
<td>$-0.091$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>0.050</td>
<td>0.003</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.056</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Lp-PLA2 mass</td>
<td>$-0.061$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Lp-PLA2 activity</td>
<td>$-0.120$</td>
<td>$&lt;0.001$</td>
</tr>
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<td>hsCRP</td>
<td>0.062</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>MCP-1</td>
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<td>0.002</td>
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<tr>
<td>PGRP-1</td>
<td>0.025</td>
<td>0.16</td>
</tr>
<tr>
<td>Troponin</td>
<td>0.036</td>
<td>0.033</td>
</tr>
<tr>
<td>BNP</td>
<td>0.070</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Table 3. Correlations of OxPL/apoB and Lp(a) With Laboratory Variables

- TC indicates total cholesterol; LDL-C, LDL cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; MRI, magnetic resonance imaging; hsCRP, high-sensitivity C-reactive protein; IL-18, interleukin-18; MCP-1, monocyte chemoattractant protein-1; PGRP-1, peptidoglycan recognition protein-1; and BNP, brain natriuretic peptide.

### Table 2. Spearman Correlation ($r$) Between Lp(a) and OxPL/ApoB by Race/Ethnicity and Sex

<table>
<thead>
<tr>
<th>Correlation</th>
<th>All</th>
<th>Black Women</th>
<th>Black Men</th>
<th>White Women</th>
<th>White Men</th>
<th>Hispanic Women</th>
<th>Hispanic Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) vs OxPL/apoB</td>
<td>0.84*</td>
<td>0.87*</td>
<td>0.87*</td>
<td>0.72*</td>
<td>0.68*</td>
<td>0.69*</td>
<td>0.53*</td>
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<tr>
<td>Correlation between major apo(a) allele and OxPL/apoB by race/ethnicity-sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo(a) vs OxPL/apoB</td>
<td>$-0.50*$</td>
<td>$-0.47*$</td>
<td>$-0.48*$</td>
<td>$-0.46*$</td>
<td>$-0.46*$</td>
<td>$-0.50*$</td>
<td>$-0.32*$</td>
</tr>
<tr>
<td>Correlation between Lp(a) and OxPL/apoB by race/ethnicity-sex stratified by No. of apo(a) isoforms in the major allele</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>12–20</td>
<td>0.85*</td>
<td>0.81*</td>
<td>0.84*</td>
<td>0.84*</td>
<td>0.85*</td>
<td>0.85*</td>
<td>0.80*</td>
</tr>
<tr>
<td>21–26</td>
<td>0.88*</td>
<td>0.86*</td>
<td>0.85*</td>
<td>0.74*</td>
<td>0.62*</td>
<td>0.80*</td>
<td>0.69*</td>
</tr>
<tr>
<td>27–41</td>
<td>0.47*</td>
<td>0.67*</td>
<td>0.71*</td>
<td>0.16†</td>
<td>0.13†</td>
<td>0.38*</td>
<td>0.25*</td>
</tr>
</tbody>
</table>

* $P<0.001$; † $P<0.05$.
However, this is not likely the explanation because a reverse L shape can be seen in all racial/ethnic groups. This is further substantiated when the data are evaluated by apo(a) isoform size, demonstrating that this reverse L relationship was evident in all racial/ethnic groups with isoforms having <26 but not >27 kringle IV repeats. Furthermore, adding both kringle sizes together from each isoform did not substantially change the findings. This also suggests that a threshold effect may occur in this relationship and that a critical value of Lp(a) or a critical number of apo(a) particles may be needed to mediate significant binding of OxPL. Further work is required to quantitatively ascertain the extent to which Lp(a) may bind and release OxPL and to identify factors such as Lp-PLA2 that may affect this relationship.31

In support of the ability of Lp(a), as opposed to other lipoproteins, to bind OxPL recognized by E06, recent data from our laboratory demonstrated that >85% of E06 reactivity (ie, OxPL) coinmunoprecipitates with Lp(a).9 In lipoprotein ultracentrifugation experiments, nearly all OxPLs associated with lipoproteins were found in fractions containing apo(a), as opposed to other apolipoproteins. Furthermore, in vitro transfer studies showed that oxidized LDL preferentially donates OxPL to Lp(a), as opposed to LDL, in a time- and temperature-dependent manner, even in aqueous buffer. Additionally, ≈50% of E06 immunoreactivity could be extracted from isolated Lp(a) after exposure of plasma to various lipid solvents. These in vitro studies lend further proof that OxPLs are strongly associated physically with Lp(a) and corroborate the insights derived from clinical populations into its physiological function and mechanisms of atherogenicity.32 Consistent with the above observations, studies from independent laboratories have demonstrated that small dense LDL contains electronegative LDL, which is enriched in lipoperoxides and has enhanced susceptibility for generating OxPLs.33 Furthermore, electronegative LDL is enriched in Lp-PLA2, which may mediate some of its atherogenic properties.34

Relationship of OxPL/apoB and apo(a) Size

It is well known that elevated Lp(a) levels are associated with increased risk of cardiovascular disease in white subjects, particularly when elevated LDL cholesterol levels also are present,35 but this association has not consistently been demonstrated in blacks.36,37 In women, Lp(a) appears to be a stronger risk factor when very high Lp(a) levels (>65 mg/dL) are present.38 However, apo(a) phenotypes, particularly those in which apo(a) contains <22 kringle IV repeats, appear to be associated with increased cardiovascular risk in both whites and blacks and are more predictive of cardiovascular disease than elevated Lp(a) levels.21,28,39,40 In the present study, OxPL/apoB levels correlated most strongly with both elevated Lp(a) levels and smaller apo(a) isoforms regardless of race/ethnicity, age, and sex, whereas they did not correlate in the larger apo(a) isoforms, suggesting that this relationship is related to underlying genetic differences in apo(a) size and/or number rather than race/ethnicity per se. However, because blacks had higher Lp(a) levels, they also had correspondingly higher OxPL/apoB levels than whites and Hispanics. Identifying the potential sites on apo(a) and Lp(a) that mediate binding of OxPL and identifying the specific OxPLs on these lipoproteins merit further exploration.

Relationship of OxPL and Lp(a) to Cardiovascular Risk Factors and Laboratory Variables

This is the largest study to examine the relationship of OxPL/apoB levels to cardiovascular risk factors and laboratory variables in an epidemiological cohort. Because of the large number of subjects, there are some statistically significant associations. However, most of them are quite weak or borderline, and these correlations are unlikely to exert a significant pathophysiological influence on cardiovascular risk. Notably, OxPL/apoB levels were either not correlated or minimally associated with several inflammatory variables (high-sensitivity C-reactive protein, interleukin-18, monocyte chemoattractant protein-1, peptidoglycan recognition protein-1), markers of myocardial damage (troponin), or increased left ventricular wall stress (brain natriuretic peptide). Interestingly, a negative association was noted between both OxPL/apoB and plasma triglyceride levels and liver fat content by magnetic resonance imaging. This observation has been made previously for Lp(a), although the reasons are not well understood and require further mechanistic insights.41,42 It is also conceivable that anti-phosphocholine antibodies may modulate plasma levels of OxPL/apoB. Although such levels were not measured in this study, previous studies have not documented any significant relationship between plasma levels of OxPL/apoB and immunoglobulin G or immunoglobulin M (IgM) autoantibodies to copper-oxidized LDL, a fraction of which may be anti-phosphocholine antibodies, or autoantibodies to malondialdehyde-LDL or to apoB-immune complexes.43 It has been reported in several studies that very low levels of IgM autoantibodies to phosphocholine-BSA,44,45 as well as IgM copper-oxidized LDL and malondialdehyde-LDL,43 are associated with higher incidence of various manifestations of atherosclerosis and clinical events. It is also possible that such antibodies may have therapeutic potential through a variety of mechanisms.46 Additional determinants of OxPL/apoB plasma levels besides genetically determined Lp(a) require future studies.

In a previous publication from the Dallas Heart Study, Lp-PLA2 mass and activity were lowest in blacks compared with whites and Hispanics.14 In the present analysis, weak inverse correlations were noted between OxPL/apoB and Lp(a) and both Lp-PLA2 mass and activity. These data are consistent with the physiological action of Lp-PLA2 in cleaving the oxidized fatty acid at the sn-2 position of OxPL and suggest that there also may be an influence of plasma levels of these measures. Furthermore, because Lp(a) is preferentially enriched in Lp-PLA2 activity on an equimolar basis compared with LDL, Lp-PLA2 activity on Lp(a) particles may serve as a mechanism to clear OxPL bound to Lp(a).31

Study Limitation

This study did not measure clinical outcomes. Therefore, it cannot be determined whether racial/ethnic differences in OxPL/apoB predict clinical outcomes.
Conclusions
This study documents that elevated levels of proinflammatory OxPLs carried primarily by Lp(a) represent a genetic predisposition to increased oxidative stress. Furthermore, the study suggests that the differences in apo(a) isoforms explain some, but not all, of the racial/ethnic differences in OxPL/apoB and Lp(a) seen in this population. These findings help us understand the mechanistic underpinnings of the potential atherogenicity and potentially the inhibition of fibrinolysis by Lp(a), which has previously been documented in vitro. Future studies should focus on apo(a) isoforms and their relationship to cardiovascular events mediated by OxPL.

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Disclosures
Dr’s Tsimikas and Witztum are named as inventors on patents and patent applications for the potential commercial use of antibodies to oxidized LDL. The other authors report no conflicts.

References
42. Tholstrup T, Samman S. Postprandial lipoprotein(a) is affected differently by specific individual dietary fatty acids in healthy young men. *J Nutr.* 2004;134:2550–2555.

**CLINICAL PERSPECTIVE**

Oxidized phospholipids (OxPLs) play a central role in mediating a variety of immune, proinflammatory, and plaque-destabilizing processes that further amplify inflammatory responses. We have found that OxPLs circulate on apolipoprotein B-100 (apoB) particles primarily on lipoprotein(a) [Lp(a)]. OxPL/apoB levels are elevated in patients with coronary, carotid, and femoral artery disease; with acute coronary syndromes; and after percutaneous coronary intervention and independently predict new cardiovascular events. OxPL/apoB levels were measured in 1831 black, 1047 white, and 603 Hispanic subjects in the Dallas Heart Study. OxPL/apoB levels were highest in blacks, followed by whites and Hispanics, and did not correlate with any traditional risk factors except Lp(a). The highest correlation between OxPL/apoB and Lp(a) was present in blacks, followed by whites and Hispanics; was dependent on apolipoprotein(a) [apo(a)] isoform size; and became progressively weaker with larger isoforms. The size of the major apo(a) isoform (number of kringle type IV repeats) was negatively associated with OxPL/apoB regardless of racial/ethnic group. This study demonstrates that elevated OxPL/apoB levels are associated with both small apo(a) isoforms and high Lp(a), which explains the differences in racial/ethnic groups, which have genetic differences in both apo(a) and Lp(a) levels. The association of OxPL with small apo(a) isoforms, in which a similar relationship is present among all racial/ethnic subgroups despite differences in Lp(a) levels, may be a key determinant of cardiovascular risk.
Relationship of Oxidized Phospholipids on Apolipoprotein B-100 Particles to Race/Ethnicity, Apolipoprotein(a) Isoform Size, and Cardiovascular Risk Factors: Results From the Dallas Heart Study

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