The association between lipoprotein-associated phospholipase A_2 and cardiovascular disease and total mortality in vascular medicine patients

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Introduction: **In some community-based studies, lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has been shown to be independently predictive of future fatal and nonfatal cardiovascular disease (CVD) events. We tested the hypothesis that Lp-PLA_2 is independently predictive of mortality in high-risk patients from a vascular laboratory.**

Methods: **Between 1990 and 1994, patients seen in the previous 10 years for noninvasive lower extremity arterial testing were invited to return for a vascular examination of the lower extremities. By medical record review, we identified 2265 eligible patients; of these, 508 returned for interviews, blood collection, and arterial examination and represent those who had survived, could be located, and were willing to participate. The 508 subjects were followed up for an average of 6.7 years until the end of the study period on December 31, 2001. Vital status was ascertained by multiple searches of the Social Security Death Index. The primary outcomes for this study were time to any, CVD, and coronary heart disease (CHD) mortality.**

Results: **The mean age was 68.2 years, 88% were men, 87% were non-Hispanic white, 39.1% were diagnosed with peripheral arterial disease only, 9.2% with other CVD only, and 28.5% with both peripheral arterial disease and other CVD. During the entire follow-up period, 299 (59.7%) patients died, 167 from CVD, of which 88 deaths were due to coronary heart disease. With adjustment for CVD risk factors and baseline peripheral arterial disease and other CVD, an increment of one standard deviation in Lp-PLA_2 activity was associated with a 40% higher risk for CHD mortality at 5 years of follow-up (P = .04). Additional adjustment for triglycerides, high-density lipoprotein, and low-density lipoprotein cholesterol reduced this association to nonsignificance (hazard risk, 1.12).**

Conclusion: **In a vascular laboratory patient population, higher levels of LpPLA_2 mass and activity were not significantly associated with total, CVD, or CHD mortality at 5 years of follow-up and after adjustment for traditional CVD risk factors and the presence of PAD and other CVD at baseline. An apparent elevated risk of CHD death associated with elevated Lp-PLA2 was largely explained by associated elevations in lipids and lipoproteins. (J Vasc Surg 2007;46:500-6.)**

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolase, is a calcium-independent enzyme that cleaves oxidized and polar phospholipids.[1,2] In the circulation, Lp-PLA_2 is bound predominately to low-density lipoprotein cholesterol (LDL-C) and has also been shown to have an affinity for small, dense fractions of this class of cholesterol.[3,4] In the arterial wall, Lp-PLA_2 is generated by monocytes, macrophages, and T-lymphocytes and has been found in intimal atherosclerotic plaques.[5-7] More important, Lp-PLA_2 has been classified as a novel inflammatory marker due to the production of oxidized free fatty acids and lysophosphatidylcholines during oxidation of LDL-C.[8]

Because the rate of action of Lp-PLA_2 on lipoproteins depends on the activity and mass of this enzyme, both of these measurements are required to understand its association with morbidity and mortality.[9] In previous community-based studies, Lp-PLA_2 activity and mass have been shown to be independently predictive of future fatal and nonfatal coronary heart disease events.[10-15] To our knowledge, however, no studies examining the potential association between mortality and both Lp-PLA_2 activity and mass have been performed in a cohort of patients examined in a vascular laboratory. Accordingly, we conducted a study to compare the associations between both activity and mass of Lp-PLA_2 and both total and cardiovascular disease (CVD) mortality in high-risk patients from a vascular laboratory.

METHODS

Subjects. Between 1990 and 1994, patients seen in the previous 10 years for noninvasive lower extremity arterial testing at the San Diego Veterans Administration Medical Center (VAMC) or the University of California, San Diego Medical Center (UCSDMC) vascular laboratories were invited to return for a vascular examination of the lower extremities using the same procedures as used previously. The invitations were derived from a listing of all patients who had visited our vascular laboratories in the previous 10 years, who had undergone a vascular examination, and who had provided permission to be contacted. The order in which the participants were contacted was

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determined by the timing of their original visit. Those seen furthest in the past were contacted first. Enrollment was not necessarily of consecutive patients.

The cohort consisted of individuals who were referred by specialist or primary care physicians and included patients with and without CVD comorbidities. Baseline CVD was defined as a history of myocardial infarction, stroke, percutaneous transluminal angioplasty or stent, coronary artery bypass graft, or carotid artery surgery. Baseline peripheral arterial disease (PAD) was defined as an ankle-brachial index (ABI) of <0.9 in either lower extremity or a history of revascularization of the arteries in the lower extremities. The visits between 1990 and 1994 are baseline visits for these analyses.

Of 2265 patients with vascular laboratory visits in the 10-year time period before 1990 to 1994, 481 had died before 1990 and another 1276 did not participate in the follow-up visit. Thus, 508 patients returned for interviews and represent those who had survived, could be located, and were willing to participate. At the 1990 to 1994 visits, fasting venous blood was collected and stored at –70°C. Subjects who agreed to undergo repeat testing for PAD signed a consent form approved by the University of California San Diego Human Research Protection Program and were interviewed in person using a structured questionnaire.

Vascular assessment. At the follow-up visit, a noninvasive vascular examination including segmental blood pressures and flow velocities was performed. Systolic blood pressures were measured in both ankles and both arms by sphygmomanometry and photoplethysmography. Photoplethysmographic assessment has been shown to give essentially identical results to Doppler signal determination in both the arms and legs.[16] The ABI for both legs was calculated using the arm with the higher systolic blood pressure. The subjects were categorized as normal or with PAD based on the ABI. PAD was defined as an ABI <0.90, the conventional cut-point in vascular laboratories. The 95% confidence interval (CI) of ABI reproducibility has been reported to range from 0.10 to 0.15.[17-19]

Laboratory assessment. Blood samples collected at the 1990 to 1994 visits were analyzed at that time for serum lipids and lipoproteins at a standardized lipid analytical laboratory using the Abbott VP Super System bichromatic analyzer (Abbott Laboratories Abbott Park, Ill), which uses direct enzymatic colorimetric assays. In 2006, Lp-PLA_2 assays were conducted using plasma samples from fasting venous blood obtained at the 1990 to 1994 and stored at –70°C.

Lipoprotein-associated phospholipase A_2 activity. Lp-PLA_2 activity (nmol/[min · mL]) was measured with a colorimetric activity method (CAM); diaDexus Inc, South San Francisco, Calif). Samples, standards, or controls are added to wells of a nonbinding 96-well microplate, followed by addition of CAM reaction buffer containing substrate. In the presence of Lp-PLA_2 enzyme, the substrate is converted upon hydrolysis by the phospholipase enzyme. The change in absorbance is immediately measured at 405 nm over 60 to 180 seconds. The level of Lp-PLA_2 activity in nmol/(min · mL) is calculated from the slope (OD_{405}/min), based on a standard conversion factor from a *P*-nitrophenol calibration curve. All samples were measured in duplicate. The intra-assay coefficient of variation (CV) was 4%. The inter-assay CV for six quality controls across plates was between 4% and 6%.

Lipoprotein-associated phospholipase A_2 mass. Lp-PLA_2 mass (ng/mL) was measured by the PLAC test, a commercially available Lp-PLA_2 enzyme-linked immunoabsorbent assay kit (diaDexus Inc). The assay is a sandwich enzyme immunoassay formulated with two specific monoclonal antibodies described by Caslake et al.[20] The assay system uses monoclonal anti-Lp-PLA_2 antibody (2C10) directed against Lp-PLA_2 for solid phase immobilization on microwell strips. After addition of assay buffer to the antibody-coated microplate, samples, calibrators, or controls are added to the wells and incubated for 2 hours. After a washing step to remove any unbound antigen, a second monoclonal anti-Lp-PLA_2 antibody (4B4) labeled with the enzyme horseradish peroxidase is used to detect the immobilized antigen. The enzyme conjugate is incubated for 60 minutes, followed by another washing step.

Addition of the substrate, tetramethylbenzidine (TMB), results in the development of a blue color which is stopped appropriately with the addition of Stop Solution (1N HCL), changing the color to yellow. The absorbance of the enzymatic turnover of the substrate is determined spectrophotometrically at 450 nm and is directly proportional to the concentration of Lp-PLA_2 present. A set of Lp-PLA_2 calibrators is used to plot a standard curve of absorbance versus Lp-PLA_2 concentration from which the Lp-PLA_2 concentration in the test sample can be determined. Duplicate assays were performed for 26% of the samples; the intra-assay coefficient of variation (CV) was 5%. The inter-assay CV for quality controls was between 6% and 10%.

Outcomes ascertainment and classification. The subjects were followed up for an average of 6.7 years until the end of the study period on December 31, 2001. Between 1998 and 2001, the vital status of the 508 subjects who participated in the follow-up visit was ascertained by multiple searches of the Social Security Death Index, and 501 had adequate blood samples available for the pertinent analysis. From this search, the place of death was recorded and then used to request the pertinent death certificate. Once received, the death certificate was reviewed and coded by a certified nosologist using the International Classification of Diseases, 9th edition (ICD-9). Mortality from coronary heart disease was defined using the ICD-9 code range of 410 to 414, and mortality from any CVD was defined as ICD-9 code range of 410 to 414 or 420 to 438. The coded underlying cause of death was used in these analyses.

Statistical analysis. Continuous data are presented as means ± standard deviations, and categoric data are shown as frequencies with percents. All variables were examined for normality. Those independent variables that deviated from a Gaussian distribution were transformed to achieve

Table I. Age and sex adjusted cohort characteristics stratified by lipoprotein-associated phospholipase A_2 activity and mass above and below the mean

	Lipoprotein-associated phospholipase A_2 (n = 506)					
Risk factor	*Activity ≥ 146.3 (nmol/[min · mL])*	*Activity < 146.3 (nmol/[min · mL])*	P	*Mass ≥ 367.7 (ng/mL)*	*Mass < 367.7 (ng/mL)*	P
BMI (kg/m^2)	27.7	26.8	.26	27.1	27.4	.67
Waist circumference (cm)	99.7	98.2	.20	99.8	98.1	.14
Blood pressure						
Systolic (mm Hg)	142.8	143.4	.73	142.2	144.0	.33
Diastolic (mm Hg)	78.3	78.3	.92	78.6	78.1	.57
Fasting plasma glucose (mg/dL)	132.4	130.1	.66	129.1	133.4	.42
Cholesterol						
Total (mg/dL)	220.8	197.6	<.01	217.0	201.3	<.01
HDL (mg/dL)	43.3	48.8	<.01	45.3	46.8	.20
LDL (mg/dL)	142.6	119.6	<.01	136.6	125.4	<.01
Triglycerides (mg/dL)	155.4	123.5	<.01	152.9	125.5	<.01
CRP (pg/mL)	0.52	0.58	.32	0.60	0.49	.08
Homocysteine (μmol/L)	13.4	14.5	.23	14.2	13.7	.57
Lipoprotein (a) (pg/mL)	20.0	19.7	.89	21.2	18.5	.23
Serum amyloid A (pg/mL)	0.80	1.13	.13	1.08	0.86	.32
D-dimer (μg/mL)	1.55	1.29	.11	1.39	1.44	.79
CD-40 ligand (pg/mL)	1314.3	1597.3	.41	1503.9	1408.4	.78
NT-pro BNP (pg/mL)	559.2	456.1	.14	579.1	436.2	.04
PAI-1 (pg/mL)	20530.3	19012.9	.68	23268.5	16285.7	.06
TNF-α (pg/mL)	99.2	193.5	.55	99.8	192.9	.55
MCP-1 (pg/mL)	1530.4	1334.5	.40	1810.9	1055.2	<.01
Interleukin-6 (pg/mL)	32.1	16.4	.34	36.3	12.2	.14
Hypertension (%)	69.5	64.9	.29	66.6	67.8	.79
Diabetes (%)	27.1	33.6	.11	27.0	33.7	.11
Past or current smoker (%)	68.5	71.1	.54	64.5	75.1	<.01
Pack-years	57.0	58.4	.71	56.0	59.5	.38

BMI, Body mass index; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *CRP*, C-reactive protein; *NT-pro BNP*, N-terminal pro-B-type natriuretic peptide; *PAI-1*, plasminogen activator inhibitor 1; *TNF-α*, tumor necrosis factor-α; *MCP-1*, monocyte chemotactic protein 1.

normality (if possible). Those that were resistant to normalization were analyzed using nonparametric methods. Differences in age-adjusted and sex-adjusted mean values of the independent variables by high vs low Lp-PLA_2 status were determined by analysis of covariance. Correlations were conducted using the Spearman rank correlation method.

To determine the risk for incident mortality, separate Cox proportional hazard models were constructed for Lp-PLA_2 activity and mass in a stepwise fashion. The first step in each analysis consisted of adjustments for age and sex as covariates of the Lp-PLA_2 variable (ie, activity or mass). The second step included the addition of traditional CVD risk factors and baseline PAD and other CVD; in the third step triglycerides were added; in the fourth step, high-density lipoprotein cholesterol (HDL-C) was added; and finally, in the fifth step LDL-C was added to the model. Hazard ratios were determined for 5 years of follow-up. All analyses were conducted using SAS 8.2 (SAS Institute, Cary, NC). A two-tailed $\alpha = 0.05$ was considered significant.

RESULTS

The age of the men (68.2 years) and women (68.1 years) in the study was not significantly different ($P = .93$). Sixty (12%) of the participants were women. A total of 196 subjects (39.1%) were diagnosed with PAD only, 46 (9.2%) were diagnosed with other CVD but not PAD, and 143 (28.5%) were diagnosed with both PAD and other CVD. The mean duration of follow-up was 6.7 ± 3.2 years. Of the 299 patients (59.7%) who died during the entire follow-up period, 167 died of CVD, and 88 of these deaths were due to coronary heart disease (CHD).

The mean Lp-PLA_2 activity was 145.2 ± 33.5 nmol/([min · mL]), and the corresponding mean for Lp-PLA_2 mass was 377.9 ± 148.0 ng/mL. Those with an Lp-PLA_2 activity level above the mean had significantly higher levels of total and LDL-C as well as triglycerides, while also having lower HDL-C (Table I). Notably, for the other CVD risk factors or inflammatory markers studied, there were no other significant differences between those with an Lp-PLA_2 activity above the mean compared with an Lp-PLA_2 activity below the mean.

For Lp-PLA_2 mass, the associations were similar. Specifically, those above the mean for Lp-PLA_2 mass had significantly higher total and LDL-C levels and triglycerides. Those above the mean did not have significantly different HDL-C levels, but they did have significantly higher levels of N-terminal pro-B-type natriuretic peptide and monocyte chemotactic protein 1 levels. There were also trends for higher C-reactive protein and plasminogen activator inhibitor 1 (PAI-1), which were of borderline significance ($P = .08$, and $P = .06$ respectively).

Table II. Mean lipoprotein-associated phospholipase A_2 activity and mass levels for different cohort characteristics

		Lipoprotein-associated phospholipase A_2			
Group	*N*	*Activity (nmol/[min · mL])*	P	*Mass (ng/mL)*	P
Sex					
Male	441	147.9	Ref	387.3	Ref
Female	60	125.4	<.01	308.9	<.01
Ethnicity					
NHW	436	147.5	Ref	381.1	Ref
Other	65	130	<.01	356.6	.21
Smoking					
Never	72	132.7	Ref	320.5	Ref
Quit	278	146	<.01	368.7	.01
Current	151	149	<.01	420.4	<.01
Diabetes					
No	348	146.4	Ref	389.2	Ref
Yes	153	142.4	.22	356.1	.02
FHx CHD					
No	217	140.5	Ref	367.9	Ref
Yes	284	148.5	<.01	384.4	.22
PAD					
No	162	144.5	Ref	385.6	Ref
Yes	339	145.7	.72	377.7	.61
CVD					
No	365	142.1	Ref	378.5	Ref
Yes	133	148.8	.02	380.9	.86

NHW, Non-Hispanic white; *FHx*, family history; *CHD*, coronary heart disease; *PAD*, peripheral arterial disease; *Ref*, reference category; *CVD*, cardiovascular disease.

Table III. Age-adjusted and sex-adjusted correlations for lipoprotein-associated phospholipase A_2 mass and activity

	Lipoprotein-associated phospholipase A_2	
Variable	*Activity*	*Mass*
Waist circumference	0.09*	0.03
Body mass index	0.08	−0.04
Body weight	0.08*	−0.01
Waist-to-hip ratio	0.06	−0.01
Fasting glucose	0.04	−0.08
Blood pressure		
Systolic	−0.05	0.04
Diastolic	0.02	0.09*
HDL cholesterol	−0.24†	−0.02
LDL cholesterol	0.45†	0.18†
Triglycerides	0.19†	0.19†
Pack-years smoking	−0.02	−0.01
Interleukin-6	−0.01	0.04
TNF-α	0.04	0.08
MCP-1	0.03	0.13†
PAI-1	0.04	0.13†
NT-pro-BNP	0.01	0.08
CD-40	−0.03	0.01
D-dimer	0.05	−0.02
C-reactive protein	−0.07	0.04
Serum amyloid A	−0.06	0.04
Lipoprotein (a)	0.04	0.15
Homocysteine	−0.01	−0.03

HDL, High-density lipoprotein; *LDL*, low-density lipoprotein; *TNF-α*, Tumor necrosis factor-α; *MCP-1*, monocyte chemotactic protein 1; *PAI-1*, plasminogen activator inhibitor 1; *NT-pro-BNP*, N-terminal pro-B-type natriuretic peptide.
*$.05 < P < .10$.
†$P < .05$.

Levels of both Lp-PLA_2 activity and mass were significantly different by sex and smoking status (Table II). Compared with all other ethnic groups combined, non-Hispanic whites had significantly higher Lp-PLA_2 activity (147.5 vs 130.0 nmol/[min · mL]). Levels of Lp-PLA_2 mass were also higher in non-Hispanic whites, but these differences did not reach significance. Conversely, those with diabetes had significantly lower Lp-PLA_2 mass but not activity levels. Of interest, those with a family history of CHD or a personal history of CVD had significantly higher Lp-PLA_2 activity but not mass levels. Mean Lp-PLA_2 activity and mass levels were not significantly different by PAD status.

After adjustment for age and sex, Lp-PLA_2 activity and mass were significantly correlated ($r = 0.55$, $P < .01$). Lp-PLA_2 activity and mass were also significantly inversely correlated with the ABI ($r = -0.12$, $P < .01$ and $r = -0.10$, $P < .03$; respectively). Table III shows the correlations between Lp-PLA_2 activity and mass with the CVD risk factors and novel biomarkers. LDL-C had the strongest age and sex-adjusted correlation with Lp-PLA_2 activity ($r = 0.45$) followed by HDL-C ($r = -0.24$) and triglycerides ($r = 0.19$). The correlations between Lp-PLA_2 activity and waist circumference and body weight were modest and of borderline significance. There were no significant associations between LP-PLA_2 activity and fasting glucose and any of the novel risk factors such as C-reactive protein, interleukin 6, and D-dimer. With the same adjustment for age and sex, Lp-PLA_2 mass was significantly correlated with LDL-C ($r = 0.18$) and triglycerides ($r = 0.19$), but not HDL-C. Lp-PLA_2 mass was also significantly correlated with MCP-1 and PAI-1 ($r = 0.13$ for both) but not with any of the other novel biomarkers.

At 5 years of follow-up and in the Cox proportional hazards models, there were no significant associations between Lp-PLA_2 mass or activity and total mortality or CVD mortality. Similarly, no significant association was found between Lp-PLA_2 mass and CHD mortality. In a multivariable Cox proportional hazard model adjusted for age and sex, however, a one-standard-deviation increment of Lp-PLA_2 activity was significantly associated with a 40% ($P = .04$) increased hazard for CHD mortality (Fig) that remained essentially unchanged with additional adjustment for smoking, hypertension, diabetes, family history of premature CHD, baseline PAD, and other CVD (hazard ratio [HR], 1.37, 95% confidence interval [CI], 1.00 to 1.89). Additional adjustment for triglycerides modestly attenuated this association (HR, 1.34; 95% CI, 0.97 to 1.86). When HDL-C was added to the model, however, there was a substantial reduction in the hazard ratio to 1.25 (95% CI, 0.90 to 1.75), which was further reduced to 1.12 with additional adjustment for LDL-C (95% CI, 0.78 to 1.60).

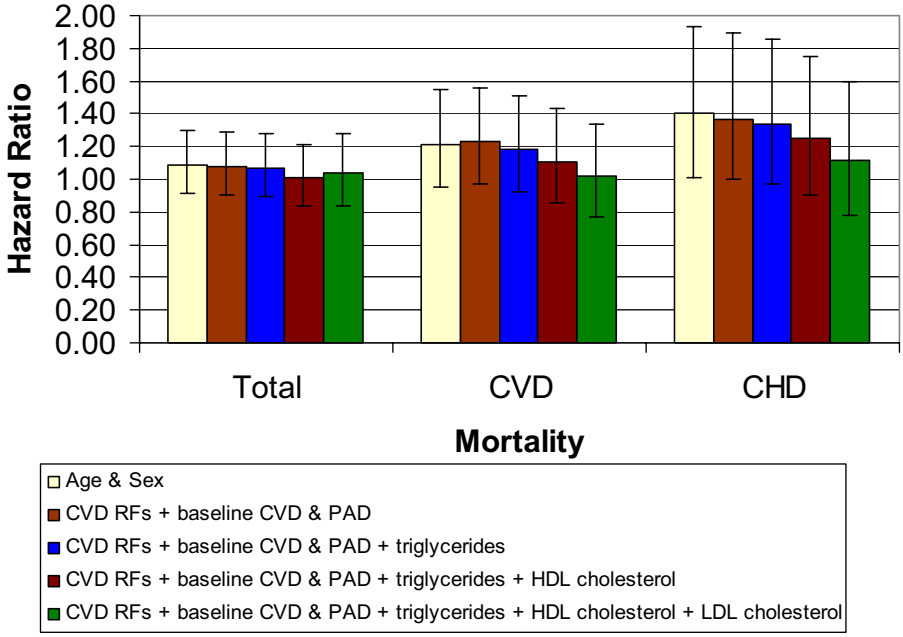

Fig. Risks at 5 years, presented with 95% confidence interval, for total, cardiovascular disease *(CVD)*, and coronary heart disease *(CHD)* mortality associated with standardized increments of lipoprotein-associated phospholipase A_2 activity. CVD risk factors: age, sex, smoking, diabetes, hypertension, and family history of CHD. *PAD*, peripheral arterial disease; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein.

Adding BMI and CRP to the models did not significantly change the nature of these associations.

To determine if the mortality risks differed by baseline comorbidity status, we stratified the cohort into the following groups: (1) other CVD only, (2) PAD only, (3) CVD and PAD, and (4) no CVD or PAD. We then conducted the same Cox regression analyses as described for the entire cohort. These exploratory analyses uncovered significant associations with Lp-PLA_2 mass and both CVD and CHD mortality in the other CVD only group that was not further attenuated by adjustment for HDL-C and LDL-C. For the PAD-only and CVD-plus-PAD groups, no significant associations were found with Lp-PLA_2 mass and activity with mortality. Because the sample size for these subsets was relatively small, these results should be interpreted with caution.

DISCUSSION

In this cohort study of patients from a vascular laboratory and after adjustment for multiple CVD risk factors and baseline PAD and other CVD, higher levels of Lp-PLA_2 activity were associated with significantly higher risks for CHD mortality. However, additional adjustment for HDL-C and LDL-C significantly attenuated this association so that it was no longer statistically significant. We also note that among those with other CVD at baseline and after adjustment for the CVD risk factors, Lp-PLA_2 mass was significantly associated with an increased hazard for CVD and CHD mortality. These associations were not attenuated with additional adjustment for HDL-C and LDL-C. These results suggest a complex relationship between both Lp-PLA_2 mass and activity and CVD events.

The lack of statistical significance between Lp-PLA_2 activity and CHD mortality after adjustment for HDL-C and LDL-C in our study may be due to our relatively small cohort. The rationale for this assertion is that several other studies have found statistically significant associations between incident CVD events and Lp-PLA_2 activity at 7 years of follow-up that were of similar magnitude to ours. Specifically, after adjustment for traditional CVD risk factors, a one-standard-deviation increment in Lp-PLA_2 was associated with hazard ratios of 1.30, 1.23, and 1.18 in the Mayo,[14] Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA),[20] and West of Scotland Coronary Prevention Study (WOSCOPS)[9] studies, respectively. Combined with our findings, these results demonstrate a relatively consistent magnitude of association between higher levels of Lp-PLA_2 and CVD risk across different study populations that is independent of multiple risk factors.

Atherosclerosis is a chronic reparative inflammatory process[21] and the underlying mechanism for the development the majority of CVD events. The immune/inflammatory pathway of this process is multifaceted and complex.[22] A key step is the oxidation of lipoproteins in the intima of the arterial wall leading to an expansion of the inflammation by the recruitment of cells that elaborate vasoactive cytokines.[23,24] Lp-PLA_2 has been proposed as an inflammatory marker involved in the atherosclerotic process.[2] Specifically, Lp-PLA_2 participates in the oxidative modifi-

cation of LDL by cleaving oxidized phosphatidylcholine and generating lysophosphatidylcholine and oxidized free fatty acids. These free fatty acids promote inflammatory processes present at every stage of atherogenesis[25] and result in diverse inflammatory effects on various cell types.[26] These properties suggest that Lp-PLA_2 would be a risk factor for the development and progression of atherosclerotic CVD.

From this context, some previous community-based studies have demonstrated Lp-PLA_2 to be a significant risk marker for CVD events. For example, Oei et al[14] reported nearly twofold higher relative risks for either incident CHD or stroke that were independent of CVD risk factors and HDL-C levels, whereas other studies have demonstrated the risk associated with higher levels of Lp-PLA_2 to be independent of both CVD risk factors and other inflammatory markers such as C-reactive protein.[13] In the Atherosclerosis Risk in Communities (ARIC) study, however, Lp-PLA_2 was significantly associated with CHD events during 6 years only in those with an LDL-C level of <130 mg/dL,[12] and we did not observe an interaction between LDL-C and Lp-PLA2 for incident CHD (data not shown). Finally, the Women's Health Study (WHS) found no significant association between Lp-PLA_2 and CVD events after adjustment for CVD risk factors.[11]

A significant association with CVD events has been demonstrated in clinical populations.[15] In the Pravastatin or Atorvastatin Evaluation and Infection Therapy – Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial, those in the highest quintile of Lp-PLA_2 activity (but not mass) had a 33% higher risk for recurrent CVD events.[27] Similarly, Brilakis et al[15] reported a 28% higher risk for major incident events during 4 years of follow-up in those undergoing clinically indicated angiography. The Thrombogenic Factors and Recurrent Coronary Events (THROMBO) postinfarction study reported significant associations between Lp-PLA_2 activity and recurrent coronary events at a magnitude similar to our findings.[28]

Other studies have investigated the potential association between Lp-PLA_2 and surrogate markers of CVD. Patients with endothelial dysfunction in the coronary arteries have been shown to have significantly higher levels of Lp-PLA_2, whereas those with Lp-PLA_2 in the highest tertile have more than three times higher odds for having coronary artery endothelial dysfunction.[29] Similarly, after adjustment for age, a one-standard-deviation increment of both Lp-PLA_2 mass and activity has been associated with 40% higher odds for the presence of coronary artery calcium. With adjustment for covariates, including LDL-C, HDL-C, triglycerides, and C-reactive protein, a statistically significant association remained for Lp-PLA_2 mass (odds ratio [OR], 1.28; 95% CI, 1.03 to 1.60) but not for activity (OR, 1.09; 95% CI, 0.84 to 1.42).[30] Finally, investigators from the Rotterdam study assessed the association between Lp-PLA_2 and carotid intimal medial thickness, carotid plaques, calcified atherosclerosis of the abdominal aorta, and the ABI. The age-adjusted OR of having atherosclerosis at any site for the highest versus the lowest tertile of Lp-PLA2 activity was 1.86 (95% CI, 1.01 to 3.43) in men and 1.60 (95% CI, 1.08 to 2.37) in women. Notably, after additional adjustment for cholesterol, these associations attenuated to nonsignificance. The ORs of having atherosclerosis at specific sites followed a similar pattern.[31]

The participants in our study were a relatively small proportion of the cohort of individuals seen in the previous 10 years; therefore, the results of this study may be influenced by selection and survival biases. Men comprised 92% of subjects who did not participate in the study (nonparticipants) versus 87% of study subjects. The mean age of the nonparticipants at the midpoint of the study was 70 years, which was similar to the study subjects' 68 years. In the nonparticipants group, the mean baseline ABI was 0.85 compared with 0.90 in the study subjects. These results suggest that the participants were modestly younger and had slightly less advanced disease than the nonparticipants but were otherwise comparable.

CONCLUSION

Our study in a vascular laboratory cohort concurs with most earlier studies suggesting that Lp-PLA_2 is a biomarker for and possibly plays a role in CVD events as well as subclinical CVD. Similarly, our data suggest that some, but likely not all, of this risk can be attributed to correlations with lipoprotein cholesterol fractions. Finally, differences exist for the associations by Lp-PLA_2 mass and activity, which at this time do not appear to follow a consistent pattern. Given the complexity of the findings to date, further studies are recommended to elucidate the relationship between this novel inflammatory marker and risk for future CVD morbidity and mortality.

AUTHOR CONTRIBUTIONS

Conception and design: MA, MC
Analysis and interpretation: MA, MC, JD, JN
Data collection: MC, JD, JN
Writing the article: MA
Critical revision of the article: MA, MC, JN, LN
Final approval of the article: MA, MC, JN, LN, JD
Statistical analysis: JD, LN, MA
Obtained funding: MC, JN
Overall responsibility: MA

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