ORIGINAL ARTICLE

Oxidized Phospholipids, Lp(a) Lipoprotein, and Coronary Artery Disease

Sotirios Tsimikas, M.D., Emmanouil S. Brilakis, M.D., Elizabeth R. Miller, B.S., Joseph P. McConnell, Ph.D., Ryan J. Lennon, M.S., Kenneth S. Kornman, Ph.D., Joseph L. Witztum, M.D., and Peter B. Berger, M.D.

ABSTRACT

BACKGROUND

Lp(a) lipoprotein binds proinflammatory oxidized phospholipids. We investigated whether levels of oxidized low-density lipoprotein (LDL) measured with use of monoclonal antibody E06 reflect the presence and extent of obstructive coronary artery disease, defined as a stenosis of more than 50 percent of the luminal diameter.

METHODS

Levels of oxidized LDL and Lp(a) lipoprotein were measured in a total of 504 patients immediately before coronary angiography. Levels of oxidized LDL are reported as the oxidized phospholipid content per particle of apolipoprotein B-100 (oxidized phospholipid:apo B-100 ratio).

RESULTS

Measurements of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were skewed toward lower values, and the values for the oxidized phospholipid:apo B-100 ratio correlated strongly with those for Lp(a) lipoprotein ($r=0.83$, $P<0.001$). In the entire cohort, the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels showed a strong and graded association with the presence and extent of coronary artery disease (i.e., the number of vessels with a stenosis of more than 50 percent of the luminal diameter) ($P<0.001$). Among patients 60 years of age or younger, those in the highest quartiles for the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels had odds ratios for coronary artery disease of 3.12 ($P<0.001$) and 3.64 ($P<0.001$), respectively, as compared with patients in the lowest quartile. The combined effect of hypercholesterolemia and being in the highest quartiles of the oxidized phospholipid:apo B-100 ratio (odds ratio, 16.8; $P<0.001$) and Lp(a) lipoprotein levels (odds ratio, 14.2; $P<0.001$) significantly increased the probability of coronary artery disease among patients 60 years of age or younger. In the entire study group, the association of the oxidized phospholipid:apo B-100 ratio with obstructive coronary artery disease was independent of all clinical and lipid measures except one, Lp(a) lipoprotein. However, among patients 60 years of age or younger, the oxidized phospholipid:apo B-100 ratio remained an independent predictor of coronary artery disease.

CONCLUSIONS

Circulating levels of oxidized LDL are strongly associated with angiographically documented coronary artery disease, particularly in patients 60 years of age or younger. These data suggest that the atherogenicity of Lp(a) lipoprotein may be mediated in part by associated proinflammatory oxidized phospholipids.

From the Divisions of Cardiovascular Diseases (S.T.) and Endocrinology and Metabolism (E.R.M., J.L.W.), University of California, San Diego; the Division of Cardiovascular Diseases (E.S.B.), the Department of Laboratory Medicine and Pathology (J.P.M.), and the Division of Biostatistics (R.J.L.), Mayo Clinic, Rochester, Minn.; Interleukin Genetics, Waltham, Mass. (K.S.K.); and the Division of Cardiovascular Diseases, Duke Clinical Research Institute, Durham, N.C. (P.B.B.). Address reprint requests to Dr. Tsimikas at the Vascular Medicine Program, University of California, San Diego, 9500 Gilman Dr., Basic Sciences Bldg., Rm. 1080, La Jolla, CA 92093-0682, or at stsimikas@ucsd.edu.

N Engl J Med 2005;353:46-57.

Copyright © 2005 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

Human coronary atherosclerosis is a chronic inflammatory disease that is superimposed on a background of lipid abnormalities. Proinflammatory oxidized low-density lipoprotein (LDL) may be a unifying link between lipid accumulation and inflammation in the vessel wall. In humans, oxidized LDL in plasma and within atherosclerotic lesions is strongly associated with coronary artery disease, acute coronary syndromes, and vulnerable plaques.[1-7]

Lp(a) lipoprotein is a lipoprotein of unknown physiologic function that is composed of apolipoprotein B-100 (apo B-100) to which apolipoprotein(a) is covalently bound. Increased plasma levels of Lp(a) lipoprotein are independent predictors of the presence of angiographically documented and clinical coronary artery disease, particularly in patients with hypercholesterolemia.[8] However, the underlying mechanisms by which Lp(a) lipoprotein contributes to the pathogenesis of atherosclerosis are not well understood. We recently showed that proinflammatory oxidized phospholipids are strongly associated with Lp(a) lipoprotein in human plasma.[5-7,9] Therefore, we hypothesized that the presence of oxidized phospholipids on apo B-100–containing lipoproteins may explain some of the atherogenic properties of Lp(a) lipoprotein, and we designed this study to evaluate the relationship between circulating oxidized LDL, Lp(a) lipoprotein, and angiographically documented coronary artery disease.

METHODS

STUDY DESIGN

We designed the current study on the basis of a previous study in which we had enrolled a total of 504 consecutive patients (97.2 percent of whom were white), 18 to 75 years of age, who were undergoing clinically indicated coronary angiography at the Mayo Clinic between June 1998 and December 1998.[10] Race was self-reported. The exclusion criteria, which have been described previously, included prior coronary revascularization and the presence of diabetes mellitus.[10] Arterial plasma samples were obtained from the femoral sheath before angiography and were placed in tubes containing EDTA and frozen at −70°C until the analyses were performed. Hypercholesterolemia was defined as a total cholesterol level of at least 250 mg per deciliter (6.5 mmol per liter), an LDL level of at least 150 mg per deciliter (3.9 mmol per liter), or ongoing treatment with lipid-lowering agents. The study was approved by the Mayo Clinic institutional review board, and all patients gave written informed consent.

ANGIOGRAPHIC ANALYSIS

The maximal stenosis in each of 27 coronary-artery segments was assessed by a cardiologist, who was unaware of risk factors, with the use of handheld calipers or in visual analysis according to the segmental classification system of the Coronary Artery Surgery Study. The extent of angiographically documented coronary artery disease was quantified as follows: normal coronary arteries (smooth, with either no stenosis or a stenosis of <10 percent of the luminal diameter), mild disease (a stenosis of 10 to 50 percent of the luminal diameter in one or more coronary arteries or their major branches), or one-vessel, two-vessel, or three-vessel disease, defined as a stenosis of more than 50 percent of the luminal diameter in one, two, or three coronary arteries or their major branches.[10]

LABORATORY ANALYSES

Analyses of apo B-100, Lp(a) lipoprotein, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were performed with the use of commercially available kits. LDL cholesterol was estimated with the use of the Friedewald formula. High-sensitivity C-reactive protein (CRP) (lower limit of detection, 0.15 mg per liter) was measured as described elsewhere.[11]

Our assay of oxidized LDL determines the content of oxidized phospholipids per particle of apo B-100 (oxidized phospholipid:apo B-100 ratio) and is performed with the use of the murine monoclonal antibody E06, which specifically binds to the phosphorylcholine moiety of oxidized but not native phospholipids.[6,7] We have previously used the term OxLDL-E06 to describe the name of this assay. In brief, a dilution of plasma at 1:50 in phosphate-buffered saline was added to microtiter wells coated with monoclonal antibody MB47, which specifically binds apo B-100 particles. Under these conditions, a saturating amount of apo B-100 was added to each well, and consequently, equal numbers of apo B-100 particles were captured in each well for all assays. The oxidized phospholipid:apo B-100 ratio was measured by chemiluminescent enzyme-linked immunosorbent assay with the use of biotinylated E06, as described elsewhere.[6,7]

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

STATISTICAL ANALYSIS

Discrete data are presented as frequencies and percentages, and continuous variables as means and standard deviations or as medians and interquartile ranges if the distributions were skewed. Spearman's correlation coefficient was used to measure the linear associations between the rank values of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels as well as lipid levels and other clinical risk factors. The association of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels with the extent of coronary artery disease was tested by one-way analysis of variance of the log-transformed values followed by a one-degree-of-freedom test for trend. The percentages of patients with obstructive coronary artery disease and the odds ratios were calculated for quartiles of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels for all patients, according to age (≤60 years or >60 years), and according to the presence or absence of hypercholesterolemia.

Logistic-regression models were used to estimate the associations between patients' characteristics and lipid measurements and obstructive coronary artery disease. Multiple logistic-regression analysis was used to estimate the partial associations between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels and obstructive coronary artery disease, with adjustment for age, sex, smoking status, the presence or absence of hypertension, and levels of LDL cholesterol, HDL cholesterol, triglycerides, and CRP. The base-2 logarithms ($\log_2$) of the oxidized phospholipid:apo B-100 ratio and the levels of Lp(a) lipoprotein, triglycerides, and CRP were used in all the logistic-regression models to account for skewness in the distributions. Thus, odds ratios for these variables reflect the change in odds for an increase of 1 $\log_2$ (the equivalent of a doubling of the value) in the measure.

RESULTS

The baseline clinical characteristics of the patients, indications for coronary angiography, lipid measurements, and CRP levels are shown in Table 1. The distributions of both the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were skewed toward lower values, with 85 percent of the patients having levels lower than 0.4 and 45 mg per deciliter, respectively (Fig. 1). In the entire population, a strong correlation (r=0.83, P<0.001) was noted between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels.

ASSOCIATION WITH THE EXTENT OF ANGIOGRAPHICALLY DOCUMENTED DISEASE

In the entire study group, the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were strongly associated with a graded increase in the extent of coronary artery disease (P<0.001 for both analyses) (data not shown). These relationships were markedly stronger for patients 60 years of age or younger than for patients older than 60 years (Fig. 2).

ASSOCIATION WITH OBSTRUCTIVE CORONARY ARTERY DISEASE

The proportion of patients with obstructive coronary artery disease increased consistently with increases in the oxidized phospholipid:apo B-100 ratio and in Lp(a) lipoprotein levels (Table 2). This association was particularly evident among patients 60 years of age or younger, among whom the highest quartiles of the oxidized phospholipid:apo B-100 ratio (odds ratio, 3.12; P<0.001) and Lp(a) lipoprotein levels (odds ratio, 3.64, P<0.001) were associated with a significantly higher risk, as compared with the lowest quartiles. This association was not present among patients older than 60 years.

The combined effects of hypercholesterolemia plus either the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels greatly increased the probability of obstructive coronary artery disease. When compared with patients in the lowest quartile who did not have hypercholesterolemia, patients in the highest quartile of the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels who had hypercholesterolemia were significantly more likely to have obstructive coronary artery disease (Table 3). These relationships were markedly accentuated among patients 60 years of age or younger (for the oxidized phospholipid:apo B-100 ratio, odds ratio, 16.8 [P<0.001]; for Lp(a) lipoprotein levels, odds ratio, 14.2 [P<0.001]), as compared with those older than 60 years (for the oxidized phospholipid:apo B-100 ratio, odds ratio, 4.95 [P=0.003]; for Lp(a) lipoprotein levels, odds ratio, 4.92 [P=0.007]).

The relationship of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels to coronary artery disease remained fundamentally similar after the exclusion from analysis of 41 patients with acute myocardial infarction within six weeks

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

before enrollment. Also, there was a stronger association between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels and coronary artery disease in patients with hypercholesterolemia who were taking statins than among such patients who were not taking statins, but differences in the odds ratios were not statistically significant (data not shown).

PREDICTORS OF OBSTRUCTIVE CORONARY ARTERY DISEASE

Without adjustment for other risk factors, the oxidized phospholipid:apo B-100 ratio was predictive of obstructive coronary artery disease (odds ratio per doubling, 1.19; 95 percent confidence interval, 1.05 to 1.34; P=0.005) as was the Lp(a) lipoprotein level (odds ratio per doubling, 1.22; 95 percent confidence interval, 1.07 to 1.40; P=0.003). Similarly, male sex (odds ratio, 4.33; 95 percent confidence interval, 2.95 to 6.35; P<0.001), age (odds ratio per decade, 1.48; 95 percent confidence interval, 1.25 to 1.75; P<0.001), current smoking (odds ratio, 1.65; 95 percent confidence interval, 1.16 to 2.35; P=0.006), hypertension (odds ratio, 1.81; 95 percent confidence interval, 1.27 to 2.58; P=0.001), LDL cholesterol (odds ratio per increase of 25 mg per deciliter [0.65 mmol per liter], odds ratio, 1.28; 95 percent confidence interval, 1.12 to 1.45; P=0.003), and triglyceride levels (odds ratio per doubling, 1.27; 95 percent confidence interval, 1.00 to 1.61; P=0.05) were also predictive, whereas HDL cholesterol (odds ratio per increase of 10 mg per deciliter [2.3 mmol per liter], 0.64; 95 percent confidence interval, 0.56 to 0.74; P<0.001) was a negative predictor. CRP (odds ratio per doubling, 1.08; 95 percent confidence interval, 0.98 to 1.19; P=0.12) was not a predictor of obstructive coronary artery disease.

Among patients 60 years of age or younger, the odds ratios per doubling for the oxidized phospholipid:apo B-100 ratio (1.43; 95 percent confidence interval, 1.20 to 1.71; P<0.001) and Lp(a) lipoprotein level (1.41; 95 percent confidence interval, 1.16 to 1.73; P<0.001) were significant, whereas among those older than 60 years they were no longer significant (for the oxidized phospholipid:apo B-100 ratio: odds ratio per doubling, 1.05; 95 percent confidence interval, 0.89 to 1.25; P=0.58; and for Lp[a] lipoprotein levels: odds ratio per doubling, 1.09; 95 percent confidence interval, 0.90 to 1.32; P=0.37).

Multivariable analysis with the use of logistic-regression models to derive adjusted odds ratios for coronary artery disease showed that an increase in the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.21; 95 percent confidence interval, 1.05 to 1.39; P=0.007) was an independent predictor of obstructive coronary artery disease, as were male sex (odds ratio, 4.27; 95 percent confidence interval, 2.59 to 7.03; P<0.001), age (odds ratio per decade, 1.72; 95 percent confidence interval, 1.41 to 2.10; P<0.001), an increase in LDL cholesterol (odds ratio per 25 mg per deciliter, 1.28; 95 percent confidence interval, 1.11 to 1.48; P<

Table 1. Baseline Characteristics and Lipid Levels in the Study Group.*

Variable	Value
Age — yr	60.1±10.9
Female sex — no. (%)	193 (38)
White race — no. (%)†	490 (97)
Hypertension — no. (%)	232 (46)
Current smoker — no. (%)	40 (8)
Previous myocardial infarction — no. (%)	77 (15)
Congestive heart failure — no. (%)	59 (12)
Family history of coronary artery disease — no. (%)	128 (25)
Hypercholesterolemia — no. (%)	286 (57)
Statin therapy — no. (%)	142 (28)
Serum creatinine level — mg/dl	
Median	1.1
Interquartile range	1.0–1.3
Indications for angiography — no. (%)‡	
Myocardial infarction within 6 wk before enrollment	41 (8)
Unstable angina	147 (29)
Dyspnea on exertion	137 (27)
Ischemia on nuclear stress test	125 (25)
Other	166 (33)
Lipid levels — mg/dl	
Total cholesterol	207±45
LDL cholesterol	124±37
HDL cholesterol	48±15
Triglycerides	
Median	153
Interquartile range	112–207
Apolipoprotein B-100	98±21
Lp(a) lipoprotein	
Median	21.1
Interquartile range	8.8–39.6
C-reactive protein — mg/liter	
Median	2.9
Interquartile range	1.2–6.7

* The study group was made up of 504 patients. Plus–minus values are means ±SD. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.
† Race was self-reported.
‡ Patients could have more than one indication for angiography.

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

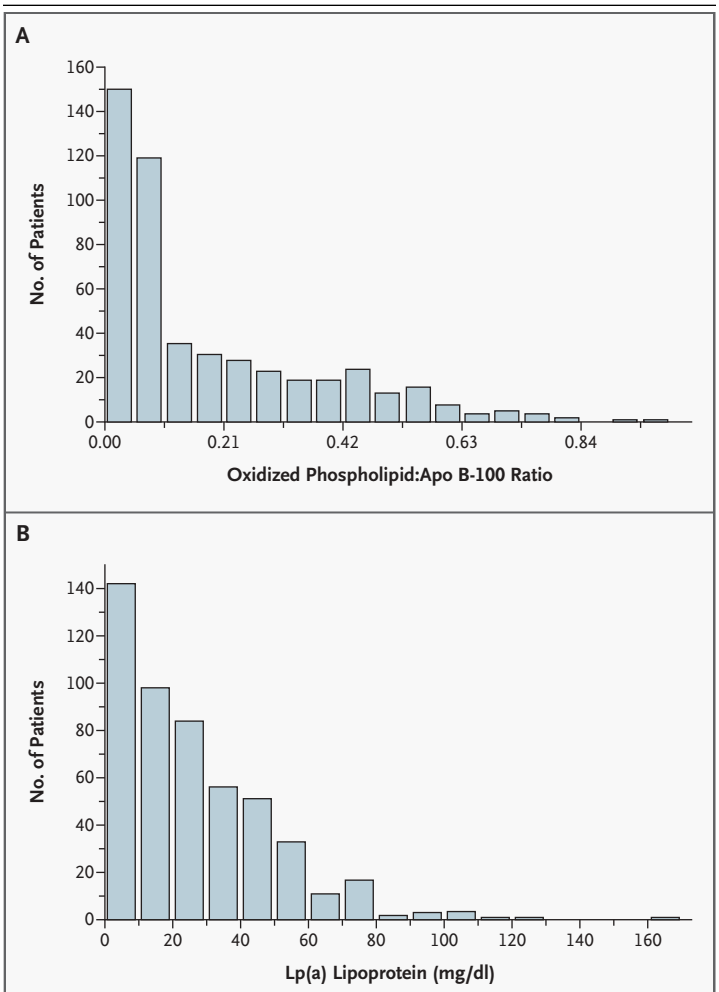

Figure 1. Frequency Distribution of the Oxidized Phospholipid:Apo B-100 Ratio (Panel A) and Lp(a) Lipoprotein Levels (Panel B).

Oxidized phospholipid:apo B-100 ratio denotes the oxidized phospholipid content per particle of apolipoprotein B-100.

0.001), and hypertension (odds ratio, 1.67; 95 percent confidence interval, 1.10 to 2.52; P=0.016), whereas an increase in HDL cholesterol levels (odds ratio per 10 mg per deciliter, 0.75; 95 percent confidence interval, 0.63 to 0.90; P=0.002) was a negative predictor. An increase in CRP (odds ratio per doubling, 1.09; 95 percent confidence interval, 0.97 to 1.22; P=0.16) was not a predictor of obstructive coronary artery disease. When Lp(a) lipoprotein was added to the model and the oxidized phospholipid:apo B-100 ratio was removed, Lp(a) lipoprotein was also an independent predictor (odds ratio per doubling, 1.20; 95 percent confidence interval, 1.02 to 1.40; P=0.02). As in the unadjusted data, the odds ratios per doubling for the oxidized phospholipid:apo B-100 ratio (1.49; 95 percent confidence interval, 1.20 to 1.84; P<0.001) and for Lp(a) lipoprotein (1.42; 95 percent confidence interval, 1.12 to 1.81; P=0.004) among patients 60 years of age or younger were significantly accentuated, whereas among those older than 60 years they were no longer significant (for the oxidized phospholipid: apo B-100 ratio: odds ratio per doubling, 1.00; 95 percent confidence interval, 0.82 to 1.22; P=0.96; for Lp(a) lipoprotein: odds ratio per doubling, 1.05; 95 percent confidence interval, 0.84 to 1.31; P=0.69).

Interestingly, in the entire study group, when Lp(a) lipoprotein was forced into the model with the oxidized phospholipid:apo B-100 ratio, there was a trend toward significance of the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.21; 95 percent confidence interval, 0.95 to 1.54; P=0.12), whereas Lp(a) lipoprotein levels no longer remained an independent predictor of coronary artery disease (odds ratio per doubling, 1.00; 95 percent confidence interval, 0.76 to 1.32; P=0.99). However, when patients were analyzed according to age, the oxidized phospholipid:apo B-100 ratio, but not Lp(a) lipoprotein levels, was an independent predictor of obstructive coronary artery disease among those 60 years of age or younger, but not among those older than 60 years (Fig. 3). CRP was also a predictor of obstructive coronary artery disease among patients 60 years of age or younger, but not among those older than 60 years. When the 41 patients with acute myocardial infarction, who also had the highest levels of CRP, were removed from the analysis, CRP was no longer a predictor of obstructive coronary artery disease (odds ratio per doubling, 1.06; 95 percent confidence interval, 0.85 to 1.33; P=0.58), but the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.55; 95 percent confidence interval, 1.05 to 2.27; P=0.03) remained a significant predictor. When the data were evaluated according to the absence of coronary artery disease, as compared with the presence of any coronary artery disease, the odds ratios were slightly smaller, but in general, the trends described were maintained, so that younger patients had higher odds ratios than older patients.

CORRELATIONS BETWEEN OXIDIZED LDL LEVELS AND OTHER BIOMARKERS

Levels of LDL cholesterol were weakly associated with levels of Lp(a) lipoprotein (r=0.17, P<0.001),

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

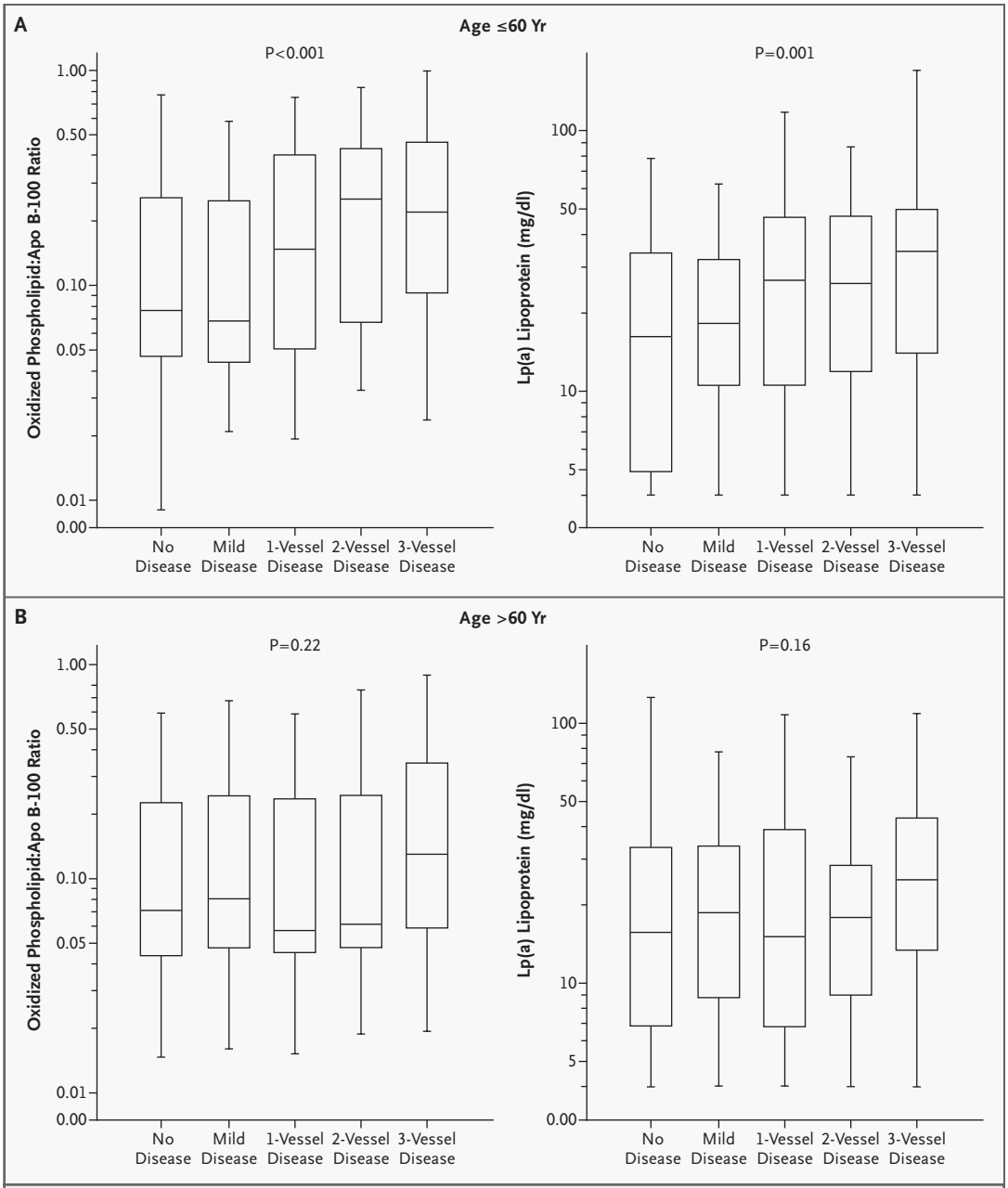

Figure 2. Association of the Oxidized Phospholipid:Apo B-100 Ratio and Lp(a) Lipoprotein Levels to the Extent of Coronary Artery Disease (CAD) in 239 Patients 60 Years of Age or Younger and 265 Patients Older Than 60 Years.

The extent of coronary artery disease is categorized as no disease (stenosis of less than 10 percent of the luminal diameter), mild disease (stenosis of 10 to 50 percent of the luminal diameter), or one-vessel, two-vessel, or three-vessel disease (all patients with a stenosis of more than 50 percent of the luminal diameter). Each box represents the median and interquartile range of values, with the I bars (whiskers) extended to the minimum and maximum. Oxidized phospholipid:apo B-100 ratio denotes the oxidized phospholipid content per particle of apolipoprotein B-100. Values for the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein are shown on a logarithmic scale.

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

Table 2. Odds Ratios for Obstructive Coronary Artery Disease (CAD) According to Quartiles for the Ratio of Oxidized Phospholipids to Apolipoprotein B-100 and Levels of Lp(a) Lipoprotein.*

Patient Group	Oxidized Phospholipid:Apo B-100 Ratio			Lp(a) Lipoprotein		
	Total No.	No. with CAD (%)	OR (95% CI)	Total No.	No. with CAD (%)	OR (95% CI)
All patients						
Quartile I	126	59 (47)	1.00	126	56 (44)	1.00
Quartile II	125	64 (51)	1.19 (0.73–1.96)	126	62 (49)	1.21 (0.74–1.99)
Quartile III	126	68 (54)	1.33 (0.81–2.18)	127	70 (55)	1.54 (0.94–2.52)
Quartile IV	125	79 (63)	1.95 (1.18–3.23)	125	83 (66)	2.47 (1.48–4.12)
P for trend			0.009			<0.001
Age ≤60 yr						
Quartile I	58	19 (33)	1.00	60	18 (30)	1.00
Quartile II	53	17 (32)	0.97 (0.44–2.15)	57	19 (33)	1.17 (0.53–2.54)
Quartile III	60	27 (45)	1.68 (0.79–3.55)	58	28 (48)	2.18 (1.02–4.63)
Quartile IV	68	41 (60)	3.12 (1.50–6.48)	64	39 (61)	3.64 (1.73–7.68)
P for trend			<0.001			<0.001
Age >60 yr						
Quartile I	68	40 (59)	1.00	66	38 (58)	1.00
Quartile II	72	47 (65)	1.32 (0.66–2.61)	69	43 (62)	1.22 (0.61–2.43)
Quartile III	66	41 (62)	1.15 (0.57–2.30)	69	42 (61)	1.15 (0.58–2.28)
Quartile IV	57	38 (67)	1.40 (0.67–2.83)	61	44 (72)	1.91 (0.91–4.01)
P for trend			0.46			0.13

* OR denotes odds ratio, and CI confidence interval. For the oxidized phospholipid:apo B-100 ratio, quartiles I through IV correspond to the following values: <0.047, 0.047 to 0.089, >0.089 to 0.294, and >0.294, respectively. For Lp(a) lipoprotein, quartiles I through IV correspond to the following values: <8.8, 8.8 to 21.1, >21.1 to 39.7, and >39.7 mg per deciliter, respectively. Samples for the oxidized phospholipid:apo B-100 ratio were unavailable for two patients.

and with the oxidized phospholipid:apo B-100 ratio ($r=0.09$, $P=0.05$). CRP levels correlated weakly with LDL cholesterol levels ($r=0.10$, $P=0.02$) and triglyceride levels ($r=0.11$, $P=0.01$). There were no significant correlations between the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels and CRP levels, age, body-mass index, blood pressure, and serum creatinine level.

DISCUSSION

This study shows an association between the oxidized phospholipid:apo B-100 ratio in plasma and the presence and extent of angiographically documented coronary artery disease. The association is independent of all clinical and lipid-related risk factors, except one, Lp(a) lipoprotein, which also has a strong association with angiographically documented coronary artery disease. The odds ratios for angiographically documented coronary artery disease associated with the Lp(a) lipoprotein level were nearly identical with those associated with the oxidized phospholipid:apo B-100 ratio. However, among patients younger than 60 years of age, the oxidized phospholipid:apo B-100 ratio remained an independent predictor of obstructive coronary artery disease. There was a strong correlation between levels of Lp(a) lipoprotein and the oxidized phospholipid:apo B-100 ratio. These observations, in conjunction with previous studies from our laboratory showing that in plasma such oxidized phospholipids are predominantly physically present on Lp(a) lipoprotein,[5-7,9] as opposed to other lipoproteins, lend strong support to the hypothesis that, in the setting of enhanced oxidative stress, proinflammatory oxidized phospholipids may, in part, mediate the atherogenicity of Lp(a) lipoprotein.

The natural murine monoclonal IgM autoantibody E06, cloned from apolipoprotein E-receptor–deficient mice,[12] is functionally identical with classic natural T15 murine antibodies that bind phosphorylcholine on the cell-wall polysaccharide of patho-

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

Table 3. Odds Ratios for Obstructive Coronary Artery Disease (CAD) According to Quartiles of the Ratio of Oxidized Phospholipids to Apolipoprotein B-100 and Levels of Lp(a) Lipoprotein in Patients without and with Hypercholesterolemia (HC).*

Patient Group	Oxidized Phospholipid:Apo B-100 ratio					LP(a) Lipoprotein				
	No HC		HC		P Value	No HC		HC		P Value
	% *with CAD*	*OR (95% CI)*	% *with CAD*	*OR (95% CI)*		% *with CAD*	*OR (95% CI)*	% *with CAD*	*OR (95% CI)*	
All patients										
Quartile I	29	1.00	67	4.93 (2.31–10.5)		31	1.00	60	3.41 (1.63–7.11)	
Quartile II	44	1.92 (0.91–4.06)	56	3.10 (1.54–6.25)		35	1.18 (0.55–2.56)	59	3.28 (1.64–6.56)	
Quartile III	38	1.47 (0.68–3.19)	64	4.36 (2.16–8.79)		39	1.42 (0.67–3.02)	67	4.57 (2.25–9.29)	
Quartile IV	39	1.54 (0.70–3.40)	77	8.13 (3.88–17.1)	<0.001	48	2.04 (0.93–4.48)	77	7.30 (3.53–15.1)	<0.001
Age ≤60 yr										
Quartile I	14	1.00	61	9.33 (2.64–33.0)		15	1.00	50	5.80 (1.71–19.7)	
Quartile II	27	2.21 (0.61–7.97)	37	3.53 (1.03–12.0)		15	1.05 (0.25–4.39)	48	5.44 (1.67–17.7)	
Quartile III	28	2.33 (0.64–8.45)	57	8.00 (2.51–25.5)		37	3.36 (1.01–11.2)	61	8.96 (2.66–30.2)	
Quartile IV	43	4.59 (1.39–15.1)	74	16.8 (5.11–55.2)	<0.001	46	4.97 (1.46–16.9)	71	14.2 (4.37–46.3)	<0.001
Age >60 yr										
Quartile I	45	1.00	71	3.00 (1.10–8.18)		47	1.00	69	2.47 (0.90–6.77)	
Quartile II	61	1.85 (0.67–5.15)	68	2.57 (1.01–6.54)		54	1.31 (0.47–3.65)	67	2.33 (0.92–5.89)	
Quartile III	48	1.11 (0.39–3.14)	71	2.90 (1.11–7.58)		42	0.80 (0.28–2.31)	71	2.77 (1.09–7.03)	
Quartile IV	31	0.55 (0.15–1.92)	80	4.95 (1.76–13.9)	0.003	50	1.12 (0.36–3.53)	81	4.92 (1.77–13.7)	0.007

* OR denotes odds ratio, and CI confidence interval. For the oxidized phospholipid:apo B-100 ratio, quartiles I through IV correspond to the following values: <0.047, 0.047 to 0.089, >0.089 to 0.294, and >0.294, respectively. For Lp(a) lipoprotein, quartiles I through IV correspond to the following values: <8.8, 8.8 to 21.1, >21.1 to 39.7, and >39.7 mg per deciliter, respectively. The P values indicate whether any two of the eight groups (defined by quartile and hypercholesterolemia status) have significantly different proportions of subjects with CAD.

gens such as pneumococcus and provide optimal protection from pneumococcal infections.[13] In vitro, E06 binds to and prevents the uptake of oxidized LDL and apoptotic cells by scavenger receptors of macrophages. Binder et al. have also shown that the immunization of mice with *Streptococcus pneumoniae* results in increased titers of IgM oxidized LDL autoantibodies and reduction in the progression of atherosclerosis.[14,15] These observations suggest that seemingly unrelated proatherogenic processes, such as oxidation, apoptosis, and infection, share molecular mimicry of the phosphorylcholine epitopes found on proinflammatory oxidized phospholipids.[16]

Although previous studies have shown that plasma oxidized LDL levels are elevated in patients with clinically manifest stable coronary artery disease[17,18] and acute coronary syndromes,[2,4,5,19] our study shows that oxidized phospholipids present on particles of apo B-100 and primarily on Lp(a) lipoprotein correlate with both the presence and extent of angiographically documented coronary artery disease. Although most of the oxidized LDL is present within the vessel wall,[20] this study suggests that the small amounts of minimally modified LDL (e.g., particles of apo B-100 that contain oxidized phospholipids) are present in the circulation. This finding is also consistent with previous studies from our laboratory showing that the oxidized phospholipid:apo B-100 ratio (with oxidized LDL measured with use of antibody E06) rises abruptly after acute coronary events[5] and immediately after percutaneous coronary intervention[6] — situations in which the release of oxidized phospholipids (or oxidized LDL, or both) from the vessel wall might be postulated.

A potential pathophysiological relationship between levels of oxidized phospholipids and Lp(a) lipoprotein is strongly supported by this study and by data from earlier studies from our laboratory showing that oxidized phospholipids are physically associated with Lp(a) lipoprotein[5-7] bound to

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

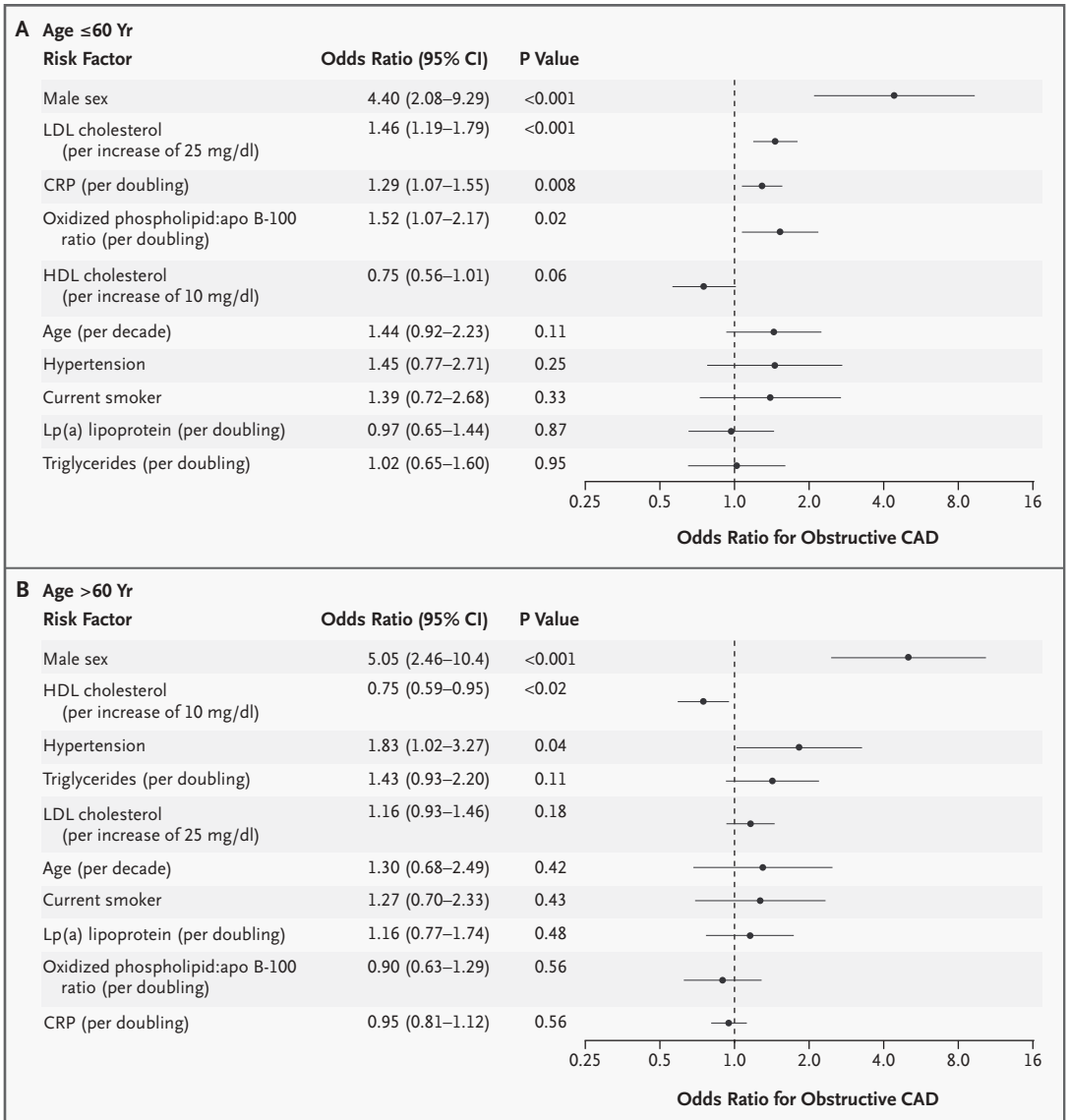

Figure 3. Odds Ratios for Obstructive Coronary Artery Disease (CAD) Associated with Selected Risk Factors among Patients 60 Years of Age or Younger and Those Older Than 60 Years, from the Multivariable Analysis.

CI denotes confidence interval, LDL low-density lipoprotein, CRP C-reactive protein, oxidized phospholipid:apo B-100 ratio the ratio of oxidized phospholipid content per particle of apolipoprotein B-100, and HDL high-density lipoprotein. Risk factors are shown in descending order of significance. In this analysis, Lp(a) lipoprotein was forced into the model with the oxidized phospholipid:apo B-100 ratio.

lysine residues on isolated fragments of kringle V of apolipoprotein(a)[9] and also in the lipid phase of Lp(a) lipoprotein (unpublished data). In addition, the kringle V fragments containing such oxidized phospholipids induce inflammatory responses by up-regulating secretion of interleukin-8 by cultured human macrophages.[9,21]

In this study, we have shown that the predictive abilities of levels of oxidized LDL and Lp(a) lipoprotein for obstructive coronary artery disease are highly interdependent. In the entire study group, when Lp(a) lipoprotein was excluded from the multivariable analysis, the odds ratios for the oxidized phospholipid:apo B-100 ratio were similar to those for traditional risk factors such as age, hypertension, and LDL cholesterol. Similarly, without the ox-

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

idized phospholipid:apo B-100 ratio in the analysis, Lp(a) lipoprotein levels stood as an independent predictor, as has been shown in a recent meta-analysis.[8] In the entire study group, with the oxidized phospholipid:apo B-100 ratio in the model, there was no added ability of Lp(a) lipoprotein levels to explain the risk of obstructive coronary artery disease, suggesting that measures of oxidized LDL and Lp(a) lipoprotein represent a common path of biologic influence on the risk for coronary artery disease. However, in patients 60 years of age or younger, the oxidized phospholipid:apo B-100 ratio maintained its independent predictive power even with Lp(a) lipoprotein in the model. This observation supports the hypothesis that much of the risk attributable to Lp(a) lipoprotein levels can be explained by the binding of oxidized phospholipids by Lp(a) lipoprotein, but that in younger patients, an additional risk associated with oxidized phospholipids may be present, perhaps through proinflammatory pathways independent of Lp(a) lipoprotein.

The physiologic role of Lp(a) lipoprotein is unknown. We and others have suggested that a potential physiologic role of Lp(a) lipoprotein may be to bind and detoxify proinflammatory oxidized phospholipids.[5-7,22] Lp(a) lipoprotein, which is present only in humans and Old World primates (although a partially related gene arose separately in hedgehogs), may have evolved to provide protection against various oxidative stressors. For example, Lp(a) lipoprotein has been shown to be involved in wound healing[23] and possibly in preventing angiogenesis in tumor models,[24] and elevated levels have been noted in centenarians in a manner consistent with human longevity.[25]

Similarly, oxidized phospholipids are generated not only during atherogenesis but also in inflammation and apoptosis,[13,26] which suggests that housekeeping functions involving the clearance of such oxidized phospholipids may have evolved for maintaining general health as well as vascular health. In this regard, Lp(a) lipoprotein may act in a way similar to CRP, which Chang et al. have shown also binds specifically to the phosphorylcholine moiety of oxidized phospholipids and apoptotic cells.[27] Indeed, we and others have shown that Lp(a) lipoprotein acts as an acute-phase reactant in patients with acute coronary syndromes.[5,6,28] It has also been reported to be highly enriched (higher by a factor of 7 than LDL) in platelet-activating factor acetyl hydrolase,[29,30] an enzyme that potentially could detoxify such oxidized phospholipids by removing the oxidized fatty acid.

Thus, when present at low levels, Lp(a) lipoprotein may serve a protective function by binding and participating in the transfer and possible degradation of oxidized phospholipids formed during normal homeostasis or in acutely stressful situations. However, when Lp(a) lipoprotein levels are chronically elevated (as determined genetically), especially in a milieu of chronically increased oxidative stress, Lp(a) lipoprotein, with its content of oxidized phospholipids, may be proatherogenic, particularly since it has enhanced binding to the extracellular matrix of the artery wall.[31-33]

The association between the oxidized phospholipid:apo B-100 ratio and angiographically documented coronary artery disease in our study was much stronger for patients 60 years of age or younger than for older patients. The reasons for this association are not entirely clear, but many previous studies have documented a strikingly similar relationship between Lp(a) lipoprotein levels and angiographically documented disease among younger patients only.[34-40] By excluding patients with diabetes and previous coronary revascularization from our study, we may have preferentially enriched the study group with younger patients with fewer traditional risk factors. In addition, increasing age, which is a surrogate for known and unknown risk factors, is itself one of the strongest risk factors for coronary artery disease. Thus, the independent effects of oxidized LDL and Lp(a) lipoprotein levels appear to diminish with age, presumably because of the cumulative contributions of additional risk factors that affect the clinical expression of atherosclerosis.

The limitations of this study include the fact that angiography is not a precise method for quantifying atherosclerosis. In addition, we have not yet defined the exact oxidized phospholipids, their physical location within Lp(a) lipoprotein, or the rates of flux, binding, and removal of oxidized phospholipids that are on Lp(a) lipoprotein.

In conclusion, we have documented that plasma levels of oxidized phospholipids present on apo B-100–containing lipoproteins and predominantly on Lp(a) lipoprotein reflect the presence and extent of angiographically documented coronary artery disease. We propose that in settings of enhanced oxidative stress and elevated Lp(a) lipoprotein levels, a proinflammatory milieu may predominate that contributes to the clinical expression of cardiovas-

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

cular disease. Further studies are needed to explore these mechanisms and to determine whether these measures of oxidation predict clinical events.

Supported by grants from the La Jolla Specialized Center of Research in Molecular Medicine and Atherosclerosis (HL56989), the National Heart, Lung, and Blood Institute (HL69646, HL57505), the Donald W. Reynolds Foundation, and the Mayo Foundation.

Dr. Tsimikas reports having served as a consultant to and on the speakers' bureau of Pfizer and General Electric and having received investigator-initiated grants from these companies. Dr. Witztum reports having served as a consultant to AtheroGenics and on the speakers' bureau of Merck. Dr. Berger reports having received research funding and honoraria from Aventis, Bristol-Myers Squib, and Sanofi and having served on scientific advisory boards for Genentech and Johnson & Johnson. Dr. Kornman is an employee of Interleukin Genetics and reports holding equity in the company. Dr. McConnell reports having received grant support from diaDexus. A patent for the potential use of the E06 antibody has been awarded to the University of California in the names of Dr. Witztum and colleagues and has been licensed by the University of California to AtheroGenics.

We are indebted to Claes Bergmark for advice on this project.

REFERENCES

1. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. Circulation 2001;103:1930-2.
2. Ehara S, Ueda M, Naruko T, et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation 2001;103:1955-60.
3. Holvoet P, Collen D, Van de Werf F. Malondialdehyde-modified LDL as a marker of acute coronary syndromes. JAMA 1999;281:1718-21.
4. Nishi K, Itabe H, Uno M, et al. Oxidized LDL in carotid plaques and plasma associates with plaque instability. Arterioscler Thromb Vasc Biol 2002;22:1649-54.
5. Tsimikas S, Bergmark C, Beyer RW, et al. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. J Am Coll Cardiol 2003;41:360-70.
6. Tsimikas S, Lau HK, Han KR, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. Circulation 2004;109:3164-70.
7. Tsimikas S, Witztum JL, Miller ER, et al. High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. Circulation 2004;110:1406-12.
8. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary artery disease: meta-analysis of prospective studies. Circulation 2000;102:1082-5.
9. Edelstein C, Pfaffinger D, Hinman J, et al. Lysine-phosphatidylcholine adducts in Kringle V impart unique immunological and potential pro-inflammatory properties to human apolipoprotein(a). J Biol Chem 2003;278:52841-7.
10. Wolk R, Berger P, Lennon RJ, Brilakis ES, Somers VK. Body mass index: a risk factor for unstable angina and myocardial infarction in patients with angiographically confirmed coronary artery disease. Circulation 2003;108:2206-11.
11. McConnell JP, Branum EL, Ballman KV, Lagerstedt SA, Katzmann JA, Jaffe AS. Gender differences in C-reactive protein concentrations — confirmation with two sensitive methods. Clin Chem Lab Med 2002;40:56-9.
12. Palinski W, Hörkkö S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice: demonstration of epitopes of oxidized low density lipoprotein in human plasma. J Clin Invest 1996;98:800-14.
13. Shaw PX, Hörkkö S, Chang MK, et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest 2000;105:1731-40.
14. Binder CJ, Horkko S, Dewan A, et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. Nat Med 2003;9:736-43.
15. Binder CJ, Hartvigsen K, Chang MK, et al. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. J Clin Invest 2004;114:427-37.
16. Binder CJ, Chang MK, Shaw PX, et al. Innate and acquired immunity in atherogenesis. Nat Med 2002;8:1218-26.
17. Toshima S, Hasegawa A, Kurabayashi M, et al. Circulating oxidized low density lipoprotein levels: a biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 2000;20:2243-7.
18. Holvoet P, Mertens A, Verhamme P, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2001;21:844-8.
19. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998;98:1487-94.
20. Tsimikas S, Glass C, Steinberg D, Witztum JL. Lipoproteins, lipoprotein oxidation and atherogenesis. In: Chien KR, ed. Molecular basis of cardiovascular disease: a companion to Braunwald's Heart Disease. Philadelphia: W.B. Saunders, 2004:385-413.
21. Klezovitch O, Edelstein C, Scanu AM. Stimulation of interleukin-8 production in human THP-1 macrophages by apolipoprotein(a): evidence for a critical involvement of elements in its C-terminal domain. J Biol Chem 2001;276:46864-9.
22. Hobbs HH, White AL. Lipoprotein(a): intrigues and insights. Curr Opin Lipidol 1999;10:225-36.
23. Yano Y, Shimokawa K, Okada Y, Noma A. Immunolocalization of lipoprotein(a) in wounded tissues. J Histochem Cytochem 1997;45:559-68.
24. Trieu VN, Uckun FM. Apolipoprotein(a), a link between atherosclerosis and tumor angiogenesis. Biochem Biophys Res Commun 1999;257:714-8.
25. Thillet J, Doucet C, Chapman J, Herbeth B, Cohen D, Faure-Delanef L. Elevated lipoprotein(a) levels and small apo(a) isoforms are compatible with longevity: evidence from a large population of French centenarians. Atherosclerosis 1998;136:389-94.
26. Chang MK, Binder CJ, Miller YI, et al. Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. J Exp Med 2004;200:1359-70.
27. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphorylcholine of oxidized phospholipids. Proc Natl Acad Sci U S A 2002;99:13043-8.
28. Maeda S, Abe A, Seishima M, Makino K, Noma A, Kawade M. Transient changes of serum lipoprotein(a) as an acute phase protein. Atherosclerosis 1989;78:145-50.
29. Blencowe C, Hermetter A, Kostner GM, Deigner HP. Enhanced association of platelet-activating factor acetylhydrolase with lipoprotein(a) in comparison with low density lipoprotein. J Biol Chem 1995;270:31151-7.
30. Karabina SA, Liapikos TA, Grekas G, Goudevenos J, Tselepis AD. Distribution of PAF-acetylhydrolase activity in human plasma low-density lipoprotein subfractions. Biochim Biophys Acta 1994;1213:34-8.
31. Dangas G, Mehran R, Harpel PC, et al. Lipoprotein(a) and inflammation in human coronary atheroma: association with the severity of clinical presentation. J Am Coll Cardiol 1998;32:2035-42.
32. Cushing GL, Gaubatz JW, Nava ML, et al. Quantitation and localization of apolipoproteins [a] and B in coronary artery bypass vein grafts resected at re-operation. Arteriosclerosis 1989;9:593-603.

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.

33. Berg K, Dahlen G, Christophersen B, Cook T, Kjekshus J, Pedersen T. Lp(a) lipoprotein level predicts survival and major coronary events in the Scandinavian Simvastatin Survival Study. Clin Genet 1997;52:254-61.
34. Hearn JA, DeMaio SJ Jr, Roubin GS, Hammarstrom M, Sgoutas D. Predictive value of lipoprotein(a) and other serum lipoproteins in the angiographic diagnosis of coronary artery disease. Am J Cardiol 1990;66:1176-80.
35. Sandkamp M, Funke H, Schulte H, Kohler E, Assmann G. Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem 1990;36:20-3.
36. Foody JM, Milberg JA, Robinson K, Pearce GL, Jacobsen DW, Sprecher DL. Homocysteine and lipoprotein(a) interact to increase CAD risk in young men and women. Arterioscler Thromb Vasc Biol 2000;20:493-9.
37. Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL. Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA 1986;256:2540-4.
38. Dahlen GH, Guyton JR, Attar M, Farmer JA, Kautz JA, Gotto AM Jr. Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 1986;74:758-65.
39. Sunayama S, Daida H, Mokuno H, et al. Lack of increased coronary atherosclerotic risk due to elevated lipoprotein(a) in women > or = 55 years of age. Circulation 1996;94:1263-8.
40. Assmann G, Schulte H, von Eckardstein A. Hypertriglyceridemia and elevated lipoprotein(a) are risk factors for major coronary events in middle-aged men. Am J Cardiol 1996;77:1179-84.
Copyright © 2005 Massachusetts Medical Society.

JOURNAL INDEX

The index to volume 352 of the *Journal* will be available on August 18, 2005. At that time, it can be downloaded free in PDF format from **www.nejm.org** or can be ordered in a printed and bound format. To order a bound copy, please call 1-800-217-7874 from the United States and Canada (call 651-582-3800 from other countries) or e-mail info@valeoip.com.

The New England Journal of Medicine
Downloaded from nejm.org at MCGILL UNIVERSITY LIBRARY on December 15, 2012. For personal use only. No other uses without permission.
Copyright © 2005 Massachusetts Medical Society. All rights reserved.