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Clinical Utility of Lipoprotein-Associated Phospholipase A_2 for Cardiovascular Disease Prediction in a Multiethnic Cohort of Women

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BACKGROUND: Findings regarding the association of lipoprotein-associated phospholipase A_2 (Lp-PLA2) activity and mass with incident cardiovascular disease (CVD) have been inconsistent, and their role in risk prediction is uncertain.

METHODS: A case–cohort sample from the Women's Health Initiative Observational Study (WHI-OS) comprised 1821 CVD cases and a subcohort of 1992 controls. We used Cox regression models with inverse sampling weights to assess the association of Lp-PLA2 mass and activity with CVD (myocardial infarction, stroke, and CVD mortality).

RESULTS: Subcohort means were 184.3 mmol/min/mL for Lp-PLA2 activity and 499.2 μg/L for Lp-PLA2 mass, with 99% having mass above 200 μg/L, the clinically recommended cut point. Both activity and mass were positively associated with incident CVD in age- and race/ethnicity-adjusted analyses. Following adjustment according to CVD risk factors, the association with activity became null (hazard ratio = 1.02 for top vs bottom quartile, 95% CI = 0.79–1.33, P for trend = 0.65), but the association with mass remained (hazard ratio = 1.84, 95% CI = 1.45–2.34, P for trend $<$ 0.0001). In contrast to blood pressure, HDL, and hsCRP, reclassification statistics for Lp-PLA2 mass did not suggest improvement for overall CVD after full adjustment.

CONCLUSIONS: In the WHI-OS Lp-PLA2 mass, but not activity, was independently associated with CVD. However, model fit did not significantly improve with Lp-PLA2 mass, and assay calibration remains a clinical concern.

Lipoprotein-associated phospholipase A_2 (Lp-PLA2)[5] is an enzyme produced by inflammatory cells and is associated with circulating atherogenic proteins *(1)*. Lp-PLA2 is expressed in the diseased vessel and considered to be a marker for plaque instability. About 80% of the enzyme is bound to LDL, and the remaining 20% is linked to HDL and VLDL *(2)*. Lp-PLA2 activity, but not enzyme mass, has also been linked to small dense LDL particles in human plasma *(3)*. Lp-PLA2 is thought to play a dual role in atherosclerosis, with both proatherogenic and antiinflammatory properties *(4)*.

Epidemiologic investigations have shown mixed results for an association of increased Lp-PLA2 with cardiovascular disease (CVD), with inconsistent associations by population (primary or secondary prevention), endpoint [myocardial infarction (MI) or stroke], and type of assay (activity or mass). Direct comparison of Lp-PLA2 levels is not available owing to a lack of calibration across studies. study by the Lp-PLA2 Studies Collaboration *(5)* revealed that both activity and mass were associated with coronary heart disease (CHD) and stroke. The main analyses, however, combined primary and secondary prevention, although the results appeared stronger for secondary prevention. Because follow-up was limited to 6 years, they did not assess prediction or the clinical utility of measurements of Lp-PLA2 in addition to traditional risk factors, a question that has not yet been addressed *(6)*.

In this study we first examined the association of both Lp-PLA2 activity and mass with subsequent CVD

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[5] Nonstandard abbreviations: Lp-PLA2, Lipoprotein-associated phospholipase A_2; LDL, low-density lipoproteins; HDL, high-density lipoproteins; CVD, cardiovascular disease; MI, myocardial infarction; CHD, coronary heart disease: WHI-OS, Women's Health Initiative Observational Study; HR, hazard ratio; CI, confidence interval; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; BMI, body mass index; IDI, integrated discrimination improvement; NRI, net reclassification improvement; HR, hazard ratio.

events (MI, stroke, and CVD mortality) in the Women's Health Initiative Observational Study (WHI-OS), an ethnically diverse cohort of postmenopausal women, among all women and within subgroups defined by race/ethnicity. We compared the associations of Lp-PLA2 with CHD and stroke, and differences in results by diabetes status and use of hormone therapy. In addition, we assessed the impact of Lp-PLA2 on prediction of CVD in an initially healthy primary prevention cohort of women, and whether Lp-PLA2 measurement can add to traditional CVD risk factors as well as other epidemiologic factors and high-sensitivity C-reactive protein (hsCRP).

Methods

Women were participants in the WHI-OS *(7)* and its long-term follow-up, the WHI Extension Study . The WHI-OS includes 93 676 ethnically diverse postmenopausal women age 50–79 years at enrollment recruited between 1994 and 1998 at 40 clinical centers with targeting of minority groups to obtain a cross-section of the US population *(8)*. Of these, 71 872 had no prior history of myocardial infarction (MI), stroke, revascularization procedures, pulmonary embolism, deep vein thrombosis, peripheral vascular disease, or cancer, and 60 890 additionally had baseline blood specimens and baseline risk factor information available.

The WHI Clinical Centers collected baseline information on sociodemographic characteristics, lifestyle factors, health behaviors, and medical history, including blood pressure measurements. Diabetes *(9)*, current smoking, medication use, and family history, defined here as MI before age 55 years in men and 65 years in women, were self-reported. Participants brought current medications to clinic visits to assess medication use.

Self-reported outcome data through September 2008 were confirmed through medical record review centrally by trained physicians *(10)*. MI and coronary death were combined for the CHD outcome. Medical records, electrocardiogram readings, and cardiac enzyme and troponin determinations were used for confirmation. Strokes were defined as rapid onset of a persistent neurologic deficit attributed to an obstruction or rupture of the brain arterial system, lasting more than 24 h and without evidence of other cause. These were classified as ischemic or hemorrhagic through review of brain-imaging study reports. Underlying cause of death was classified on the basis of death certificates, medical records, and other records such as autopsy reports. The primary endpoint for this analysis was a combined endpoint of major CVD, including MI, any stroke, and death due to cardiovascular causes. This project was approved by the institutional review board at the Brigham and Women's Hospital, Boston, MA.

SAMPLE SELECTION

Because of the large size of the WHI, a prospective case–cohort design *(11)* was employed. Selected cases included all eligible CVD cases from black (n = 200), Hispanic (n = 53), and Asian (n = 55) women, and women with other/unknown ethnicity (n = 55). For efficiency, the remaining 1637 of 2000 cases were randomly selected from 2370 cases among white women. A subcohort of approximately 2000 women was selected using the same eligibility criteria and stratified to match cases by race/ethnicity and 5-year age groups.

Further exclusion of those with other prior CVD conditions, including transient ischemic attack, CVD surgery, or congestive heart failure, and those with no valid measures of Lp-PLA2 (n = 2) led to a final sample size of 1821 cases [746 cases of clinical MI or CHD death, 914 fatal or nonfatal strokes (754 ischemic and 160 hemorrhagic) and 161 cardiovascular deaths] and a subcohort of size 1992, of whom 132 were also cases. Among those in the subcohort who did not develop CVD, the median (25%, 75%) follow-up time was 9.9 (8.6, 11.8) years.

For selected women, plasma samples collected and stored at −70 C^o at study entry were assayed centrally for total cholesterol, HDL cholesterol, hsCRP, and hemoglobin A_{1c} (Hb A_{1c}) (among diabetics) using standardized procedures. Lp-PLA_2 activity was measured in a 96-well microplate with a colorimetric substrate that is converted on hydrolysis by the phospholipase enzyme. Lp-PLA_2 mass concentration was measured with the lipoprotein-associated phospholipase A2 (PLAC) test using a latex bead–based immunoturbidimetric assay, which uses 2 monoclonal antibodies specific to Lp-PLA_2 in a sandwich assay format. Reagents for both assays were provided by diaDexus. CVs based on laboratory standards and on 201 blind duplicate WHI samples were 4.0% and 4.7%, respectively, for Lp-PLA2 activity and 5.9% and 12.6%, respectively, for Lp-PLA2 mass. The core laboratory at Children's Hospital, Boston, is certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program.

STATISTICAL ANALYSIS

The first analysis estimated means and proportions for CVD risk factors among cases and in the subcohort sample, both in crude analysis and after reweighting to reflect the total WHI cohort. Because sampling was stratified by race/ethnicity and age, stratum-specific weighting was used throughout *(12)*. Statistical tests were used to compare means and proportions of cases to noncases in the subcohort sample both in crude

analysis and after reweighting using the Satterthwaite approximation for the *t*-test and the Rao-Scott F-statistic for weighted proportions. hsCRP was log transformed to normalize the data before testing. We examined the weighted population association of Lp-PLA2 mass and activity with CVD risk factors, grouping the Lp-PLA2 variables into quartiles. We estimated and tested overall population associations using survey procedures in SAS 9.2.

We examined the association of Lp-PLA2 and CVD using previously described methods for proportional hazards regression in case–cohort samples *(13)* with appropriate weighting of the observations. We used weighted Cox regression *(14)* to estimate hazard ratios (HRs) using Proc Phreg of SAS, and we computed asymptotic variance *(15)*. We examined the association of Lp-PLA2 with CVD in both crude models, adjusting for age and race/ethnicity only, and adjusted models. The latter included (*a*) prior conditions, including diabetes and angina, and medication use, including statins and current and past hormone therapy; (*b*) the previous variables plus traditional cardiovascular risk factors, including current smoking and the natural logs of systolic blood pressure (SBP), total and HDL cholesterol; and (*c*) the previous variables plus factors in the Reynolds risk score *(16)*, including the natural log of hsCRP, family history of premature MI, and Hb A_{1c} among diabetic patients. We estimated the associations both in quartiles of Lp-PLA2 and using continuous terms, expressed per SD unit for easy comparison. We explored nonlinear relations with CVD using quadratic terms and log transformations, but found no deviation from linearity. Because levels of Lp-PLA2 are typically lower among users of hormone therapy *(17, 18)* and higher among those with components of the metabolic syndrome *(19)*, we examined interactions with hormone therapy as well as with age, race/ethnicity, diabetes, and levels of body mass index (BMI), cholesterol, and hsCRP.

Absolute risk for each woman was estimated according to the method reported by Langholz and Jiao *(15)*. To directly compare models with and without Lp-PLA2, we examined changes in the *c*-statistic, the integrated discrimination improvement (IDI) *(20)*, and reclassification statistics *(21)*, including reclassification calibration χ-squares *(22)*, and the net reclassification improvement (NRI) *(20)*. For the reclassification tables, clinically based cut points of 5%, 10%, and 20% for estimated 10-year risk of CVD were used *(23)*. Survival methods were used throughout *(24, 25)*, and measures were reweighted to reflect the distribution in the overall cohort. We derived statistical tests of reweighted measures sing bootstrap samples.

Results

The mean age of the 1821 CVD cases and the frequency-matched subcohort sample of 1992 women was 68 years, with 10% of black race (Table 1). Population estimates after reweighting to the total WHI cohort were a mean of 63 years with 7% black individuals. The age- and race/ethnicity-matched cases and noncases differed by weight, smoking status, blood pressure, total and HDL cholesterol, hsCRP, diabetes, and family history of early MI. The range of values in the total sample were 0–539.5 mmol/min/mL for Lp-PLA2 activity, and 30.3–1678.0 μg/L for Lp-PLA2 mass, both with a relatively normal distribution. Only 47 women in the total sample (1.3%) had values for Lp-PLA2 mass below 200 μg/L. Mean Lp-PLA2 activity was 191.5 mmol/min/mL among cases and 184.3 mmol/min/mL in the subcohort sample, whereas that for Lp-PLA2 mass was 531.2 and 499.2 μg/L in cases and subcohort, respectively. The estimated means of Lp-PLA2 activity and mass after reweighting to the overall WHI cohort were 182.0 mmol/min/mL and 488.2 μg/L, respectively. The crude correlation of Lp-PLA2 mass and activity in the subcohort was 0.69 and became 0.72 after reweighting to the total WHI-OS cohort.

Higher quartiles of both Lp-PLA2 activity and mass were associated with increasing age, white race, higher total cholesterol, and lower hsCRP (Table 2). Both activity and mass were strongly inversely associated with hormone therapy use, with lower levels among current users. In addition, increased Lp-PLA2 activity but not mass showed a modest association with increased weight, Hb A_{1c} among diabetics, and family history of early MI, and Lp-PLA2 mass showed a modest association with current smoking. There was no overall association of either variable with diabetes itself. Lp-PLA2 activity was also strongly inversely associated with HDL cholesterol, although Lp-PLA2 mass was not. There was also little association with statin use in these crude data. Among women not on hormone therapy, both activity and mass retained the same associations with lipids, but there was no longer an association with hsCRP (data not shown).

In Cox proportional hazards models adjusted for age and race/ethnicity, quartiles of Lp-PLA2 activity were associated with risk of incident CVD, with an HR of 1.55 for comparison of the top to bottom quartile (95% CI = 1.27–1.88, *P*-trend <0.0001), which remained after adjustment for prior conditions and statin use (Table 3). After further adjustment for traditional cardiovascular risk factors, however, the association disappeared and became null (HR = 1.02, 95% CI = 0.79–1.33, *P*-trend =0.65). The primary driver of the change was the addition of HDL cholesterol to the model. The association remained null after

Table 1. Comparisons of baseline characteristics of total CVD cases and random subcohort in the sample and reweighted to the total WHI cohort.[a]

	Crude			Reweighted to population		
Variables	Subcohort (n = 1992)	Cases (n = 1821)	*P*	Cohort (N = 58 324)	Cases (N = 2438)	*P*
Age, y	67.7	67.8	0.11	62.7	67.9	<0.0001
Black race, %	204 (10.2)	186 (10.2)	0.96	4207 (7.2)	186 (7.6)	0.61
White race, %	1622 (81.4)	1489 (81.8)	0.65	49410 (84.7)	2106 (86.4)	0.12
Height, cm	160.9	160.8	0.78	161.5	160.9	0.095
Weight, kg	70.0	72.2	<0.0001	72.0	72.1	0.85
BMI, kg/m^2	26.9	27.9	<0.0001	27.3	27.8	0.057
Current smoking, %	96 (4.8)	161 (8.8)	<0.0001	3,310 (5.7)	207 (8.5)	0.010
Hormone therapy, %						
Current	830 (41.7)	719 (39.6)	0.12	27,986 (48.0)	977 (40.1)	<0.0001
Past	286 (14.4)	276 (15.2)	0.53	7,051 (12.1)	375 (15.4)	0.013
BP, mmHg						
SBP	129.7	135.5	<0.0001	126.3	135.3	<0.0001
Diastolic BP	74.3	75.8	<0.0001	74.8	75.7	0.012
Antihypertensive medication use, %	529 (26.6)	687 (37.7)	<0.0001	13,091 (22.4)	909 (37.3)	<0.0001
Total cholesterol, mg/dL	230.9	228.0	0.047	231.0	228.4	0.17
HDL, mg/dL	56.8	51.1	<0.0001	56.6	51.1	<0.0001
Cholesterol-lowering medication, %	178 (8.9)	176 (9.7)	0.55	4,359 (7.5)	236 (9.7)	0.037
Statin use, %	161 (8.1)	134 (7.4)	0.30	3,921 (6.7)	180 (7.4)	0.54
hsCRP, mg/L[b]	2.3	3.0	<0.0001	2.4	3.0	<0.0001
Diabetes, %	93 (4.7)	189 (10.4)	<0.0001	2,151 (3.7)	238 (9.7)	<0.0001
Hb A_{1c} (if diabetic), %	7.4	7.9	0.013	7.5	7.9	0.11
Angina, %	55 (2.8)	83 (4.6)	0.004	1,314 (2.3)	112 (4.6)	0.0007
Family history of early MI, %	352 (17.7)	403 (22.1)	0.0002	11,413 (19.6)	552 (22.7)	0.042
Lp-PLA2 activity, mmol/min/mL (μmol · min^{-1} · L^{-1})	184.3	191.5	<0.0001	182.0	191.9	<0.0001
Lp-PLA2 mass, ng/mL	499.2	531.2	<0.0001	488.2	533.1	<0.0001

[a] Mean or percentage except as noted.
[b] Median. Test is based on natural logs.

further adjustment for hsCRP, family history, and Hb A_{1c} in diabetic patients.

The associations of Lp-PLA2 mass with CVD were similarly strong in crude models, with an HR of 1.74 for comparison of extreme quartiles (95% CI = 1.43–2.10, *P*-trend <0.0001). In contrast to activity, however, the association of Lp-PLA2 mass remained after further adjustment. In the fully adjusted model, the HR for extreme quartiles was 1.84 (95% CI = 1.45–2.34, *P*-trend <0.0001).

Subgroup analyses by hormone therapy use, in nondiabetic individuals, and in those not on statin therapy revealed similar results (Table 4), with a crude association for Lp-PLA2 activity that became null after full adjustment, but with continued association for Lp-PLA2 mass. Among nonusers of hormone therapy and statins, without prior diabetes or angina the association remained significant (HR for extreme quartiles = 1.65, 95% CI = 1.09–2.50, *P*-trend = 0.01).

For Lp-PLA2 as a continuous variable, the HR for activity was 1.19 (95% CI = 1.11–1.28, $P < 0.0001$) per SD (50.5 mmol/min/mL) after adjustment for only age and race/ethnicity, but became null (HR = 1.04, 95% CI = 0.95–1.14, $P = 0.40$) after full adjustment. There were no significant effects in any subgroups considered (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue9). The effect of Lp-

Table 2. Baseline characteristics by quartile of Lp-PLA2 in the subcohort sample reweighted to the population.[a]

Variables	Quartile of Lp-PLA2 activity or mass				
	Q1	Q2	Q3	Q4	*P* for trend[b]
Lp-PLA2 activity, mmol/min/mL (μmol · min^{-1} · L^{-1})[c]	128.4	163.7	194.4	236.7	
Age, y	61.2	62.5	63.6	63.3	0.0014
Black race, %	9.6	9.2	5.7	4.4	0.0002
White race, %	79.9	84.2	85.4	89.3	0.0007
Height, cm	161.9	160.2	162.0	162.1	0.28
Weight, kg	70.0	71.7	72.7	73.4	0.023
BMI, kg/m^2	26.7	27.4	27.4	27.8	0.047
Current smoking, %	5.1	3.9	6.3	7.5	0.19
Hormone therapy, %					
Current	74.2	50.9	42.4	24.7	<0.0001
Past	6.8	11.5	17.7	12.4	0.0017
BP, mmHg					
SBP	127.0	125.8	125.0	127.6	0.81
DBP	75.3	74.6	74.0	75.2	0.73
Antihypertensive medication use, %	24.0	22.7	20.8	22.3	0.53
Total cholesterol, mg/dL	209.8	222.8	232.8	258.4	<0.0001
HDL, mg/dL	63.9	58.3	53.6	50.5	<0.0001
Cholesterol-lowering medication, %	6.1	8.7	8.4	6.7	0.79
Statin use, %	4.5	8.5	8.2	5.7	0.52
hsCRP, mg/L[c]	3.5	2.4	2.0	2.0	<0.0001
Diabetes, %	4.2	2.1	4.2	4.2	0.73
Hb A_{1c} (if diabetic), %	6.6	7.0	8.5	7.5	0.030
Angina, %	1.4	3.0	2.1	2.5	0.47
Family history of early MI, %	16.4	19.5	17.4	25.1	0.040
Lp-PLA2 mass, ng/mL[c]	329.9	432.3	515.8	649.2	
Age, y	61.3	62.9	62.7	63.7	0.0019
Black race, %	10.9	8.0	4.5	5.4	0.0003
White race, %	76.0	86.0	88.6	88.2	<0.0001
Height, cm	161.9	161.1	161.4	161.8	>0.99
Weight, kg	72.2	70.5	72.9	72.3	0.60
BMI, kg/m^2	27.5	27.3	27.0	27.5	0.85
Current smoking, %	4.0	3.9	7.1	7.7	0.046
Hormone therapy, %					
Current	62.7	55.5	43.3	30.5	<0.0001
Past	8.1	12.7	10.6	17.0	0.0029
Blood pressure, mmHg					
SBP	127.0	126.0	124.9	127.5	0.95
DBP	75.2	74.5	74.3	75.2	0.87
Antihypertensive medication use, %	24.9	25.6	16.8	22.5	0.13
Total cholesterol, mg/dL	205.5	224.9	230.7	262.6	<0.0001
HDL, mg/dL	56.7	57.9	55.1	56.7	0.53
Cholesterol-lowering medication, %	9.2	9.1	5.3	6.3	0.038

Continued on page XX

Table 2. Baseline characteristics by quartile of Lp-PLA2 in the subcohort sample reweighted to the population.[a]
(Continued from page XX)

Variables	Quartile of Lp-PLA2 activity or mass				
	Q1	Q2	Q3	Q4	*P* for trend[b]
Statin use, %	7.4	8.7	5.2	5.5	0.10
hsCRP, mg/L[c]	3.2	2.8	1.8	2.1	<0.0001
Diabetes, %	5.9	3.7	1.3	3.8	0.17
Hb A_{1c} (if diabetic), %	6.9	7.9	7.8	7.8	0.19
Angina, %	1.4	3.3	1.8	2.5	0.63
Family history of early MI, %	18.1	19.8	19.3	21.0	0.48

[a] Mean or percentage except as noted.
[b] Test for trend in means using linear regression or in proportions using logistic regression.
[c] Median. Test for hsCRP is based on natural logs.

PLA2 mass was predictive after adjustment for age and race/ethnicity only (HR = 1.22, 95% CI = 1.14–1.31, $P < 0.0001$) per SD (152.0 μg/L) or after full adjustment (HR = 1.24, 95% CI = 1.14–1.35, $P < 0.0001$) (see online Supplemental Table 2). The estimated effects were comparable in magnitude on the SD unit scale to those seen for other CVD risk factors such as SBP, HDL, hsCRP, and Hb A_{1c} among those with diabetes (see online Supplemental Table 3). The same associations were seen in all subgroups considered, with no effect modification by race, hormone therapy use, diabetes, angina, statin use, or levels of total or HDL cholesterol, BMI or hsCRP (see online Supplemental Table 2).

Table 3. Association of Lp-PLA2 with total CVD.

Variables	HR (95% CI) by quartile of Lp-PLA2[a]				
	Q1	Q2	Q3	Q4	*P* for trend
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.23 (1.00–1.50)	1.22 (1.00–1.49)	1.55 (1.27–1.88)	<0.0001
MV1[b]	1.00	1.27 (1.03–1.56)	1.19 (0.97–1.47)	1.55 (1.26–1.92)	0.0002
MV2	1.00	1.18 (0.95–1.48)	0.96 (0.76–1.21)	1.02 (0.79–1.33)	0.65
MV3	1.00	1.21 (0.97–1.51)	1.00 (0.79–1.27)	1.08 (0.83–1.40)	0.95
ATP variables[c]	1.00	1.16 (0.93–1.44)	0.93 (0.74–1.17)	0.99 (0.77–1.28)	0.48
RRS variables[d]	1.00	1.21 (0.97–1.50)	1.00 (0.80–1.27)	1.09 (0.85–1.40)	0.96
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.12 (0.91–1.37)	1.40 (1.15–1.72)	1.74 (1.43–2.10)	<0.0001
MV1	1.00	1.14 (0.93–1.41)	1.49 (1.21–1.84)	1.77 (1.45–2.17)	<0.0001
MV2	1.00	1.26 (1.00–1.57)	1.42 (1.14–1.79)	1.75 (1.39–2.21)	<0.0001
MV3	1.00	1.25 (1.00–1.57)	1.47 (1.17–1.86)	1.84 (1.45–2.34)	<0.0001
ATP variables	1.00	1.25 (1.00–1.56)	1.40 (1.12–1.76)	1.74 (1.38–2.18)	<0.0001
RRS variables	1.00	1.24 (0.99–1.56)	1.46 (1.16–1.84)	1.86 (1.47–2.35)	<0.0001

[a] Quartile cut points are 146.5, 179.3, and 212.1 $\mu mol \cdot min^{-1} \cdot L^{-1}$ for activity and 387.3, 475.1, and 572.4 ng/mL for mass.
[b] MV1, adjusted for age and race/ethnicity, plus current conditions and medication, including prior diabetes, angina, statin use, and current and past hormone therapy. MV2, additionally adjusted for traditional cardiovascular risk factors, including current smoking and the natural logs of SBP, total and high-density lipoprotein cholesterol. MV3, additionally adjusted for the natural log of CRP, family history of premature MI, and Hb A_{1c} among diabetics.
[c] ATP variables, adjusted for age, current smoking, prior diabetes, and the natural logs of SBP and total and HDL cholesterol.
[d] RRS variables, adjusted for age, current smoking, family history of premature MI, prior diabetes, and Hb A_{1c} among diabetics, and the natural logs of SBP, total and HDL cholesterol, and CRP.

Table 4. Association of Lp-PLA2 with total CVD among subgroups.

Variables	HR (95% CI) by quartile of Lp-PLA2[a]				*P* for trend
	Q1	Q2	Q3	Q4	
Individuals not on hormone therapy (1098 cases/1162 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.07 (0.80–1.44)	1.35 (1.01–1.81)	1.70 (1.26–2.28)	0.0001
Fully adjusted[b]	1.00	0.98 (0.70–1.37)	1.06 (0.74–1.51)	1.17 (0.97–1.72)	0.37
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.12 (0.84–1.52)	1.28 (0.95–1.73)	1.65 (1.23–2.20)	0.0004
Fully adjusted	1.00	1.28 (0.90–1.81)	1.24 (0.86–1.80)	1.75 (1.20–2.54)	0.005
Individuals on hormone therapy (719 cases/830 subcohort)					
Lp-PLA2 Activity					
Adjusted for age and race/ethnicity	1.00	0.99 (0.67–1.47)	1.12 (0.77–1.64)	1.24 (0.84–1.83)	0.22
Fully adjusted[a]	1.00	1.03 (0.66–1.63)	0.99 (0.63–1.55)	0.90 (0.53–1.52)	0.66
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	0.98 (0.65–1.48)	1.38 (0.92–2.07)	1.57 (1.06–2.33)	0.007
Fully adjusted[a]	1.00	1.12 (0.71–1.76)	1.43 (0.90–2.26)	1.66 (1.03–2.69)	0.022
Nondiabetic individuals (1632 cases/ 1899 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.21 (0.98–1.50)	1.16 (0.94–1.43)	1.49 (1.21–1.82)	0.0004
Fully adjusted[b]	1.00	1.15 (0.92–1.45)	0.97 (0.76–1.24)	0.99 (0.76–1.31)	0.57
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.13 (0.91–1.40)	1.42 (1.15–1.76)	1.78 (1.45–2.18)	<0.0001
Fully adjusted	1.00	1.22 (0.97–1.54)	1.35 (1.06–1.71)	1.75 (1.37–2.24)	<0.0001
Individuals not on statins (1687 cases/1831 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.30 (1.05–1.60)	1.25 (1.02–1.55)	1.54 (1.25–1.90)	0.0002
Fully adjusted	1.00	1.26 (0.99–1.59)	1.00 (0.78–1.28)	1.02 (0.77–1.36)	0.58
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.10 (0.88–1.37)	1.44 (1.16–1.78)	1.71 (1.39–2.10)	<0.0001
Fully adjusted	1.00	1.23 (0.96–1.57)	1.47 (1.15–1.88)	1.74 (1.35–2.25)	<0.0001
Nonusers of hormone therapy and statins, without prior diabetes or angina (876 cases/978 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.12 (0.80–1.57)	1.38 (0.99–1.94)	1.60 (1.13–2.26)	0.003
Fully adjusted	1.00	1.01 (0.68–1.48)	1.05 (0.70–1.58)	0.98 (0.62–1.56)	0.98
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.06 (0.74–1.50)	1.34 (0.95–1.89)	1.72 (1.23–2.40)	0.0004
Fully adjusted	1.00	1.07 (0.72–1.59)	1.21 (0.81–1.80)	1.65 (1.09–2.50)	0.013

[a] Cutpoints per subgroup-specific quartile are 166.5, 194.3, 227.4 mmol/min/mL (166.5, 194.3, 227.4 μmol · min^{-1} · L^{-1}) for activity and 417.2, 506.5, 615.4 ng/mL for mass among those not on hormone therapy; 133.6, 158.6, 193.8 mmol/min/mL (133.6, 158.6, 193.8 μmol · min^{-1} · L^{-1}) for activity and 363.1, 436.6, 528.7 ng/mL for mass among those on hormone therapy; 146.6, 179.0, 211.9 mmol/min/mL (146.6, 179.0, 211.9 μmol · min^{-1} · L^{-1}) for activity and 388.2, 476.6, 572.4 ng/mL for mass among nondiabetics; 145.3, 179.1, 212.5 mmol/min/mL (145.3, 179.1, 212.5 μmol · min^{-1} · L^{-1}) for activity and 388.1, 477.7, 576.4 ng/mL for mass among those not on statins; and 166.8, 195.1, 228.0 mmol/min/mL (166.8, 195.1, 228.0 μmol · min^{-1} · L^{-1}) for activity and 429.9, 511.9, 620.1 ng/mL for mass among the healthy nonusers of hormone therapy.

[b] Fully adjusted, adjusted for age, race/ethnicity, prior diabetes, angina, statin use, current smoking, the natural logs of SBP, total and HDL cholesterol and CRP, family history of premature MI, and Hb A_{1c} among diabetic patients. Models are additionally adjusted for current and past use of hormone therapy as appropriate.

Table 5. Association of Lp-PLA2 with MI and total stroke (including later events).

Variables	HR (95% CI) by quartile of Lp-PLA2				*P* for trend
	Q1	Q2	Q3	Q4	
MI (776 cases/1992 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.25 (0.96–1.64)	1.37 (1.05–1.79)	2.06 (1.60–2.66)	<0.0001
Fully adjusted[a]	1.00	1.20 (0.90–1.61)	1.08 (0.80–1.48)	1.40 (1.00–1.95)	0.084
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.04 (0.79–1.35)	1.32 (1.02–1.72)	1.72 (1.34–2.20)	<0.0001
Fully adjusted	1.00	1.09 (0.81–1.47)	1.33 (0.99–1.79)	1.61 (1.19–2.18)	0.0006
Stroke (952 cases/1992 subcohort)					
Lp-PLA2 activity					
Adjusted for age and race/ethnicity	1.00	1.24 (0.98–1.56)	1.11 (0.88–1.41)	1.24 (0.98–1.56)	0.19
Fully adjusted	1.00	1.24 (0.96–1.61)	0.93 (0.71–1.23)	0.88 (0.64–1.20)	0.13
Lp-PLA2 mass					
Adjusted for age and race/ethnicity	1.00	1.18 (0.92–1.52)	1.59 (1.24–2.03)	1.89 (1.49–2.39)	<0.0001
Fully adjusted	1.00	1.43 (1.09–1.88)	1.81 (1.38–2.39)	2.32 (1.75–3.07)	<0.0001

[a] Fully adjusted, adjusted for age, race/ethnicity, prior diabetes, angina, statin use, current smoking, the natural logs of SBP, total and HDL cholesterol and CRP, family history of premature MI, and Hb A_{1c} among diabetic patients. Models were additionally adjusted for current and past use of hormone therapy as appropriate.

There were 776 cases of clinical MI or CHD death and 952 cases of fatal or nonfatal stroke. Lp-PLA2 activity showed a marginal association with MI even after full adjustment (HR for extreme quartiles = 1.40, 95% CI = 1.00–1.95, *P*-trend = 0.08), but no association with total stroke (Table 5). The HRs per SD unit were 1.13 (95%CI = 1.01–1.28, $P = 0.037$) for MI and 0.98 (95% CI = 0.88–1.09, $P = 0.68$) for total stroke. In contrast, Lp-PLA2 mass showed a strong association with both MI and stroke even after full adjustment, with an HR across extreme quartiles of 1.61 (95% CI = 1.19–2.18, *P*-trend = 0.0006) for MI and 2.32 (95% CI = 1.75–3.07, *P*-trend <0.0001) for total stroke. The HRs per SD unit were 1.18 (95% CI = 1.06–1.31, $P = 0.003$) for MI and 1.33 (95%CI = 1.21–1.47, $P < 0.0001$) for total stroke. The latter relationships were similar among those not using hormone therapy.

To determine the clinical utility of adding Lp-PLA2 mass to predictive models for CVD, we evaluated models with and without this variable, including Adult Treatment Panel III (ATP-III) variables, Reynolds risk score (RRS) variables, and a full model with all variables (Table 6). The addition of Lp-PLA2 mass improved the *c*-statistic for all models by 0.005–0.006. When reclassification measures were examined (see online Supplemental Tables 4–6), model improvement varied depending on the reference model. When the ATP III or RRS variables were included, the reclassification calibration improved, and the NRI was 0.035 (95% CI = 0.009–0.063, $P = 0.005$) compared to the ATP-III variables and 0.027 (95% CI = 0.001–0.052, $P = 0.038$) compared to the RRS variables (Table 6). The IDI was significant only for the RRS variables, however. When the full model, additionally including race/ethnicity, angina, statin use, and current and past hormone therapy, was used as the reference, there was little improvement in fit. Although the *c*-statistic improved by 0.006, the reclassification calibration statistics, the NRI (0.020, $P = 0.11$), and the IDI (0.0033, $P = 0.08$) did not suggest significant improvement. This is in contrast to other cardiovascular risk factors, including SBP (NRI = 0.068, $P < 0.0001$), HDL cholesterol (NRI = 0.079, $P < 0.0001$), and hsCRP (NRI = 0.032, $P = 0.003$), which did improve model accuracy in these data, both with and without Lp-PLA2 mass included (see online Supplemental Table 7). Finally, when reclassification was considered among only those not on statins or hormone therapy and without diabetes or prior angina, the results were similar. Although it was still associated with CVD (Table 4; also see online Supplemental Table 2), Lp-PLA2 mass did not add to either the ATP-III or RRS variables in terms of risk prediction (Table 6).

Discussion

In these postmenopausal women, Lp-PLA2 activity and mass were correlated with several other cardiovas-

Table 6. Comparison of models for total CVD with and without Lp-PLA2 mass, based on weighted survival estimates for case–cohort studies.

Model comparison	C-index without Lp-PLA2	C-index with Lp-PLA2	Change in C-index	RC[a] χ^2 without Lp-PLA2	RC χ^2 with Lp-PLA2	NRI	IDI
All women							
ATP variables[b]							
Lp-PLA2 mass	0.766	0.771	0.005	88.9	48.7	0.0349	0.0018
P			0.0006			0.005	0.24
RRS variables[c]							
Lp-PLA2 mass	0.769	0.775	0.006	36.3	31.4	0.0267	0.0037
P			0.0004			0.038	0.028
Full model[d]							
Lp-PLA2 mass	0.770	0.776	0.006	33.9	31.2	0.0199	0.0033
P			0.0003			0.11	0.082
Healthy nonusers of hormone therapy[e]							
ATP variables							
Lp-PLA2 mass	0.763	0.767	0.004	44.9	37.1	0.0069	0.0009
P			0.027			0.68	0.59
RRS variables							
Lp-PLA2 mass	0.764	0.768	0.004	48.0	35.7	0.0117	0.0019
P			0.056			0.49	0.28

[a] Reclassification calibration χ^2.
[b] ATP variables include age, prior diabetes, current smoking, and the natural logs of SBP, and total and HDL cholesterol.
[c] RRS variables additionally include the natural log of CRP, family history of premature MI, and Hb A_{1c} among diabetics.
[d] The full model additionally includes race/ethnicity, angina, statin use, and current and past hormone therapy.
[e] Those not on hormone therapy or statins, without diabetes or prior angina.

cular risk factors, particularly lipids. Both were associated with total cholesterol and influenced by hormone therapy use. Lp-PLA2 activity was also strongly associated with HDL. Although hormone use explained the crude relationship with hsCRP, the associations with lipids remained among nonusers. Other reported data have revealed that Lp-PLA2 activity levels are positively correlated with total and LDL cholesterol, but not with inflammatory markers, such as hsCRP and interleukin-6 or with BMI *(26, 27)*. Lp-PLA2 has also been found to be higher among those individuals with components of the metabolic syndrome *(19)*. Although Lp-PLA2 activity is generally negatively correlated with HDL, the relationship of Lp-PLA2 mass to HDL has varied across studies. Data from the Atherosclerosis Risk in Communities (ARIC) study showed a negative correlation *(27)*, but controls from a large case-control study *(26)*, from the Pravastatin in the Elderly at Risk (PROSPER) study *(28)*, and from the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial *(29)* showed a null or positive correlation with HDL.

Both Lp-PLA2 activity and mass were associated with later CVD in this primary prevention cohort, but results differed after multivariable adjustment. Because of its strong correlation with HDL, Lp-PLA2 activity was no longer associated with CVD after full adjustment, or with MI or stroke. Lp-PLA2 mass, however, remained consistently associated with CVD regardless of adjustment or subgroup restriction.

Other epidemiologic data support a role for Lp-PLA2 in predicting both CHD and stroke, although results are inconsistent. Some nested case-control studies, including WOSCPOS (West of Scotland Coronary Prevention Study) *(30)*, WHS (Women's Health Study) *(17)*, and ARIC *(27)*, revealed a modest increased risk of CHD with higher Lp-PLA2 mass. In ARIC the overall association was null, with a positive association only for those with LDL <130 mg/dL (<3.36 mmol/L). Other cohort studies of healthy individuals have revealed more sizeable effects on CHD, including for mass in the MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) study *(31)* and for activity in the Rotterdam study *(32)*. ARIC (mass) *(33)* and Rotterdam

study (activity) *(32)* have also revealed associations with stroke. Data from the Malmo study *(34)* suggested that Lp-PLA2 activity was a stronger predictor of total CVD than Lp-PLA2 mass. In a previous nested case-control study from the WHI-OS, Lp-PLA2 mass was associated with risk of ischemic stroke only among women not currently using hormone therapy *(18)*. The current WHI sample and analysis found effects of Lp-PLA2 mass, but not activity, on total stroke regardless of hormone therapy use.

A recent metaanalysis of individual patient data from the Lp-PLA2 Studies Collaboration found overall associations of both activity and mass on vascular disease, including CHD, ischemic stroke, and vascular as well as nonvascular mortality *(5)*. Although results for presence of prior vascular disease were not significantly different, there was some apparent difference in results, with weaker effects among those with no prior history. For Lp-PLA2 activity, the HRs per SD among those with no history were 1.03 (95% CI = 0.95–1.12) for CHD and 1.01 (95% CI = 0.71–1.43) for ischemic stroke. The corresponding estimates for Lp-PLA2 mass were 1.09 (95%CI = 1.02–1.16) for CHD and 1.22 (95% CI = 0.99–1.51) for ischemic stroke. These estimates are consistent with the current results, which showed stronger associations of mass than activity after adjustment in this primary prevention cohort. However, these data are not consistent with recent analyses of Lp-PLA2 in the JUPITER trial, in which activity but not mass was predictive of incident vascular events *(29)*. Furthermore, among those randomly allocated to statin therapy in JUPITER, residual risk was not associated with on-treatment levels of Lp-PLA2, nor did baseline levels modify the effect of therapy.

Although Lp-PLA2 mass was associated with CVD in these and other data, whether it has clinical utility and should be added to risk prediction models is another question *(6)*. The addition of Lp-PLA2 mass increased the *c*-statistic significantly for all comparisons, but only by 0.005–0.006. The estimated improvement with the use of reclassification methods depended on the reference model. As the comparison model became stronger, the utility of adding Lp-PLA2 mass weakened. When it was added to models with the ATP-III variables, reclassification measures suggested an improvement, with a significant NRI of 0.035. When compared to the RRS variables, the improvement was smaller. However, when compared to a model with all risk factors, including hormone therapy and statins, there was little improvement for major CVD based on reclassification measures. Previous analyses of ischemic stroke, however, did show an improvement in prediction among subtypes of ischemic stroke *(35)*. In contrast, other biomarkers, including SBP, HDL, and hsCRP, showed significant model improvement for CVD in these data.

Another major consideration for clinical utility is the reproducibility of the assay, a problem found in many studies *(6, 36)*. As noted by the Lp-PLA2 Studies Collaboration *(5)*, mean levels vary greatly by study and particular assay used. In these data the levels of both activity and mass [182.0 mmol/min/mL and 488.2 μg/L] were higher than the average over other studies *(5)* for activity [mean = 151 mmol/min/mL using the CAM (chick chorioallantoic membrane) assay] or mass (mean = 312 μg/L with the PLAC II assay). The Rancho Bernardo study *(37)* reported similarly high levels for Lp-PLA2 mass using the PLAC test (494 μg/L among noncases), but most other studies have had lower levels, including CARDIA (Coronary Artery Risk Development in Young Adults) study *(38)*, PROSPER *(28)*, Cardiac Health Study *(39)*, and ARIC *(27)*, with medians of 267, 300, 335, and 373 μg/L, respectively. Measures of Lp-PLA2 mass in a previous sample of WHI participants had a mean of 296.3 μg/L among controls *(18)*, lower than the estimated cohort mean of 488.2 μg/L in our sample. About 99% of our cohort of women had values above 200 μg/L, the clinical reference point proposed to designate high vascular risk *(40)*. The recent metaanalysis revealed that levels for both activity and mass varied greatly by assay type and study, and that standardization was necessary to combine data *(5)*. Others have also noted an unusual stability profile for this analyte, with differences by preanalytic conditions *(41)*. The lack of standard values for this test indicates a lack of calibration of the assay and limits clinical interpretation of individual levels.

A limitation of this work is that the cohort is comprised solely of women, but other studies have not found sex-specific differences *(5)*. Another limitation is that only baseline samples were used; serial measurements could have increased the size of the association. Samples were also stored for many years before analysis, which may affect the assay.

We have thus found that although the association of Lp-PLA2 activity can be explained by other traditional risk factors, Lp-PLA2 mass continued to show an association with later CVD. Despite this association, the clinical utility of Lp-PLA2 mass remains questionable due to a lack of model improvement. More important are the high absolute levels for Lp-PLA2 mass seen in these data. The lack of standardization for this assay severely limits its potential clinical use.

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