Lipoprotein-Associated Phospholipase A₂ Mass and Activity and Risk of Cardiovascular Disease in a Population With High Prevalences of Obesity and Diabetes

The Strong Heart Study

OBJECTIVE—To investigate the association of lipoprotein-associated phospholipase A₂ (LpPLA₂) 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. LpPLA₂ mass and activity were measured using commercially available assays.

RESULTS—LpPLA₂ mass and activity were moderately correlated with each other (r = 0.30), but only LpPLA₂ activity exhibited moderate correlations with lipid fractions. After extensive adjustment for covariates, both LpPLA₂ measures were significantly associated with incident CVD, but the relationship was inverse for LpPLA₂ mass (highest versus lowest tertile, relative risk [RR] 0.55 [95% CI 0.39–0.79]) and positive for LpPLA₂ 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, LpPLA₂ mass and activity showed divergent associations with CVD. The inverse relationship for LpPLA₂ mass is contrary to observations from predominantly nondiabetic populations and will require independent replication. Whether this finding relates to redistribution of LpPLA₂ to lipoprotein classes where it is less atherogenic or reflects incomplete measurement of LpPLA₂ mass associated with altered lipoprotein composition in insulin resistance warrants further investigation.
high prevalences of insulin resistance and diabetes, disorders where vascular inflammation and oxidative stress are particularly pronounced (6). We examined the relations between LpPLA2 mass and activity and incident cardiovascular disease (CVD) in a population-based cohort of American Indians enriched with dysmetabolic 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 ≥140/90 mmHg or antihypertensive therapy, diabetes as fasting glucose ≥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 (µU/mL) × fasting glucose (mmol/L)/22.5 (9). Insulin resistance was defined according to a previous classification scheme as BMI >28.9 kg/m² and HOMA-IR >4.65, or BMI >27.5 kg/m² 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 nonfatal 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**
LpPLA2 mass and activity were measured at the SHS Core Laboratory in serum samples stored at −80°C. In prior studies (5), determinations of LpPLA2 mass and activity after long-term storage have yielded values whose distributions and correlations to other analytes have been consistent with recently drawn samples. LpPLA2 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 LpPLA2 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 LpPLA2 mass, additional funding was requested for determination of LpPLA2 activity, which was measured in the same samples previously thawed once for the LpPLA2 mass determinations. LpPLA2 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 Claus method (13), respectively. HbA1c 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 χ² test, and continuous variables by the Wilcoxon rank sum test. Correlations were evaluated by Spearman coefficients. Effect modification of LpPLA2’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 LpPLA2 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 HbA1c (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 m²) and macroalbuminuria (UACR ≥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 m²; and 26.7 and 18.3% had micro- and macroalbuminuria. Diabetes participants had median HbA1c 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 higher hypertension, current smoking, insulin use, and duration of diabetes than control subjects. Case subjects also had higher lipids, serum creatinine, UACR, and fibrinogen.
with and without insulin resistance (338 and 334 vs. 338 patients). LpPLA2 activity was higher in patients without diabetes than control subjects (median 254 [194–438] vs. 222 [321–340] mmol/min/L, P = 0.001). Findings were similar for LpPLA2 mass 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 LpPLA2 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 LpPLA2 mass and LpPLA2 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 LpPLA2 mass with hsCRP (positive), and with UACR, HbA1c, and diabetes duration (negative). By contrast, LpPLA2 activity showed a modest negative correlation with hsCRP and fibrinogen, though not HbA1c in diabetic subjects, but was again negatively correlated with diabetes duration. LpPLA2 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 LpPLA2 mass or activity with LDL, HDL, and total/HDL cholesterol, hsCRP, HbA1c, 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 LpPLA2 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.

**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 (4–13)                  | <0.001|
| Impaired fasting glucose, %     | 21.0                   | 21.2                      | 0.912 |
| Hypertension, %                 | 54.4                   | 44.6                      | 0.082 |
| BMI, kg/m²                      | 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 m²           | 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|
| HbA1c, %                        | 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 LpPLA2 mass and activity**

Valid measurements were obtained in 491 case subjects and 492 control subjects for LpPLA2 mass, and 494 case subjects and 499 control subjects for LpPLA2 activity. LpPLA2 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). LpPLA2 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 LpPLA2 measures and baseline covariates are shown in Table 2. For LpPLA2 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 LpPLA2 mass and systolic blood pressure, serum creatinine, UACR, and, as did women, diabetes duration. In turn, LpPLA2 activity showed modest positive correlations in women with age and HbA1c, and modest negative correlations with BMI and hsCRP. In men, the correlations of LpPLA2 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 LpPLA2 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 LpPLA2 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 LpPLA2 mass and LpPLA2 activity were moderate in both sexes.

**LpPLA2 mass and outcome**

Table 3 shows the relationship between tertiles of LpPLA2 mass and major CVD. There was a significant inverse association across increasing tertiles of LpPLA2 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.
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 LpPLA2 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 non-diabetic participants, risk estimates were attenuated as compared with age- and sex-adjusted values in diabetic subjects, albeit with broad 95% CIs.

**LpPLA2 activity and outcome**

As also shown in Table 3, LpPLA2 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 LpPLA2 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 LpPLA2 activity and major CVD was similar to the entire cohort’s. The risk estimates strengthened, however, with further adjustment for diabetes duration, HbA$_1c$, and diabetes medications. Likewise, a significant positive association was observed in the non-diabetic 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 LpPLA2 mass and activity and first-ever CVD. Specifically, LpPLA2 mass and LpPLA2 activity showed directionally opposite relationships with incident CVD, wherein LpPLA2 mass was inversely associated, but LpPLA2 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 LpPLA2 and CVD events in diabetes**

Although previous population-based studies of LpPLA2 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 LpPLA2 activity and incident CHD, but no significant relationship was detected for LpPLA2 mass (16). Interestingly, the correlation between LpPLA2 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 LpPLA2 activity for non-fatal 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 LpPLA2 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 LpPLA2 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 LpPLA2 mass or
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 LpPLA2 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 LpPLA2 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 LpPLA2 mass and CVD detailed here is unanticipated. Not only is it divergent from the positive relationship documented for LpPLA2 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 LpPLA2 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 LpPLA2 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 LpPLA2 function is closely dependent on the type of lipoprotein to which it is complexed (18,19), the measurable fraction of LpPLA2 mass may vary for different lipoprotein particles (19). Specifically, immunosassay methods for determination of LpPLA2 mass appear not to detect all enzymatically active enzyme in LDL, as they do for HDL (19). The same problem, however, does not apply to LpPLA2 activity, plasma measurement of which is not influenced by its companion lipoprotein.

Approximately 75% of plasma LpPLA2 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 LpPLA2 mass, but not activity, was lower in participants with diabetes than without in our

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**Table 3—Associations of tertiles of LpPLA2 mass and activity with CVD**

|                     | LpPLA2 mass (µg/L) |                     | LpPLA2 activity (µmol/min/L) |                     |
|---------------------|-------------------|-------------------|-------------------------------|-------------------|
|                     | Tertile 1         | Tertile 2         | Tertile 3 | $P_{\text{Trend}}$ | Tertile 1         | Tertile 2         | Tertile 3 | $P_{\text{Trend}}$ |
| **Level* in women** |                   |                   |                     |                   |                   |                   |                     |
| 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, HbA1c, 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.

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LpPLA2 in people at high cardiometabolic risk

Table 4—Associations of tertiles of LpPLA2 mass and activity with CVD in subgroups with and without diabetes

|                     | LpPLA2 mass |                         | LpPLA2 activity |                         |
|---------------------|-------------|-------------------------|-----------------|-------------------------|
|                     | Tertile 1   | Tertile 2 | Tertile 3 | \(P_{\text{Trend}}\) | Tertile 1 | Tertile 2 | Tertile 3 | \(P_{\text{Trend}}\) |
| 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, HbA1c, 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.

The results reported here are consistent with prior findings from LpPLA2 Studies Collaboration, which also documented lower levels of LpPLA2 mass in diabetic individuals (5). Because redistribution of LpPLA2 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 LpPLA2 mass and activity. The higher the absolute level of small LDL particles and the proportion of LpPLA2 complexed with them, the lower the measurable concentration of LpPLA2 mass but the greater the measurable LpPLA2 activity, and with it, the heightened atherogenicity resulting from increased concentrations of LpPLA2—small-LDL complexes. This explanation might seem at odds with the weak correlation between LpPLA2 mass and total LDL observed here in men only, but LpPLA2 mass, unlike activity, has been reported to be a poor measure of smaller LDL particles (19).

Lower levels of LpPLA2 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, LpPLA2 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 LpPLA2 toward HDL in the setting of diabetes (19,24), and the observation that LpPLA2 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 LpPLA2 to HDL is difficult to invoke as an explanation for our results, however, given the nonexistent correlation documented between LpPLA2 mass and HDL. The lack of correlation between LpPLA2 mass and lipid fractions in our cohort remains puzzling, and suggests the need for future studies to isolate lipoprotein subclasses and determine their respective LpPLA2 content in the context of dysglycemia.

An additional consideration is that determination of LpPLA2 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 LpPLA2 mass and activity observed here could reflect contributions of enzymes beyond LpPLA2 itself.

Apart from these possibilities, we considered the potential influence of...
medications, which have been shown to affect LpPLA2 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 LpPLA2 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 LpPLA2 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 LpPLA2 mass relates to the dysmetabolic phenotype of our population is partly supported by the negative, if modest, correlations observed for LpPLA2 mass with HbA1c and disease duration among diabetic participants. Although the inverse LpPLA2 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 m² (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 LpPLA2 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, LpPLA2 mass and activity exhibited opposite relationships with incident CVD. LpPLA2 mass showed an inverse independent association and LpPLA2 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 LpPLA2, our findings support measurement of LpPLA2 activity, instead of mass, to guide identification of subjects with increased LpPLA2-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. The authors thank the SHS participants, staff, and coordinators.

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