

# Lipoprotein-Associated Phospholipase A<sub>2</sub> Mass and Activity and Risk of Cardiovascular Disease in a Population With High Prevalences of Obesity and Diabetes

The Strong Heart Study

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**OBJECTIVE**—To investigate the association of lipoprotein-associated phospholipase A<sub>2</sub> (LpPLA<sub>2</sub>) mass and activity with incident cardiovascular disease (CVD) in a population with high prevalences of insulin resistance and diabetes, conditions for which epidemiological data remain sparse.

**RESEARCH DESIGN AND METHODS**—We conducted a nested, case-control study ( $n = 1,008$ ) within a population-based cohort of American Indians. Case subjects were defined by incidence of first-ever CVD up to 10 years later. Control subjects comprised participants free of CVD events during the follow-up period who were frequency matched to case subjects by age, sex, and diabetes status. LpPLA<sub>2</sub> mass and activity were measured using commercially available assays.

**RESULTS**—LpPLA<sub>2</sub> mass and activity were moderately correlated with each other ( $r = 0.30$ ), but only LpPLA<sub>2</sub> activity exhibited moderate correlations with lipid fractions. After extensive adjustment for covariates, both LpPLA<sub>2</sub> measures were significantly associated with incident CVD, but the relationship was inverse for LpPLA<sub>2</sub> mass (highest versus lowest tertile, relative risk [RR] 0.55 [95% CI 0.39–0.79]) and positive for LpPLA<sub>2</sub> activity (highest versus lowest tertile, 1.65 [1.12–2.42]). These associations were similar when participants with and without diabetes were examined separately.

**CONCLUSIONS**—In this population-based cohort enriched with dysmetabolic phenotypes, LpPLA<sub>2</sub> mass and activity showed divergent associations with CVD. The inverse relationship for LpPLA<sub>2</sub> mass is contrary to observations from predominantly nondiabetic populations and will require independent replication. Whether this finding relates to redistribution of LpPLA<sub>2</sub> to lipoprotein classes where it is less atherogenic or reflects incomplete measurement of LpPLA<sub>2</sub> mass associated with altered lipoprotein composition in insulin resistance warrants further investigation.

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Recognition of the seminal role of inflammation in atherogenesis (1) has led to a search for key molecular drivers of the vascular inflammatory response as potential therapeutic targets or markers of prognosis in at-risk individuals. One such candidate is the enzyme lipoprotein-associated phospholipase A<sub>2</sub> (LpPLA<sub>2</sub>), a secretory product of inflammatory cells that binds primarily to apolipoprotein B-containing lipoproteins such as LDL (2). Because excess LDL accumulates within the subendothelial space during atherogenesis, LpPLA<sub>2</sub> function may have particular specificity for inflammation within the vascular compartment (3).

LpPLA<sub>2</sub> catalyzes the hydrolysis of oxidized phospholipids to lysophosphatidyl choline and oxidized, nonesterified fatty acids, mediators that can trigger inflammatory cell activation and retard clearance of apoptotic cells within the atheroma's necrotic core (4). Such proinflammatory actions would act to foster progression of atherosclerosis and plaque instability (4). The pathophysiologic role of LpPLA<sub>2</sub> in atherosclerosis remains controversial, however, because the enzyme's removal of proatherogenic oxidized phospholipids can itself have an atheroprotective effect (3). Moreover, in vitro LpPLA<sub>2</sub> can also hydrolyze platelet-activating factor (PAF), which is a potent mediator of thrombotic, allergic, and inflammatory responses (3). Although degradation of PAF would serve an anti-inflammatory function, the extent to which LpPLA<sub>2</sub> can effect PAF breakdown in vivo remains uncertain (3,4).

Against such experimental observations is a large body of epidemiological studies that have predominantly found a positive association between LpPLA<sub>2</sub> mass and activity and cardiovascular outcomes (5). Few studies to date, however, have addressed the link between LpPLA<sub>2</sub> and clinical events in populations with

high prevalences of insulin resistance and diabetes, disorders where vascular inflammation and oxidative stress are particularly pronounced (6). We examined the relations between LpPLA<sub>2</sub> mass and activity and incident cardiovascular disease (CVD) in a population-based cohort of American Indians enriched with dys-metabolic phenotypes.

## RESEARCH DESIGN AND METHODS

### Study population

The Strong Heart Study (SHS) is a population-based longitudinal survey of CVD in 13 American Indian communities, as detailed previously (7). Herein, we conducted a nested, case-control study of participants without prevalent CVD or dialysis dependence at the second SHS exam ( $n = 2,941$ ). Prevalent CVD comprised definite coronary heart disease (CHD), definite stroke, congestive heart failure (CHF), and atrial fibrillation. The second SHS exam entailed clinical and laboratory evaluation between 1993 and 1995, as previously described (7). Case subjects consisted of 504 participants who developed CVD during follow-up through December 2003. Control subjects included participants who remained free of clinical CVD during this period and were frequency matched (1:1) to case subjects by age, sex, and diabetes status.

### Definition of clinical variables and determination of end points

Hypertension was defined as blood pressure  $\geq 140/90$  mmHg or antihypertensive therapy, diabetes as fasting glucose  $\geq 126$  mg/dL or glucose-lowering treatment, and BMI as the ratio of weight (kilograms) to the square of height (meters). Metabolic syndrome was defined according to National Cholesterol Education Program criteria (8). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin ( $\mu\text{U/mL}$ )  $\times$  fasting glucose (mmol/L)/22.5 (9). Insulin resistance was defined according to a previous classification scheme as BMI  $>28.9$  kg/m<sup>2</sup> and HOMA-IR  $>4.65$ , or BMI  $>27.5$  kg/m<sup>2</sup> and HOMA-IR  $>3.60$ , among participants without diabetes (10). Glomerular filtration rate was estimated (eGFR) using the modification of diet in renal disease equation (11).

The primary end point of major CVD events was defined as definite, nonfatal myocardial infarction (MI), CHD, or stroke and

cardiovascular death. Criteria for non-fatal CVD events have been previously described (7). Deaths were identified, verified, and classified as attributable to CVD if caused by MI, sudden death from CHD, stroke, or CHF using standardized procedures (7).

### Laboratory methods

LpPLA<sub>2</sub> mass and activity were measured at the SHS Core Laboratory in serum samples stored at  $-80^{\circ}\text{C}$ . In prior studies (5), determinations of LpPLA<sub>2</sub> mass and activity after long-term storage have yielded values whose distributions and correlations to other analytes have been consistent with recently drawn samples. LpPLA<sub>2</sub> mass was measured in duplicate from May to September 2008 on never-thawed specimens by ELISA (PLAC III; diaDexus, Inc.). The intra-assay and interassay coefficients of variation (CVs) were both 8%. These were determined by testing four human serum pools with LpPLA<sub>2</sub> concentrations distributed throughout the assay's calibration range. For intra-assay CV, the samples were tested 20 times on one plate. For interassay CV, the samples were tested in duplicate, two separate assays per day, for 5 days. In view of the unexpected association with CVD observed for LpPLA<sub>2</sub> mass, additional funding was requested for determination of LpPLA<sub>2</sub> activity, which was measured in the same samples previously thawed once for the LpPLA<sub>2</sub> mass determinations. LpPLA<sub>2</sub> activity was measured in singlicate in January 2011 using a colorimetric assay (diaDexus, Inc.) for hydrolysis of labeled PAF (GlaxoSmithKline, Research Triangle Park, NC). Intra-assay CVs were 1.3 and 0.8%, whereas interassay CVs were 2.0 and 1.0%, for low and high activity standards, respectively.

The concentrations of high-sensitivity C-reactive protein (hsCRP) and fibrinogen were determined by ELISA (12) and a modified Clauss method (13), respectively. HbA<sub>1c</sub> was assessed by high-pressure liquid chromatography (14). The urine albumin/creatinine ratio (UACR) was obtained from a single spot urine (7). Lipoprotein (a) mass, measured by immunoassay during the first SHS exam, was available in 710 participants (15).

### Statistical analysis

Categorical variables were compared by the  $\chi^2$  test, and continuous variables by the Wilcoxon rank sum test. Correlations were evaluated by Spearman coefficients. Effect modification of LpPLA<sub>2</sub>'s relations

with lipid fractions by diabetes was examined by including cross-product terms in linear regression models. Unconditional logistic regression was used to evaluate multivariable-adjusted associations of sex-specific tertiles of LpPLA<sub>2</sub> mass and activity with incident CVD. This involved fitting sequential models that adjusted first for age (continuous), sex, and diabetes (yes/no); and then additionally for hypertension (yes/no), BMI (continuous), LDL and HDL (both continuous), smoking status (current versus ever/never), and serum creatinine, UACR, and hsCRP (all continuous); and subsequently for HbA<sub>1c</sub> (continuous), diabetes duration (continuous), oral hypoglycemics (yes/no), and insulin therapy (yes/no). Tests for linear trend used an ordinal indicator variable for tertiles. Subsequent models also assessed alcohol use, antihypertensive medication, estrogen therapy, and fibrinogen, as well as exclusion of participants receiving lipid-lowering therapy. Moreover, associations were examined among participants with and without diabetes separately. We also assessed the impact of excluding participants with advanced kidney disease (eGFR  $<30$  mL/min/1.73 m<sup>2</sup>) and macroalbuminuria (UACR  $\geq 300$  mg/g). All analyses were conducted with SPSS version 19.0 (SPSS Inc., Chicago, IL).

## RESULTS

### Baseline characteristics

Of the 504 case subjects, 239 had fatal/nonfatal MI, 90 fatal/nonfatal stroke (87 ischemic), and 12 both; 175 had fatal/nonfatal CHD, 4 fatal CHF, and 8 other fatal CVD. Overall, 56.8% of the study cohort was obese; 72.6% had the metabolic syndrome; 21.1 and 65.5% had impaired fasting glucose and diabetes, respectively; 17.2 and 2.7% had eGFR  $<60$  and  $<30$  mL/min/1.73 m<sup>2</sup>; and 26.7 and 18.3% had micro- and macroalbuminuria. Diabetic participants had median HbA<sub>1c</sub> of 8.6% and duration of diabetes of 10 years; 33.6% were on insulin therapy. Of those without diabetes, 59.1% were insulin resistant. These and other characteristics are presented in Table 1 according to case/control subject status. Case subjects were similar to control subjects in age, sex, and diabetes as a result of frequency matching, but had greater hypertension, current smoking, insulin use, and duration of diabetes than control subjects. Case subjects also had higher lipids, serum creatinine, UACR, and fibrinogen.

Table 1—Baseline characteristics of study cohort

Characteristic	Case subjects (n = 504)	Control subjects (n = 504)	P
Age*, years	62 ± 8	61 ± 7	0.216
Women*, %	56.5	56.5	1.00
Diabetes*, %	65.5	65.5	1.00
Oral hypoglycemic therapy†, %	51.7	49.4	0.518
Insulin therapy†, %	57.7	42.3	0.010
Duration of diabetes†, years	13 (6–21)	7 (4–15)	<0.001
Impaired fasting glucose, %	21.0	21.2	0.912
Hypertension, %	54.4	44.6	0.002
BMI, kg/m <sup>2</sup>	31.1 ± 5.8	31.9 ± 7.0	0.067
Waist circumference, cm	107 ± 14	108 ± 16	0.131
Total cholesterol, mg/dL	194 ± 41	185 ± 37	<0.001
LDL cholesterol, mg/dL	120 ± 35	114 ± 31	0.007
HDL cholesterol, mg/dL	39 ± 13	41 ± 14	0.063
Triglycerides, mg/dL	144 (103–219)	129 (87–191)	<0.001
Total/HDL cholesterol	5.4 ± 1.9	4.9 ± 1.9	<0.001
Lipoprotein (a), mg/dL (first exam)	7.0 (2.0–19.0)	5.5 (3.0–16.0)	0.548
Lipid-lowering therapy, %	3.2	2.4	0.443
Metabolic syndrome, %	76.8	72.4	0.111
Metabolic syndrome‡, %	49.4	39.8	0.073
HOMA-IR‡	2.91 (1.73–5.26)	2.79 (1.69–5.03)	0.782
Insulin resistance‡, %	60.7	57.5	0.548
Current smoker, %	35.3	27.8	0.011
Alcohol consumption, %	31.5	36.9	0.068
Estrogen therapy, %	17.4	13.4	0.194
Serum creatinine, mg/dL	1.0 ± 0.7	0.9 ± 0.3	<0.001
eGFR, mL/min/1.73 m <sup>2</sup>	81 ± 57	85 ± 29	0.164
UACR, mg/g	37 (9–369)	16 (7–78)	<0.001
hsCRP, mg/L	4.1 (2.1–7.1)	3.8 (2.0–7.1)	0.501
Fibrinogen, mg/dL	386 ± 95	361 ± 82	<0.001
HbA <sub>1c</sub> , %	7.2 (5.5–9.6)	6.8 (5.5–9.2)	0.078

For continuous variables, values are given as mean ± SD or median (interquartile range). \*Matching factors. †In subgroup with diabetes. ‡In subgroup without diabetes.

### Levels and correlations of LpPLA<sub>2</sub> mass and activity

Valid measurements were obtained in 491 case subjects and 492 control subjects for LpPLA<sub>2</sub> mass, and 494 case subjects and 499 control subjects for LpPLA<sub>2</sub> activity. LpPLA<sub>2</sub> mass levels were lower in case subjects than control subjects (median 306 [IQR 235–409] vs. 342 [264–452] μg/L, *P* < 0.001), in women than men (317 [241–408] vs. 332 [245–450] μg/L, *P* = 0.049), and in participants with diabetes than without (313 [239–422] vs. 342 [264–438] μg/L, *P* = 0.034), but did not differ among nondiabetic participants with and without insulin resistance (338 [251–443] vs. 352 [267–440] μg/L, *P* = 0.523). LpPLA<sub>2</sub> activity was higher in case subjects than control subjects (228 [194–280] vs. 216 [178–260] μmol/min/L, *P* < 0.001), lower in women than men (209 [178–249] vs. 246 [199–290] μmol/min/L, *P* < 0.001), and similar in diabetic and

nondiabetic subjects (222 [188–271] vs. 223 [183–270] μmol/min/L, *P* = 0.676) or in nondiabetic individuals with and without insulin resistance (231 [188–276] vs. 213 [177–265] μmol/min/L, *P* = 0.156).

Correlations between LpPLA<sub>2</sub> measures and baseline covariates are shown in Table 2. For LpPLA<sub>2</sub> mass, there were modest positive correlations with total cholesterol and hsCRP in women, and with LDL in men. Men also exhibited modest negative correlations between LpPLA<sub>2</sub> mass and systolic blood pressure, serum creatinine, UACR, and, as did women, diabetes duration. In turn, LpPLA<sub>2</sub> activity showed modest positive correlations in women with age and HbA<sub>1c</sub>, and modest negative correlations with BMI and hsCRP. In men, the correlations of LpPLA<sub>2</sub> activity with BMI and waist circumference were instead modestly positive, whereas for systolic blood

pressure and fibrinogen, they were modestly negative. Men without diabetes showed a moderate positive correlation for LpPLA<sub>2</sub> activity with HOMA-IR, but those with diabetes exhibited a moderately negative correlation with diabetes duration. Moreover, there were moderately strong, positive correlations in both sexes between LpPLA<sub>2</sub> activity and total cholesterol, LDL, triglycerides, and total/HDL cholesterol, along with a moderate negative correlation with HDL. These correlations tended to be stronger in men than women. Furthermore, the correlations between LpPLA<sub>2</sub> mass and LpPLA<sub>2</sub> activity were moderate in both sexes.

When correlations were examined in subgroups with and without diabetes (Supplementary Table), modest correlations were observed only among diabetic participants for LpPLA<sub>2</sub> mass with hsCRP (positive), and with UACR, HbA<sub>1c</sub>, and diabetes duration (negative). By contrast, LpPLA<sub>2</sub> activity showed a modest negative correlation with hsCRP and fibrinogen, though not HbA<sub>1c</sub> in diabetic subjects, but was again negatively correlated with diabetes duration. LpPLA<sub>2</sub> activity was also modestly negatively correlated with UACR in nondiabetic subjects, in whom its correlations with lipid fractions tended to be stronger than among diabetic participants.

We next tested whether the associations of LpPLA<sub>2</sub> mass or activity with LDL, HDL, and total/HDL cholesterol, hsCRP, HbA<sub>1c</sub>, and UACR were modified by diabetes status in age- and sex-adjusted linear models. There was no significant effect modification by diabetes (*P* ≥ 0.13) except in the case of LpPLA<sub>2</sub> activity's relation to total/HDL cholesterol (*P* = 0.007), for which the partial coefficient was modestly higher for nondiabetic than diabetic participants (*r* = 0.59 vs. 0.47). These findings were not influenced by diabetes medications.

### LpPLA<sub>2</sub> mass and outcome

Table 3 shows the relationship between tertiles of LpPLA<sub>2</sub> mass and major CVD. There was a significant inverse association across increasing tertiles of LpPLA<sub>2</sub> mass with incident CVD after adjustment for matching variables, which persisted after controlling for other clinical and laboratory covariates. After full adjustment for potential confounders, there was a 33% lower RR of CVD for the middle versus the lower tertile (*P* = 0.022), and a 45% lower risk for the upper versus the lower tertile (*P* = 0.001). Findings were similar

Table 2—Cross-sectional correlates\* of LpPLA<sub>2</sub> mass and activity

Clinical covariates	LpPLA <sub>2</sub> mass		LpPLA <sub>2</sub> activity	
	Women	Men	Women	Men
Age	0.01 (0.750)	−0.03 (0.538)	0.09 (0.032)	−0.07 (0.131)
BMI	−0.08 (0.068)	−0.04 (0.397)	−0.10 (0.025)	0.15 (0.002)
Waist circumference	−0.04 (0.366)	−0.05 (0.291)	−0.06 (0.129)	0.12 (0.013)
Systolic blood pressure	0.07 (0.122)	−0.12 (0.015)	0.04 (0.396)	−0.14 (0.004)
Diastolic blood pressure	0.08 (0.048)	−0.06 (0.247)	0.06 (0.156)	−0.01 (0.872)
Total cholesterol	0.09 (0.036)	0.09 (0.054)	0.31 (<0.001)	0.41 (<0.001)
LDL cholesterol	0.02 (0.659)	0.15 (0.003)	0.30 (<0.001)	0.49 (<0.001)
HDL cholesterol	0.05 (0.243)	0.003 (0.943)	−0.31 (<0.001)	−0.40 (<0.001)
Triglycerides	0.03 (0.504)	−0.02 (0.736)	0.23 (<0.001)	0.32 (<0.001)
Total/HDL cholesterol	0.02 (0.715)	0.07 (0.148)	0.45 (<0.001)	0.60 (<0.001)
Lipoprotein (a)†	0.02 (0.856)	0.03 (0.536)	0.05 (0.290)	−0.03 (0.571)
Serum creatinine	−0.07 (0.085)	−0.10 (0.039)	0.04 (0.336)	0.05 (0.322)
UACR	−0.06 (0.136)	−0.13 (0.015)	0.05 (0.271)	−0.09 (0.080)
hsCRP	0.09 (0.037)	0.07 (0.174)	−0.08 (0.048)	0.03 (0.523)
HOMA-IR‡	−0.02 (0.803)	−0.01 (0.879)	0.11 (0.206)	0.23 (0.001)
Fibrinogen	−0.06 (0.183)	−0.03 (0.599)	−0.04 (0.316)	−0.11 (0.028)
HbA <sub>1c</sub>	−0.07 (0.089)	−0.07 (0.160)	0.11 (0.009)	0.05 (0.346)
Duration of diabetes§	−0.12 (0.020)	−0.21 (0.001)	−0.02 (0.636)	−0.20 (0.003)
LpPLA <sub>2</sub> mass	—	—	0.30 (<0.001)	0.29 (<0.001)
LpPLA <sub>2</sub> activity	0.30 (<0.001)	0.29 (<0.001)	—	—

\*Values are for correlation coefficients and *P* in parentheses. †In a subset of participants from the first SHS exam. ‡In participants without diabetes. §In participants with diabetes.

when waist circumference replaced BMI, total/HDL cholesterol replaced LDL and HDL, and eGFR replaced serum creatinine. Nor were effect estimates influenced by additional adjustment for antihypertensive medication, alcohol, estrogen therapy, fibrinogen, or lipoprotein (a) (data not shown). Moreover, exclusion of participants with kidney disease did not affect the results.

A similar relationship was observed for LpPLA<sub>2</sub> mass when the outcome was limited to MI (Table 3). For ischemic stroke, risk estimates were also suggestive of a negative association, but there was lack of precision reflecting fewer events.

Likewise, when analyses were stratified by diabetes status, there was an inverse association among diabetic participants that was comparable to that seen overall (Table 4). The relationship was similar among participants without diabetes at different levels of adjustment, although it became nonsignificant after inclusion of HOMA-IR. To further assess the influence of dysglycemia on the inverse relationship, we explored the age- and sex-adjusted association among participants without diabetes or insulin resistance. As in nondiabetic participants, risk estimates were attenuated as compared with age- and sex-adjusted values in diabetic subjects, albeit with broad 95% CIs.

### LpPLA<sub>2</sub> activity and outcome

As also shown in Table 3, LpPLA<sub>2</sub> activity manifested the opposite association with incident CVD, with a significant graded increase in risk across increasing tertiles. The association was attenuated after adjustment for clinical and laboratory factors, but there was still a 65% higher risk of major CVD for the upper tertile in the fully adjusted model (*P* = 0.012). These findings were not meaningfully altered with consideration of waist circumference, total/HDL cholesterol, or eGFR as alternative covariates. Nor did adjustment for additional baseline covariates or exclusion of participants with kidney disease materially affect results.

The association of LpPLA<sub>2</sub> activity with MI alone was also positive, with numerically stronger risk estimates than for the composite CVD end point (Table 3). A stronger positive association was also present for ischemic stroke, but precision was again low.

Among diabetic participants, the association between LpPLA<sub>2</sub> activity and major CVD was similar to the entire cohort's. The risk estimates strengthened, however, with further adjustment for diabetes duration, HbA<sub>1c</sub>, and diabetes medications. Likewise, a significant positive association was observed in the nondiabetic subset after adjustment for

matching factors. The relationship was attenuated and ceased to be significant, however, with more extensive adjustment in this smaller subgroup or in its subset without insulin resistance.

## CONCLUSIONS

### Main findings

In this cohort with high prevalences of glycometabolic abnormalities, we found paradoxical associations between LpPLA<sub>2</sub> mass and activity and first-ever CVD. Specifically, LpPLA<sub>2</sub> mass and LpPLA<sub>2</sub> activity showed directionally opposite relationships with incident CVD, wherein LpPLA<sub>2</sub> mass was inversely associated, but LpPLA<sub>2</sub> activity was positively associated, with this outcome. These associations did not show significant differences between subgroups with and without diabetes and were, moreover, directionally consistent for the component end points of MI and ischemic stroke.

### Previous studies of LpPLA<sub>2</sub> and CVD events in diabetes

Although previous population-based studies of LpPLA<sub>2</sub> and CVD have included variable proportions of participants with diabetes, few investigations have focused on cohorts composed exclusively or predominantly of individuals with dysglycemia. In a study that combined male and female diabetic participants in the Health Professionals Follow-up Study and the Nurses' Health Study, there was a significant positive association between LpPLA<sub>2</sub> activity and incident CHD, but no significant relationship was detected for LpPLA<sub>2</sub> mass (16). Interestingly, the correlation between LpPLA<sub>2</sub> mass and activity in that subgroup of white health professionals was identical to the one observed in our cohort, and their adjusted risk estimate for the comparison of extreme tertiles of LpPLA<sub>2</sub> activity for nonfatal MI and fatal CHD was of similar, if modestly lower, magnitude to ours for the same outcome (RR 1.75 [95% CI 1.05–2.92] vs. 1.95 [1.22–3.09]). Because the risk estimates of LpPLA<sub>2</sub> mass for CHD were not reported (16), however, we are unable to compare the relationship therein with the inverse association observed here.

A second study also examined the prospective association of LpPLA<sub>2</sub> mass and activity with CVD specifically in a diabetic subset (17). In this sample of mostly white older adults from the Cardiovascular Health Study, no significant association between LpPLA<sub>2</sub> mass or

Table 3—Associations of tertiles of LpPLA<sub>2</sub> mass and activity with CVD

	LpPLA <sub>2</sub> mass (μg/L)				LpPLA <sub>2</sub> activity (μmol/min/L)			
	Tertile 1	Tertile 2	Tertile 3	P <sub>Trend</sub>	Tertile 1	Tertile 2	Tertile 3	P <sub>Trend</sub>
Level* in women	225 (82–267)	317 (267–376)	462 (376–1,000)		168 (45–190)	209 (190–235)	269 (235–522)	
Level* in men	229 (51–273)	331 (273–415)	551 (415–1,000)		184 (102–216)	246 (216–276)	309 (276–457)	
Major CVD								
Model 1								
OR (95% CI)	Referent	0.69 (0.51–0.94)	0.56 (0.41–0.77)	<0.001	Referent	1.38 (1.02–1.88)	1.86 (1.36–2.53)	<0.001
P		0.019	<0.001			0.039	<0.001	
Model 2								
OR (95% CI)	Referent	0.65 (0.46–0.91)	0.53 (0.38–0.75)	<0.001	Referent	1.21 (0.86–1.72)	1.64 (1.11–2.40)	0.012
P		0.011	<0.001			0.277	0.012	
Model 3								
OR (95% CI)	Referent	0.67 (0.48–0.94)	0.55 (0.39–0.79)	0.001	Referent	1.21 (0.85–1.72)	1.65 (1.12–2.42)	0.011
P		0.022	0.001			0.294	0.012	
Myocardial infarction								
Model 1								
OR (95% CI)	Referent	0.69 (0.47–1.01)	0.57 (0.39–0.84)	0.004	Referent	1.59 (1.07–2.37)	2.36 (1.59–3.49)	<0.001
P		0.057	0.004			0.022	<0.001	
Model 2								
OR (95% CI)	Referent	0.64 (0.42–0.98)	0.54 (0.35–0.84)	0.006	Referent	1.37 (0.86–2.18)	2.09 (1.27–3.43)	0.003
P		0.040	0.005			0.180	0.004	
Model 3								
OR (95% CI)	Referent	0.63 (0.41–0.97)	0.53 (0.34–0.82)	0.005	Referent	1.33 (0.83–2.11)	2.00 (1.21–3.29)	0.006
P		0.034	0.004			0.234	0.007	
Ischemic stroke†								
Model 1								
OR (95% CI)	Referent	0.68 (0.38–1.23)	0.87 (0.50–1.50)	0.675	Referent	2.25 (1.25–4.08)	2.16 (1.16–4.01)	0.015
P		0.205	0.616			0.007	0.015	
Model 2								
OR (95% CI)	Referent	0.69 (0.36–1.31)	0.82 (0.45–1.50)	0.578	Referent	2.47 (1.28–4.78)	2.18 (1.01–4.69)	0.050
P		0.253	0.525			0.007	0.047	

OR, odds ratio. Model 1 adjusted for age, sex, and diabetes. Model 2 adjusted for age, sex, diabetes, hypertension, BMI, LDL, HDL, current smoking, serum creatinine, UACR, and hsCRP. Model 3 adjusted for covariates in model 2 plus diabetes duration, HbA<sub>1c</sub>, oral hypoglycemic treatment, and insulin therapy. \*Median (range). †Model 3 was not run for the ischemic stroke end point owing to insufficient number of events for model fitting.

activity and CVD, including CHF, was observed (adjusted RRs of 1.24 [95% CI 0.90–1.71] and 0.95 [0.69–1.32], respectively, for values above versus below the 75th percentile) (17).

Data from the above investigations, however, were included in the LpPLA<sub>2</sub> Studies Collaboration, a meta-analysis of prospective studies that also evaluated cohorts with prevalent CVD (5). Pooling of the findings from diabetic participants across studies (*n* = 8,427 participants; 18% of total) revealed a significant positive association between LpPLA<sub>2</sub> mass and CHD (RR 1.16 per SD [95% CI 1.05–1.28]), which was comparable to that observed in the larger sample without diabetes (5).

### Potential explanations for findings

The inverse association between LpPLA<sub>2</sub> mass and CVD detailed here is unanticipated. Not only is it divergent from the

positive relationship documented for LpPLA<sub>2</sub> activity in the same cohort, but also runs counter to available epidemiological evidence of positive or null associations (5). Such evidence, however, comes from the predominantly nondiabetic populations of European descent included in the LpPLA<sub>2</sub> Studies Collaboration, although subgroup analysis of diabetic participants showed a consistent association (5). Our American Indian population differs from such cohorts in its greater severity and duration of diabetes, and in its predominance of women, rather than men. The current study also excluded prevalent CVD, which did not significantly influence the overall associations of LpPLA<sub>2</sub> mass with outcomes in the above meta-analysis but was associated with higher risk estimates, particularly for vascular death (5).

A central consideration in attempting to account for our findings is that, much

as LpPLA<sub>2</sub> function is closely dependent on the type of lipoprotein to which it is complexed (18,19), the measurable fraction of LpPLA<sub>2</sub> mass may vary for different lipoprotein particles (19). Specifically, immunoassay methods for determination of LpPLA<sub>2</sub> mass appear not to detect all active enzyme in LDL, as they do for HDL (19). The same problem, however, does not apply to LpPLA<sub>2</sub> activity, plasma measurement of which is not influenced by its companion lipoprotein.

Approximately 75% of plasma LpPLA<sub>2</sub> circulates bound to apolipoprotein-B-containing particles, principally LDL, with the remainder associating with HDL. Furthermore, the enzyme has particular affinity for smaller LDL particles (20,21), levels of which are increased in the setting of insulin resistance and type 2 diabetes (22). We observed that LpPLA<sub>2</sub> mass, but not activity, was lower in participants with diabetes than without in our

Table 4—Associations of tertiles of LpPLA<sub>2</sub> mass and activity with CVD in subgroups with and without diabetes

	LpPLA <sub>2</sub> mass				LpPLA <sub>2</sub> activity			
	Tertile 1	Tertile 2	Tertile 3	P <sub>Trend</sub>	Tertile 1	Tertile 2	Tertile 3	P <sub>Trend</sub>
Diabetes (n = 660)								
Major CVD								
Model 1								
OR (95% CI)	Referent	0.64 (0.44–0.94)	0.54 (0.37–0.79)	0.001	Referent	1.59 (1.08–2.35)	1.83 (1.24–2.69)	0.003
P		0.023	0.001			0.019	0.002	
Model 2								
OR (95% CI)	Referent	0.57 (0.37–0.88)	0.53 (0.35–0.81)	0.003	Referent	1.47 (0.94–2.30)	1.79 (1.11–2.90)	0.018
P		0.010	0.003			0.088	0.017	
Model 3								
OR (95% CI)	Referent	0.61 (0.39–0.95)	0.59 (0.38–0.91)	0.016	Referent	1.57 (0.99–2.48)	2.00 (1.22–3.27)	0.006
P		0.028	0.017			0.053	0.006	
No diabetes (n = 348)								
Major CVD								
Model 1								
OR (95% CI)	Referent	0.79 (0.46–1.34)	0.62 (0.36–1.06)	0.078	Referent	1.06 (0.63–1.77)	2.03 (1.20–3.43)	0.011
P		0.382	0.079			0.830	0.008	
Model 2								
OR (95% CI)	Referent	0.78 (0.44–1.39)	0.54 (0.30–0.98)	0.040	Referent	0.90 (0.50–1.61)	1.42 (0.73–2.78)	0.321
P		0.406	0.042			0.719	0.306	
Model 3								
OR (95% CI)	Referent	0.80 (0.45–1.44)	0.57 (0.31–1.03)	0.061	Referent	0.85 (0.47–1.55)	1.52 (0.76–3.05)	0.260
P		0.459	0.063			0.600	0.239	
No diabetes or insulin resistance* (n = 137)								
Major CVD								
Model 1								
OR (95% CI)	Referent	0.84 (0.36–2.00)	0.65 (0.27–1.57)	0.327	Referent	1.17 (0.54–2.52)	1.46 (0.58–3.70)	0.424
P		0.697	0.333			0.694	0.424	

OR, odds ratio. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, hypertension, BMI, LDL, HDL, current smoking, serum creatinine, UACR, and hsCRP. Model 3 adjusted for covariates in model 2 plus diabetes duration, HbA<sub>1c</sub>, oral hypoglycemic therapy, and insulin therapy in subgroup with diabetes or HOMA-IR in subgroup without diabetes. \*Models 2 and 3 were not run in the subgroup without diabetes or insulin resistance owing to an insufficient number of CVD events (n = 66) for model fitting.

cohort, consistent with prior findings from the LpPLA<sub>2</sub> Studies Collaboration, which also documented lower levels of LpPLA<sub>2</sub> mass in diabetic individuals (5). Because redistribution of LpPLA<sub>2</sub> to small LDL particles might amplify its proatherogenic effects, yet make enzyme mass less available for detection by immunoassay, such redistribution could provide an explanation for the divergent associations observed for LpPLA<sub>2</sub> mass and activity. The higher the absolute level of small LDL particles and the proportion of LpPLA<sub>2</sub> complexed with them, the lower the measurable concentration of LpPLA<sub>2</sub> mass but the greater the measurable LpPLA<sub>2</sub> activity, and with it, the heightened atherogenicity resulting from increased concentrations of LpPLA<sub>2</sub>–small-LDL complexes. This explanation might seem at odds with the weak correlation between LpPLA<sub>2</sub> mass and total LDL observed here in men only, but LpPLA<sub>2</sub> mass, unlike activity, has been

reported to be a poor measure of smaller LDL particles (19).

Lower levels of LpPLA<sub>2</sub> mass were also reported in a cross-sectional comparison of patients with diabetes and healthy control subjects who underwent determinations of carotid intima-media thickness (23). Of further relevance to our findings, LpPLA<sub>2</sub> mass (activity was not measured) was positively associated with carotid intima-media thickness in control subjects, but not among diabetic patients (23). Previous studies have documented a redistribution of LpPLA<sub>2</sub> toward HDL in the setting of diabetes (19,24), and the observation that LpPLA<sub>2</sub> mass was lower in diabetic than nondiabetic subjects for any given level of total/HDL cholesterol was deemed consistent with such redistribution as a potential explanation for the findings in that study (23). Redistribution of LpPLA<sub>2</sub> to HDL is difficult to invoke as an explanation for our results, however, given the nonexistent correlation

documented between LpPLA<sub>2</sub> mass and HDL. The lack of correlation between LpPLA<sub>2</sub> mass and lipid fractions in our cohort remains puzzling, and suggests the need for future studies to isolate lipoprotein subclasses and determine their respective LpPLA<sub>2</sub> content in the context of dysglycemia.

An additional consideration is that determination of LpPLA<sub>2</sub> activity is based on the rate of hydrolysis of PAF in vitro, yet several other plasma enzymes, such as lecithin-cholesterol acyltransferase, are capable of hydrolyzing PAF (25). Because such catalytic activity may be upregulated in the setting of heightened oxidative stress (26), a hallmark of insulin resistance and diabetes (6), it is possible that the disjunction between the associations of LpPLA<sub>2</sub> mass and activity observed here could reflect contributions of enzymes beyond LpPLA<sub>2</sub> itself.

Apart from these possibilities, we considered the potential influence of

medications, which have been shown to affect LpPLA<sub>2</sub> levels (27), but these had no measurable impact on our findings. Nor did the presence of kidney disease appear to account for our results. Finally, we entertained the possibility that redistribution to lipoprotein (a), a carrier of oxidized phospholipids for which LpPLA<sub>2</sub> also has close affinity (28), could explain our findings. Given the low concentration of lipoprotein (a) in American Indians (15), its lack of correlation with LpPLA<sub>2</sub> mass or activity, and the absence of a detectable influence on the associations observed, this does not appear likely.

The premise that the inverse association with CVD observed for LpPLA<sub>2</sub> mass relates to the dysmetabolic phenotype of our population is partly supported by the negative, if modest, correlations observed for LpPLA<sub>2</sub> mass with HbA<sub>1c</sub> and disease duration among diabetic participants. Although the inverse LpPLA<sub>2</sub> mass–CVD relationship was attenuated in the subgroup without diabetes and insulin resistance, the RRs were still <1.0, which does not provide evidence of a strong gradient across categories of dysglycemic severity. Yet even the nondiabetic, noninsulin-resistant subgroup was characterized by considerable prevalences of impaired fasting glucose (43.1%), metabolic syndrome (16.8%), eGFR <60 mL/min/1.73 m<sup>2</sup> (13.1%), and albuminuria (12.4%). These observations suggest that not only hyperglycemia per se but also insulin resistance and oxidative stress, or other as yet undefined factors, may be responsible for the findings in this cohort.

### Limitations

Several limitations must be acknowledged. First, we did not have determinations of lipoprotein subclasses, or apolipoprotein levels themselves, which could have helped to elucidate the basis for the associations observed. Second, despite a focus on atherosclerotic/thrombotic events, we did include fatal CHF in the primary end point to avoid misclassification of deaths from unrecognized ischemic CHF. This was a minor constituent of the primary outcome, however, and relations with specific atherothrombotic components proved directionally consistent with the main results. Last, our findings derive from a population of American Indians with more pervasive and severe glycometabolic abnormalities than studied heretofore. These results at the upper end of the cardiometabolic risk spectrum are at variance with the preponderance of existing

data for LpPLA<sub>2</sub> mass and do not necessarily apply to other ethnic groups. Nevertheless, many prior reports from SHS (29–31) have yielded risk factor associations that have been subsequently confirmed in other populations at high cardiometabolic risk.

In this cohort of American Indians with highly prevalent glycometabolic disturbances, LpPLA<sub>2</sub> mass and activity exhibited opposite relationships with incident CVD. LpPLA<sub>2</sub> mass showed an inverse independent association and LpPLA<sub>2</sub> activity a positive independent association with this outcome. The present findings in a cohort enriched with dysmetabolic phenotypes are of particular importance in light of the widening epidemics of obesity and diabetes affecting modern societies (32,33), and will require independent replication in similar populations. In the context of ongoing randomized trials assessing the potential clinical benefits of pharmacologic inhibition of LpPLA<sub>2</sub>, our findings support measurement of LpPLA<sub>2</sub> activity, instead of mass, to guide identification of subjects with increased LpPLA<sub>2</sub>-associated risk in view of the greater consistency of its relationship with CVD across the spectrum of cardiometabolic risk.

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J.R.K. participated in study conception and design, acquired data, performed statistical analysis, interpreted data, drafted the manuscript, and critically revised the manuscript for important intellectual content. J.G.U. and B.V.H. participated in study conception and design, acquired data, interpreted data, and critically revised the manuscript for important intellectual content. J.Z., R.B.D., and E.T.L. acquired data and critically revised the manuscript for important intellectual content. R.L.W. interpreted data and critically revised the manuscript for important intellectual content. J.R.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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