

Oxidized Phospholipids, Lipoprotein(a), Lipoprotein-Associated Phospholipase A2 Activity, and 10-Year Cardiovascular Outcomes

Prospective Results From the Bruneck Study

Stefan Kiechl, Johann Willeit, Manuel Mayr, Brigitte Viehweider, Martin Oberhollenzer, Florian Kronenberg, Christian J. Wiedermann, Sabine Oberthaler, Qingbo Xu, Joseph L. Witztum, Sotirios Tsimikas

Background—Oxidized phospholipids (OxPL) circulate on apolipoprotein B-100 particles (OxPL/apoB), and primarily on Lp(a) lipoprotein (a) [Lp(a)]. The relationship of OxPL/apoB levels to future cardiovascular events is not known.

Methods and Results—The Bruneck study is a prospective population-based survey of 40- to 79-year-old men and women recruited in 1990. Plasma levels of OxPL/apoB and lipoprotein (a) [Lp(a)] were measured in 765 subjects in 1995 and incident cardiovascular disease (CVD), defined as cardiovascular death, myocardial infarction, stroke, and transient ischemic attack, was assessed from 1995 to 2005. During the follow-up period, 82 subjects developed CVD. In multivariable analysis, which included traditional risk factors, high sensitivity C-reactive protein (hsCRP), and lipoprotein-associated phospholipase A2 (Lp-PLA2) activity, subjects in the highest tertile of OxPL/apoB had a significantly higher risk of cardiovascular events than those in the lowest tertile (hazard ratio[95% CI] 2.4[1.3 to 4.3], $P=0.004$). The strength of the association between OxPL/apoB and CVD risk was amplified with increasing Lp-PLA2 activity ($P=0.018$ for interaction). Moreover, OxPL/apoB levels predicted future cardiovascular events beyond the information provided by the Framingham Risk Score (FRS). The effects of OxPL/apoB and Lp(a) were not independent of each other but they were independent of all other measured risk factors.

Conclusions—This study demonstrates that OxPL/apoB levels predict 10-year CVD event rates independently of traditional risk factors, hsCRP, and FRS. Increasing Lp-PLA2 activity further amplifies the risk of CVD mediated by OxPL/apoB. (*Arterioscler Thromb Vasc Biol.* 2007;27:1788-1795.)

Key Words: lipoproteins ■ oxidation ■ atherosclerosis ■ lipoprotein (a) ■ oxidized phospholipids ■ lipoprotein-associated phospholipase A2

Genetic, environmental, and lifestyle risk factors drive the lipid abnormalities, oxidative stress, inflammation, and thrombogenicity that ultimately determine the progression of atherosclerosis. These factors mediate chronic inflammatory responses and the transition of quiescent plaques to vulnerable plaques and finally to clinical sequelae.¹⁻³

Oxidized lipids play a central role in amplifying the inflammatory response by mediating a variety of immune, proinflammatory, and plaque destabilizing processes.⁴ Plasma levels of specific oxidized phospholipids (OxPL) 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 disease,^{5,6} acute coronary syndromes,⁷ and

after percutaneous coronary intervention.⁸ Interestingly, in human plasma, OxPL are preferentially carried by Lp(a) lipoprotein (a) [Lp(a)], compared with other apoB-100 particles.⁵⁻¹¹

In the Bruneck study, it was previously demonstrated 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 2000.⁶ Elevated OxPL/apoB and Lp(a) levels measured in 1995 also predicted the presence of symptomatic cardiovascular disease at the 1995 time point. However, the relationship of OxPL/apoB levels in predicting new cardiovascular events is not known. In this study, subjects were prospectively followed for 10 years, and the relationship between OxPL/apoB and

Original received January 29, 2007; final version accepted April 23, 2007.

From the Department of Neurology (S.K., J.W.), the Division of General Internal Medicine (C.J.W., S.O., B.V., M.O.), and the Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology (F.K.), Medical University of Innsbruck, Austria; the Cardiovascular Division (Q.X., M.M.), King's College London, University of London, UK; and the Department of Medicine (J.L.W., S.T.), University of California San Diego, La Jolla.

Correspondence to Sotirios Tsimikas, Vascular Medicine Program, University of California San Diego, 9500 Gilman Drive, BSB 1080, La Jolla, CA 92093-0682. E-mail stsimikas@ucsd.edu

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Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

DOI: 10.1161/ATVBAHA.107.145805

Lp(a) levels measured in 1995 and the development of new cardiovascular disease (CVD) events accrued up to the year 2005 is reported.

Methods

Study Subjects

The Bruneck Study is a prospective population-based survey of the epidemiology and pathogenesis of atherosclerosis.¹² The study population was recruited as a sex- and age-stratified random sample of all inhabitants of Bruneck, Italy (125 women and 125 men in the 5th to 8th decades each, $n=1000$). At the 1990 baseline, 93.6% of recruited subjects participated, with data assessment completed in 919 subjects. The current study focuses on the 1995 reexamination and the follow-up period for clinical events between 1995 and 2005. In 1995, the study population still consisted of 826 subjects (96.5% of those alive), and plasma samples for assessment of OxPL/apoB and Lp(a) were available in a random subsample of 765 subjects.¹² Detailed information about cardiovascular events developing between 1995 and 2005 was available in all of these subjects (100% follow-up). The study protocol was approved by the appropriate ethics committees, and all study subjects gave their written informed consent before entering the study.

Study Methodology and Laboratory Methods

All risk factors were assessed by validated standard procedures. Study methodology and laboratory methods for factors listed in Table 1 were previously described in detail.^{6,12,13}

Determination of OxPL/apoB and Lp(a) Levels

The content of OxPL per apoB-100 particle (OxPL/apoB) was measured as previously described in detail by chemiluminescent ELISA using the murine monoclonal antibody E06, which binds to the phosphorylcholine (PC) headgroup of oxidized but not native phospholipids.^{5,6,9} As previously described, equal numbers of apoB-100 particles are captured from each plasma sample and 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 LDL-cholesterol) levels.⁶ It is to be emphasized that the "apoB" measure depicted in the denominator of the OxPL/apoB parameter does not represent the plasma apoB level, but instead reflects the amount of apoB captured on each microtiter well plate. Lp(a) was measured as previously described.⁶ The intra- and interassay coefficients of variation were: OxPL/apoB: 6% to 10%,⁶ Lp(a): 3.6 to 6.3%, and apoB-100: 2.4%.

Measurement of Lipoprotein-Associated Phospholipase A2

The enzyme Lp-PLA2, also known as platelet activating factor acetylhydrolase, cleaves oxidized fatty acid moieties at the sn-2 position of OxPL to generate a free oxidized fatty acid and lysophosphatidylcholine. Lp-PLA2 activity was measured with a commercially available kit (Azwell Inc) based on the method of Kosaka et al.¹⁴ Reference normal values are <800 IU/L ($\mu\text{mol}/\text{min/L}$) as given by the manufacturer.

Assessment of Future Cardiovascular Events

In the primary analysis the CVD end point comprised all incident cases of cardiovascular death, myocardial infarction, ischemic stroke, and transient ischemic attack TIA ($n=82$). Sensitivity analyses focused on individual diseases and extended composite outcomes. Extended composite end points additionally included revascularization procedures which increased the number of individuals affected from 82 to 98 and new onset symptomatic peripheral arterial disease which further increased the number of individuals affected from 98 to 108. Myocardial infarction was deemed confirmed when World Health Organization criteria for definite disease status were met. Stroke and TIA were classified according to the criteria of the National Survey of Stroke. The diagnosis of symptomatic peripheral

arterial disease required a positive response to the Rose questionnaire (typical claudication), with the vascular nature of complaints confirmed by standard diagnostic procedures (ankle-brachial pressure index or angiography), or an acute peripheral artery occlusion requiring revascularization. All other revascularization procedures (angioplasty and surgery) were carefully recorded. Ascertainment of events or procedures did not rely on hospital discharge codes or the patient's self-report but on a careful review of medical records provided by the general practitioners and files of the Bruneck Hospital and the extensive clinical and laboratory examinations performed as part of the study protocols.^{6,12,13} A major advantage of the Bruneck Study cohort is that virtually all subjects living in the area of Bruneck were referred to the local Bruneck Hospital, and that the network existing between the local hospital and the general practitioners allowed the retrieval of virtually all medical information on people living in the area.

Statistics

All calculations were performed using the SPSS 12.0 and BMDP software packages. Continuous variables were presented as means \pm SD or medians (interquartile range), and dichotomous variables as percentages. Differences in baseline levels of vascular risk attributes between subjects with and without subsequent CVD (1995 to 2005) were analyzed with the Student *t* test and χ^2 test. Variables with a skewed distribution were \log_{eB} -transformed to satisfy the assumption of normality and constant variance of the residuals. Cox proportional hazard models were used to assess whether baseline OxPL/apoB levels were independent risk predictors for incident CVD. For this purpose OxPL/apoB was either modeled as a categorical (tertile or sextile groups) or as a continuous variable. Several models were run: the first model included age, sex, previous cardiovascular disease, and OxPL/apoB; the second model was additionally adjusted for systolic blood pressure, smoking, diabetes, ferritin level, fibrinogen level, LDL-C and HDL-C, waist-hip ratio, alcohol consumption, social status, sports activity, and \log_{eB} -transformed level of hsCRP, urinary albumin, uric acid, \log_{e} -transformed $\alpha 1$ -antitrypsin, \log_{e} -transformed homeostasis model of insulin resistance (HOMA-IR), and Lp-PLA₂ activity. ApoB was not included because of the high correlation with LDL-C and potential problem of collinearity. If apoB was used instead of LDL-C, however, results remained virtually unchanged. Furthermore, alternative models built by a forward stepwise selection procedure (allowing for all variables in Table 1) yielded very similar results with respect to the OxPL/apoB-CVD association; the third model included previous cardiovascular disease, the Framingham Risk Score, and \log_{eB} -transformed level of hsCRP. To test for linear trend, we used the median level in each tertile group of OxPL/apoB as a continuous variable. All analyses were repeated with Lp(a) concentration included instead of OxPL/apoB level. Proportional hazard assumptions were tested for OxPL/apoB and Lp(a) and satisfied in all models. Differential associations in subgroups were analyzed by inclusion of appropriate interaction terms. All reported probability values are 2-sided.

Results

Demographics

Baseline demographic, clinical, and laboratory characteristics of the study subjects in 1995 are shown in Table 1. All subjects are of Caucasian origin. The data are presented for subjects with (CVD+) and without (CVD-) incident (future) CVD over the 10-year follow-up period. Subjects with incident CVD had increased baseline levels of OxPL/apoB, Lp(a), and Lp-PLA2 activity (Table 1). As expected, subjects with incident CVD were more likely to be older, male, physically inactive, and had higher levels of systolic blood pressure, homeostasis model of insulin resistance (HOMA-IR), waist-to-hip ratio, urinary albumin (microalbuminuria),

TABLE 1. Characteristics of Study Subjects (n=765)

Variable	Mean±SD, Median (IQR) * or %		P Value
	CVD- (n=683)	CVD+ (n=82)	
Age, y	61.8±10.9	70.2±10.3	<0.001
Female sex, %	51.0%	37.8%	0.024
OxPL/apoB*	0.051 (0.033–0.125)	0.070 (0.040–0.224)	0.008
Lipoprotein(a), mg/dl*	11.6 (4.5–34.4)	20.8 (7.7–50.5)	0.010
Lp-PLA2 activity, μmol/min/L	770.7±192.1	884.1±196.0	<0.001
Vascular risk factors			
Hypertension, %	67.5%	69.5%	0.71
Systolic BP, mm Hg	147.4±20.3	152.3±22.4	0.041
Diastolic BP, mm Hg	86.9±9.0	87.1±9.5	0.83
Current smoking, %	20.1%	18.3%	0.71
Smoking, cigarettes/d	2.6±6.1	2.8±7.1	0.72
Diabetes (WHO), %	8.8%	14.6%	0.087
Fasting glucose, mg/dl	101.7±24.6	108.0±28.3	0.033
Ferritin, μg/liter	131.6±153.2	163.8±171.0	0.076
HOMA-IR*	1.98 (1.41–2.96)	2.4 (1.65–4.05)	0.022
Microalbuminuria, g/liter*	9.0 (7.0–16.0)	11.5 (8.0–54.8)	0.003
Uric acid, mg/dl	4.7±1.3	5.1±1.4	0.006
Coagulation			
Fibrinogen, mg/dl	285.9±72.8	307.0±70.6	0.013
Antithrombin III, %	99.6±11.5	97.3±11.6	0.094
Activated protein C ratio	3.2±0.6	3.1±0.6	0.12
Lipids and lipoproteins			
Total cholesterol, mg/dl	228.4±42.0	240.7±43.8	0.013
Triglycerides, mg/dl*	109 (80–157)	118 (90–164)	0.23
HDL cholesterol, mg/dl	59.2±16.2	56.9±18.2	0.24
LDL cholesterol, mg/dl	143.8±37.5	155.8±39.5	0.007
LDL cholesterol corr‡, mg/dl	135.2±36.6	142.4±38.8	0.093
Apolipoprotein A-I, mg/dl	166.2±27.4	164.9±29.7	0.70
Apolipoprotein B-100, mg/dl	114.6±30.9	124.9±33.6	0.005
Infection and inflammation			
α ₁ antithrypsin, mg/dl	197.4±36.9	206.6±33.0	0.031
C-reactive protein, mg/liter	2.7±7.1	3.7±6.6	0.030
Nutrition, activity and body composition			
Sports index, Beacke ³²	2.4±0.9	2.1±0.8	0.001
Energy intake, Kcal	2836±871	2782±810	0.59
Fat intake, g/day	148.0±48.6	145.2±45.3	0.62
Alcohol, g/day	4.0±31.4	23.9±29.4	0.98
Body-mass index, kg/m ²	25.6±3.7	26.0±4.5	0.30
Waist-hip ratio, cm/cm	0.928±0.071	0.948±0.075	0.017
Pre-existent CVD			
CVD§, %	8.2%	25.6%	<0.001

To convert values for cholesterol to mmol/L, multiply by 0.02586. To convert values for triglycerides to mmol/L, multiply by 0.01129. BP denotes blood pressure.

*Median and interquartile range (IQR) is presented for skewed variables.

†P values for difference in variable levels between subjects with and without incident CVD (1995–2005).

‡LDL cholesterol corrected for the contribution of Lp(a) cholesterol.

§Preexistent cardiovascular disease (CVD) subsumes previous fatal and nonfatal myocardial infarction, fatal and nonfatal ischemic stroke, transient ischemic attacks, revascularization procedures, and symptomatic peripheral artery disease.

TABLE 2. Hazard Ratios (HRs) of Incident Myocardial Infarction, Stroke, and TIA (1995–2005) by Tertile Groups for OxPL/apoB and Lp(a) (n=765)

	Cases, n	Noncases, n	Model 1	Model 2
OxPL/apoB [ratio]				
Tertile 1 [< 0.0379]	18	237	1.0 (Reference)	1.00 (Reference)
Tertile 2 [$0.0379-0.0878$]	26	229	1.6 (0.9–2.9)	1.5 (0.8–2.8)
Tertile 3 [> 0.0878]	38	217	2.3 (1.3–4.1)	2.4 (1.3–4.3)
<i>P</i> for trend			0.005	0.004
Lipoprotein(a) [mg/dl]				
Tertile 1 [< 6.9]	18	236	1.00 (Reference)	1.00 (Reference)
Tertile 2 [$6.9-23.9$]	24	232	1.4 (0.8–2.6)	1.5 (0.8–2.8)
Tertile 3 [≥ 24.0]	40	215	2.6 (1.4–4.6)	2.8 (1.6–5.0)
<i>P</i> for trend			<0.001	<0.001

Model 1 was adjusted for age, sex, and previous cardiovascular disease. Model 2 was additionally adjusted for systolic blood pressure, smoking, diabetes, ferritin level, fibrinogen level, LDL and HDL cholesterol, waist-to-hip ratio, alcohol consumption, social status, sports activity (Beacke score), glucose level, uric acid level, Lp-PLA₂ activity, and log_e-transformed levels of HOMA-IR, α₁-antithrypsin, C-reactive protein, and urinary albumin.

uric acid, fasting glucose, total cholesterol, LDL-C, apoB-100, fibrinogen, and hsCRP (Table 1). Subjects with future CVD events also had a higher prevalence of preexisting CVD.

Levels of OxPL/apoB and Lp(a) were highly correlated ($r=0.87$, $P<0.001$), whereas weak correlations emerged between OxPL/apoB and Lp-PLA₂ activity ($r=0.074$, $P=0.040$), and Lp(a) and Lp-PLA₂ activity ($r=0.065$, $P=0.072$).

Relationship of OxPL/apoB and Lp(a) Levels to Incident Cardiovascular Disease

In Cox regression analysis, the risk of incident CVD gradually increased across tertile groups for OxPL/apoB and Lp(a) level (Table 2). This finding applied to base models adjusted for age, sex, and previous cardiovascular disease (model 1: hazard ratio [HR] [95%CI] 1.6[0.9 to 2.9] and 2.3[1.3 to 4.1], $P=0.005$ for trend for OxPL/apoB, and HR 1.4[0.8 to 2.6] and 2.6[1.4 to 4.6] $P<0.001$ for trend for Lp(a), both for comparison between the middle and highest versus lowest tertile group) and to multivariable models additionally ad-

justed for a broad palette of established and putative vascular risk factors (model 2: HR 1.5[0.8 to 2.8] and 2.4[1.3 to 4.3], $P=0.004$ for trend for OxPL/apoB and HR 1.5[0.8 to 2.8] and 2.8[1.6 to 5.0], $P<0.001$ for trend for Lp(a); Table 2). Cumulative hazard plots (model 2) are depicted in Figure 1 and indicate a progressive divergence in event frequency in OxPL/apoB and Lp(a) across tertiles. Lp-PLA₂ activity was also a significant risk predictor of CVD in model 2 (HR[95%CI] for a 1-SD unit increase 1.4[1.1 to 1.4], $P=0.008$). Of interest, the strength of the association between OxPL/apoB and Lp(a) and CVD risk significantly increased with increasing Lp-PLA₂ activity ($P=0.018$ and $P=0.008$, respectively, for interaction; Figure 2).

Alternative models adjusted for the Framingham Risk Score, log_{eB}-transformed hsCRP, and prior CVD yielded nearly identical results (HR 2.2[1.2 to 3.8] and 1.4[0.8 to 2.5]) for a comparison of the highest and middle, respectively, versus bottom tertile group for OxPL/apoB ($P=0.006$ for trend) and 2.3[1.3 to 4.0] and 1.2[0.7 to 2.3] for a comparison of the highest and middle versus bottom tertile

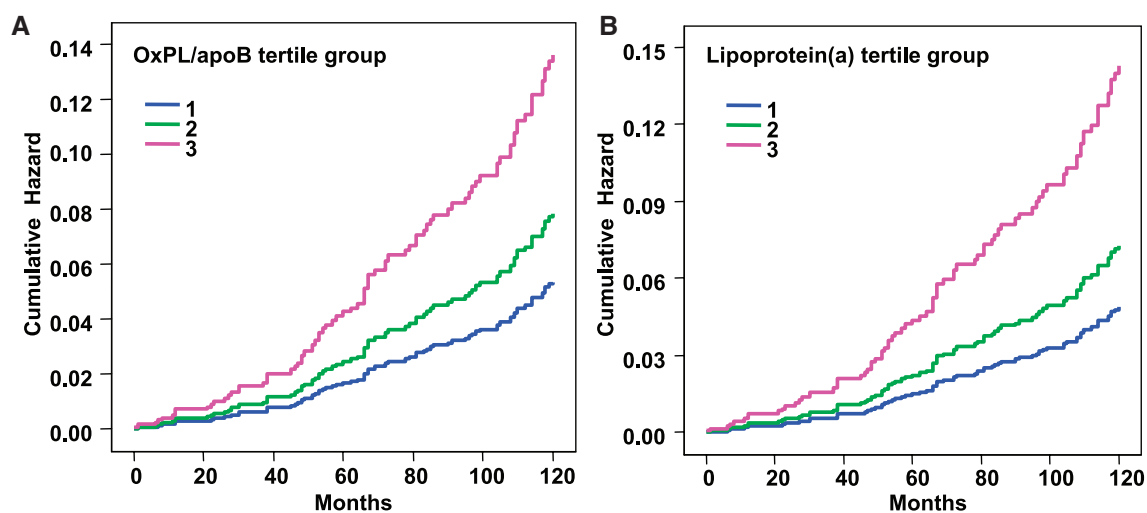


Figure 1. Cumulative hazard curves (model 2) of incident CVD from 1995 to 2005 for tertiles of OxPL/apoB (A) and Lp(a) (B).

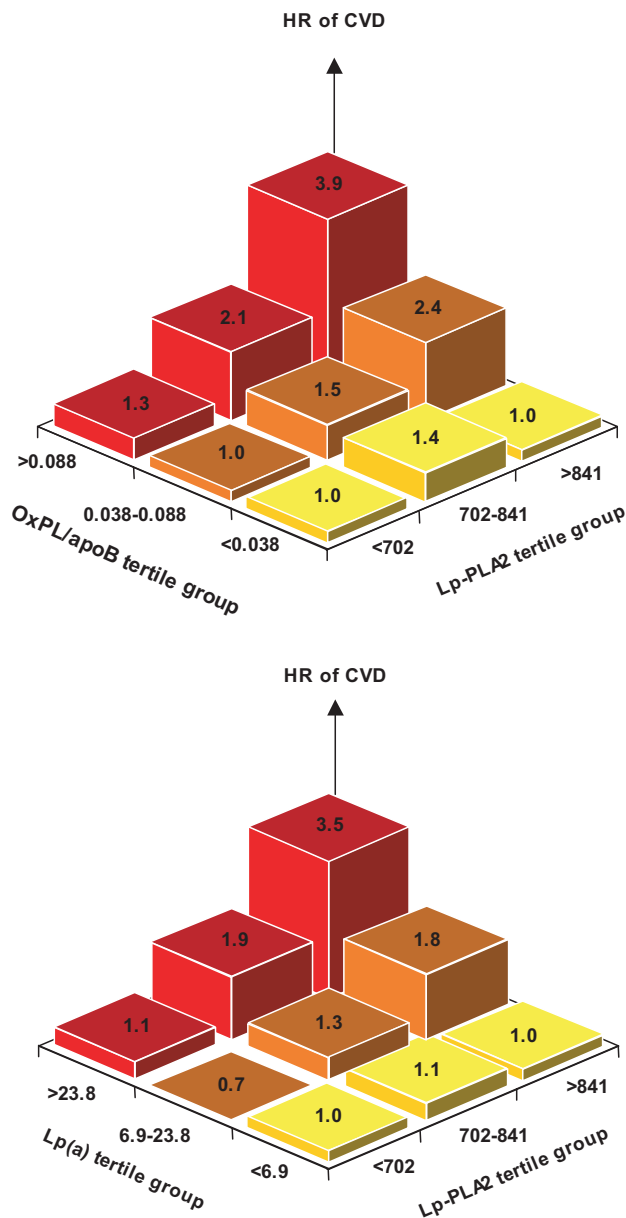


Figure 2. Relationship between OxPL/apoB (A) and Lp(a) (B) tertile groups and CVD risk according to tertiles of Lp-PLA2 activity ($P=0.018$ and $P=0.008$ for interaction of OxPL/apoB and Lp(a), respectively).

group for Lp(a) ($P=0.001$ for trend). As visualized in Figure 3A, the graded increase in CVD risk across OxPL/apoB tertile groups was evident in the low-risk, moderate-risk, and high-risk groups as defined by the Framingham Risk Score. Findings were similar but less consistent for Lp(a) tertile groups (Figure 3B).

The associations of OxPL/apoB and Lp(a) with CVD were consistent in 5 additional sensitivity analyses: (1) When OxPL/apoB and Lp(a) were treated as continuous variables, HR [95%CI]s calculated for a 1-SD unit increase in \log_{eB} -transformed variable levels were 1.4[1.1 to 1.7] ($P=0.002$) and 1.4[1.1 to 1.8] ($P=0.003$), respectively, in model 1 and 1.4[1.1 to 1.7] ($P=0.004$) and 1.4[1.1 to 1.8] ($P=0.003$), respectively, in model 2. When both OxPL/apoB and Lp(a) were entered into the

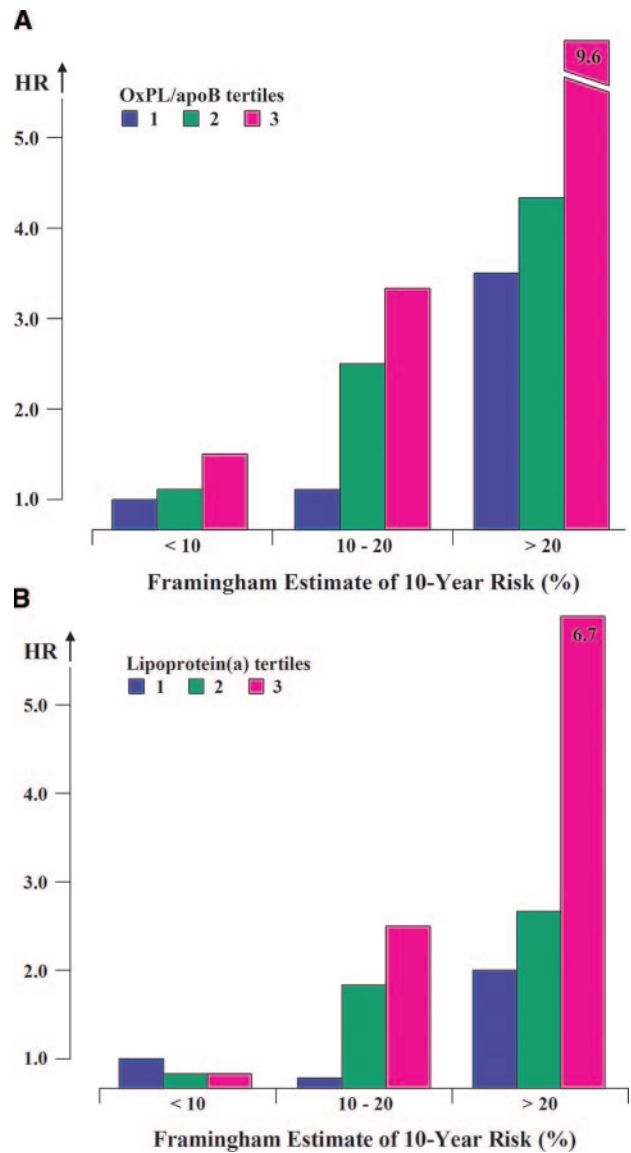


Figure 3. Relationship between tertile groups of OxPL/apoB (<0.0379, 0.0379 to 0.0878, >0.0878; A) and Lp(a) (<6.9, 6.9 to 23.8, >23.8; B) and CVD risk within each Framingham Risk Score Group. Framingham Risk Score was calculated as low risk (<10% risk of events over 10 years), moderate risk (10% to 20%), and high risk (>20%).

same model (stepwise selection) OxPL/apoB was slightly superior to Lp(a) in predicting cardiovascular events but they were not independent of each other; (2) Exclusion of the 77 subjects with preexistent CVD had little effect on the results (HR 2.3[1.1 to 4.6] and 1.9[0.9 to 3.8] for a comparison of the highest and middle versus bottom tertile group for OxPL/apoB, P for trend 0.049). There was no differential effect of OxPL/apoB on CVD in this group (HR[95%CI] for a 1-SD unit increase in \log_{eB} -transformed OxPL/apoB 1.3[1.1 to 1.7]; $P=0.017$) and in subjects with preexistent disease (HR[95%CI] for a 1-SD unit increase in \log_{eB} -transformed OxPL/apoB 1.3[1.0 to 2.1], $P=0.085$); (3) In subgroup analyses, the association tended to be slightly more pronounced in subjects with LDL cholesterol above the median (143 mg/dL), but this did not achieve statistical significance (no effect-measure modification); (4)

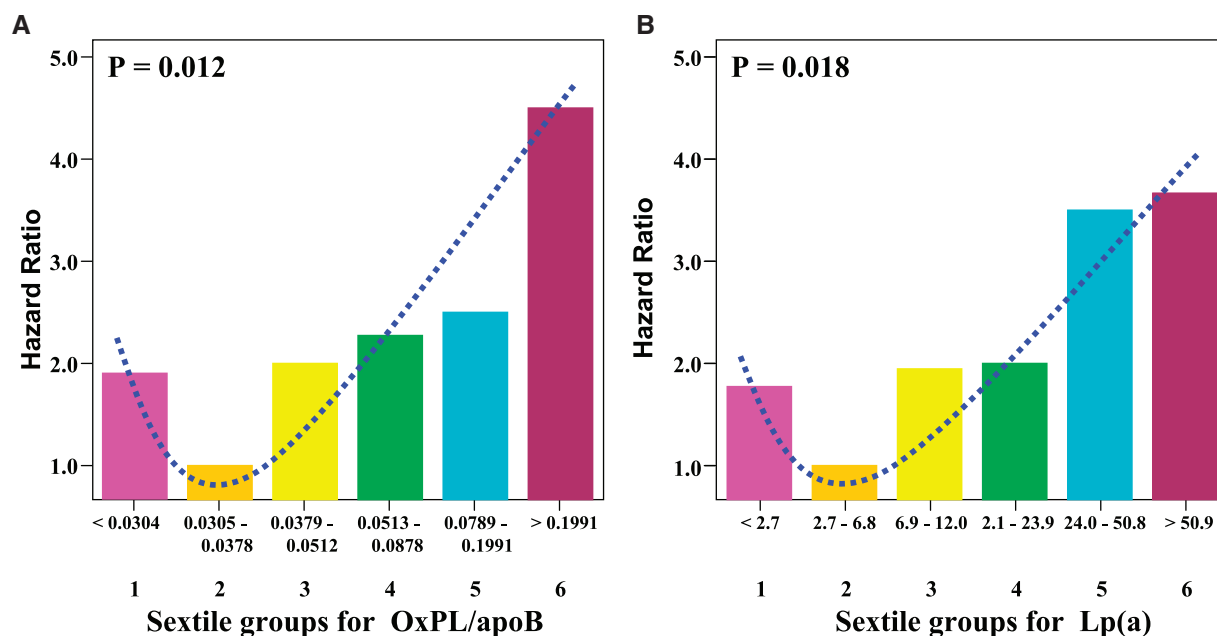


Figure 4. Plots of hazard ratios for incident CVD according to OxPL/apoB (A) and Lp(a) (B) sextile groups.

Associations of OxPL/apoB were present both for individual cardiovascular end points as well as composite end points when revascularization procedures and new onset peripheral arterial disease were included (supplemental Figure I, available online at <http://atvb.ahajournals.org>). Similar data were noted for Lp(a) (supplemental Figure I); (5) The HR [95% CI] for CVD calculated for a 1-SD unit increase in \log_e -transformed OxPL/apoB level was 1.36[1.00 to 1.85] $P=0.049$ in subjects with low-molecular-weight apo(a) phenotypes (≤ 22 Kringle-IV repeats) and 1.55[1.14 to 2.11] $P=0.005$ in subjects with high-molecular-weight apo(a) phenotypes (> 22 Kringle-IV repeats; $P=0.48$ for effect modification). There were no significant differences in any of the above analyses in men versus women.

Exploratory Analysis to Assess Whether a J-Shaped Curve Exists Between OxPL/apoB and Lp(a) Levels and CVD

To further evaluate the relationship between OxPL/apoB or Lp(a) and CVD risk, hazard ratios were computed for sextile groups of given variables suggesting the presence of a J-shaped curve in the relationship of OxPL/apoB and Lp(a) and CVD (Figure 4). However, this study was not adequately sized statistically to test the hypothesis that a J-shaped scale fits the data better than a linear dose-response relationship.

Discussion

This study demonstrates that OxPL/apoB measured at baseline in an unselected population derived from the general community predicts the development of cardiovascular events over a 10-year prospective follow-up period. The predictive value of OxPL/apoB was independent of traditional risk factors and hsCRP, and further amplified with increasing Lp-PLA2 activity. Furthermore, elevated OxPL/apoB levels provided predictive information within each Framingham Risk Score estimate of 10-year cardiovascular risk. Similar findings were noted for Lp(a). OxPL/apoB and

Lp(a) were not independent of each other but were independent of all other measured risk factors. The close relationship of OxPL/apoB and Lp(a) in predicting cardiovascular events strongly supports the hypothesis that the atherogenicity of Lp(a) may be attributable, in part, to its ability to preferentially bind proinflammatory oxidized phospholipids compared with other apoB-containing lipoproteins.⁵⁻¹¹

The OxPL/apoB assay quantitates the content of OxPL per apoB particle and was originally designed to measure minimally-oxidized LDL in plasma. However, it was later determined that most, but not all, of the OxPL/apoB are actually a subset of apoB-100 particles, namely Lp(a) particles, and thus this assay represents a novel biological measure of oxidized phospholipids that are primarily on Lp(a) particles.⁵⁻¹¹ It is also noteworthy to emphasize that the term "OxLDL" is not a single molecular entity but describes a broad array of biological, chemical, and immunologic changes that result in generation of oxidation-specific epitopes. The antibodies and assays used for measuring OxLDL described in the literature are not necessarily comparable nor are they expected to provide similar results, primarily because of differences in epitope recognition and fundamentally different assay methodologies (reviewed by Tsimikas¹⁵).

Although several studies have shown associations of OxLDL with various cardiovascular disease entities, relatively little information is available on prediction of cardiovascular events. This is the first prospective epidemiological study to suggest a role of oxidized phospholipids in predicting future myocardial infarction, stroke, and TIA. Consistent with the current report, Shimada and colleagues¹⁶ showed in patients preselected with coronary artery disease that elevated OxLDL levels measured with antibody DLH3 were associated with recurrent cardiac events. Similarly, Meisinger et al¹⁷ showed in a case-control study that OxLDL measured by antibody 4E6 predicted increased risk of future acute coronary events. However, a recent case-control study from a combined

analysis from the Health Professionals Follow-Up Study and the Nurses' Health Study showed that OxLDL levels measured by 4E6 did not independently predict increased cardiovascular events when apoB levels were included in the multivariable model. Furthermore, Tsouli et al¹⁸ have recently shown that when normal unoxidized LDL is added to plasma samples with known amounts of OxLDL measured by 4E6, the OxLDL levels rose in proportion to the amount of added LDL, suggesting that this particular assay, as opposed to the others, may not be entirely specific for OxLDL. In fact, it has been shown in multiple studies that OxLDL levels measured with 4E6 correlates strongly with LDL-C levels (reviewed by Tsimikas¹⁵). These data in composite suggest that available measures of OxLDL are not necessarily equivalent and that comparative studies of the various assays are needed to assess which assays and assay formats and which oxidation-specific epitopes provide the most clinically useful information.

Lp(a) is associated with enhanced atherogenic potential, particularly at levels >30 mg/dL, and has generally been shown to be an independent predictor (odds ratio \approx 1.5 to 2) of cardiovascular risk,^{19,20} particularly in younger subjects (<60 years old) and those with elevated LDL cholesterol levels.^{5,21–25} Because it appears that the atherogenicity of Lp(a) may be mediated in part by its association with OxPL, it would be interesting to assess the risk of CVD events of OxPL/apoB in younger patients to assess whether it provides enhanced predictive value for CVD compared with Lp(a), as has been shown for angiographically-determined CAD.⁵ This study also provides preliminary evidence that a J-shaped relationship may exist between Lp(a) levels and cardiovascular events. In the Scandinavian Simvastatin Survival Study, Berg et al²⁶ showed that subjects with elevated Lp(a) levels had a higher risk of death and major cardiac events, and interestingly, the greatest benefit with simvastatin was not derived in the lowest Lp(a) quartile, but in the next to lowest, which is consistent with the current data. Because of the relatively low number of events in the current study, this observation requires confirmation in larger studies.

This is also the first study to evaluate the relationship between OxPL and Lp-PLA2 activity in predicting CVD. Higher baseline levels of Lp-PLA2 activity were present in subjects with incident CVD, as shown previously, but primarily in studies measuring Lp-PLA2 mass.²⁷ Both OxPL/apoB and Lp-PLA2 activity were significant predictors of vascular risk in multivariable models. However, the prediction of CVD by OxPL/apoB or Lp(a) was significantly accentuated by increasing Lp-PLA2 activity (effect modification, *P* for interaction 0.018 and 0.008 for OxPL/apoB and Lp(a), respectively), but when either OxPL/apoB (or Lp(a)) and Lp-PLA2 levels were low, there was essentially no association of either with CVD. This finding implies that these measures may be related pathophysiologically and provide complementary information in predicting new CVD events.

It has not been determined whether the physiological role of Lp-PLA2 is beneficial or detrimental in atherogenesis and whether the substrates (OxPL) of Lp-PLA2 are more, less, or equally atherogenic than the byproducts (free oxidized fatty

acids and lysophosphatidylcholine).²⁷ Animal studies of over-expression of Lp-PLA2 suggest that it has a beneficial role in reducing oxidative stress and atherogenesis, but human studies have shown that Lp-PLA2 is a modest independent predictive biomarker of CVD.²⁷ Interestingly, the enzyme Lp-PLA2 is mostly associated with LDL, but when assayed at equimolar protein concentrations, Lp(a) contains 1.5- to 2-fold higher mass and several-fold greater Lp-PLA₂ activity compared with LDL, even though Lp-PLA2 is associated with the apoB but not the apo(a) moiety of Lp(a).^{28,29} Furthermore, smaller apo(a) isoforms exhibit higher apparent Lp-PLA2 *K_m* and *V_{max}* values, compared with large ones, suggesting that the apo(a) may influence the association of Lp-PLA₂ with Lp(a).³⁰ We have previously suggested that under normal physiological conditions Lp(a) may function in binding and facilitating degradation of OxPL because it is enriched in Lp-PLA2. This would be consistent with the observation that at low levels Lp(a) may have a beneficial effect, as evidenced by the J-shaped curve (Figure 4). However, in patients with elevated Lp(a) levels, this potential physiological function may be overwhelmed. In support of this concept, it was shown that, compared with those without CAD, patients with CAD have significantly lower Lp-PLA2 mass and activity on isolated Lp(a) particles, but normal LDL-associated Lp-PLA2 mass and activity is present in both patients with and without CAD.³¹ Interestingly, removal of apo(a) from the Lp(a) particle resulted in a significant increase in the Lp-PLA₂ activity.³¹ Because the current study measured Lp-PLA2 activity in plasma rather than on isolated Lp(a), it is not possible to assess whether Lp(a)-associated Lp-PLA2 activity provides additional information on CVD risk prediction beyond measuring plasma levels. Future studies measuring Lp-PLA2 mass and activity on isolated Lp(a) particles will be needed to address the hypothesis that Lp(a)-associated Lp-PLA2 activity may influence atherogenesis and CVD through degradation of OxPL.

Conclusions

This study documents that OxPL/apoB levels independently predict 10-year cardiovascular events in an unselected population and in a manner strikingly similar to Lp(a), and suggests that the atherogenicity of Lp(a) may be derived in part from its association with OxPL. The synergistic association between OxPL/apoB, Lp(a), and Lp-PLA2 activity on CVD risk suggests that these particles may be pathophysiologically linked in the normal metabolism of OxPL and in mediating atherogenesis.

Sources of Funding

This investigation was supported by NHLBI grant HL56989 (La Jolla Specialized Center of Research in Molecular Medicine and Atherosclerosis), the Donald W. Reynolds Foundation, the Fondation Leducq, and by grants from the Austrian Heart Fund and the Austrian Nationalbank (Project 9331) and by the "Pustertaler Verein zur Prävention von Herz- und Hirngefäßerkrankungen", "Sanitaetseinheit Ost", and "Assessorat fuer Gesundheit", Province of Bolzano, Italy.

Disclosures

Drs Tsimikas and Witztum are coinventors of patents and patent applications on the use of antibodies to oxidized LDL owned by the

University of California, and Dr Witztum was a consultant to Atherogenics, Inc, Atlanta, Ga.

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