Lipoprotein-Associated Phospholipase A_2 Activity and Incident Coronary Heart Disease Among Men and Women With Type 2 Diabetes

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OBJECTIVE—Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has been shown to be associated with increased risk of coronary heart disease (CHD) in general adult populations. Because men and women with type 2 diabetes are at particularly high risk for CHD, the aim of this study was to assess the association of Lp-PLA_2 with future coronary events among diabetic men and women.

RESEARCH DESIGN AND METHODS—We measured Lp-PLA_2 activity among 740 men and 777 women with confirmed diabetes enrolled in the Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS). Participants were free of all cardiovascular disease and cancer at baseline.

RESULTS—During 10 years of follow-up among men and 14 years among women, we documented 178 and 146 cases of CHD, respectively. We defined CHD as coronary artery bypass graft, angioplasty, nonfatal myocardial infarction, and fatal CHD. After adjustment for age, smoking, medical history, and biomarkers including C-reactive protein, HDL, and LDL, the relative risk of total CHD comparing extreme tertiles of Lp-PLA_2 was 1.39 (95% CI 1.01–1.90; P trend = 0.03). When we restricted analyses to only nonfatal myocardial infarction and fatal CHD, the relative risk was 1.75 (95% CI 1.05–2.92; P for trend = 0.001). LDL, HDL, C-reactive protein, hormone replacement therapy use, and diabetes duration did not modify these relationships.

CONCLUSIONS—Levels of Lp-PLA_2 activity were significantly associated with incident CHD among men and women with type 2 diabetes, independent of traditional and inflammatory risk factors. This positive association was strongest for more severe clinical end points. ***Diabetes*** **59:1239–1243, 2010**

Diabetic subjects have a two- to fourfold increased risk of heart disease (1), but the mechanism through which this increased risk is mediated is not fully understood. Inflammatory processes have been increasingly recognized as a critical step in the pathogenesis of both diabetes and heart disease and may offer a biological link between the two diseases (2). One newly recognized inflammatory biomarker is lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an enzyme that may influence atherogenesis and plaque rupture without altering the general immune response (3).

Recent evidence from large long-term epidemiological studies suggests that elevated levels of Lp-PLA_2 are associated with risk of coronary heart disease (CHD) (4–10). To date, no study has had sufficient power to examine this association among diabetic subjects, who may represent a particularly high-risk group. The aim of this study is to examine the effect of Lp-PLA_2 activity on future coronary heart disease among male and female type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS

Study population. The Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS) are prospective cohort studies of 51,529 men and 121,700 women, respectively. The present study includes 740 men and 777 women with diabetes who were free of cancer and cardiovascular disease at blood draw, and who had detectable Lp-PLA_2 activity values. These cohorts have been described in detail elsewhere (11,12).

Definition of diabetes and cardiovascular end points. Participants who indicated a diagnosis of diabetes on any biennial follow-up questionnaire were sent a supplementary questionnaire to confirm diabetes. We used the National Diabetes Data Group criteria (13) to define diabetes because our participants were diagnosed before the American Diabetes Association released their criteria in 1997. Diabetes was confirmed in subsamples from the HPFS (97% confirmed) and the NHS (98% confirmed) (14,15).

CHD end points were coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty (CABG/PTCA), nonfatal myocardial infarction (MI), and fatal CHD. If participants experienced multiple CHD events during follow-up, they were assigned the more severe clinical event, but censored at the date of the first event. Further information about diabetes and CHD diagnoses has been provided previously (11,12) and is provided in the supplementary information (available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0730/DC1).

Blood collection and laboratory measurements. Lp-PLA_2 activity was measured by a colorimetric activity test automated assay performed on a clinical chemistry analyzer using a colorimetric substrate for Lp-PLA_2 (7). All samples were run in duplicate with a mean coefficient of variation (CV) of 4.9%. Lp-PLA_2 concentration was measured with an enzyme-linked immunoassay (PLAC test; diaDexus, San Francisco, CA). Other biomarker measurements are described in the supplementary methods. In this study, there was a moderate and statistically significant correlation between concentration and activity ($r = 0.30$, $P < 0.001$, among women; $r = 0.29$, $P < 0.001$, among men). In preliminary analyses, Lp-PLA_2 concentration was not associated with incident coronary heart disease (data not shown), indicating that Lp-PLA_2 activity may be the more biologically relevant parameterization of Lp-PLA_2. All subsequent analyses therefore focused on Lp-PLA_2 activity.

Statistical analysis. Follow-up began at the month of blood draw and continued either until June 2004, CHD event, or death. We used Cox proportional hazards analysis stratified by 5-year age categories over each 2-year follow-up period to estimate relative risks (RRs) and 95% CIs for tertiles of Lp-PLA_2 activity. Tests for trend were estimated across median values of Lp-PLA_2 tertile. To assess predictive ability, we calculated the area under the receiver-operating characteristic curves for multivariable-adjusted models with and without Lp-PLA_2 activity included. We assessed potential effect modification by modeling joint effects of Lp-PLA_2 tertile and tertiles of LDL, HDL, C-reactive protein, hormone replacement therapy use, and duration of diabetes. We then compared models with and without a cross-classification

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TABLE 1
Age-adjusted baseline characteristics across tertiles of Lp-PLA$_2$ activity among 777 female type 2 diabetic subjects from the NHS and 740 male type 2 diabetic subjects from the HPFS

	Tertile of Lp-PLA$_2$					
	Tertile 1		Tertile 2		Tertile 3	
	Men	Women	Men	Women	Men	Women
Lp-PLA$_2$ mean (nmol · min^{-1} · ml^{-1})	126.2	119.9	159.3	157.3	199.7	200.0
Lp-PLA$_2$ range	60.4–146.0	56.4–141.2	146.1–173.1	141.3–172.5	173.2–391.2	172.6–343.0
Age at baseline (years)	61.4	57.0	62.1	58.3	61.4	58.7
BMI (kg/m^2)	27.9	29.4	27.6	30.4	27.8	30.4
Physical activity (MET)	28.9	43.7	28.9	20.8	27.4	28.1
Current smoker (%)	6.2	9.9	7.0	10.5	8.4	21.1
Regular aspirin use (%)	37.6	24.6	37.1	21.4	31.2	24.2
Duration of diabetes (years)	7.8	9.4	7.9	8.2	6.7	6.2
Family history of CHD (%)	36.3	23.0	35.0	21.3	35.4	28.3
History of hypertension (%)	42.6	55.1	45.2	62.4	45.9	64.4
History of high serum cholesterol (%)	33.3	47.0	39.5	52.9	49.6	51.8
Cholesterol-lowering medication use (%)	5.9	3.9	8.5	4.3	6.2	4.2
Postmenopausal hormone use (%)	N/A	36.1	N/A	23.0	N/A	18.3
Insulin use (%)	18.8	20.1	19.9	21.1	15.5	17.7
Alcohol (g/day)	8.7	3.4	8.9	2.5	9.1	3.4
C-reactive protein (median; mg/dl)	1.4	5.0	1.9	4.8	1.8	5.4
A1C (%)	7.4	6.7	7.4	6.8	7.2	7.0
Intercellular adhesion molecule (ng/ml)	344.7	290.2	351.8	303.4	370.1	347.2
Triglycerides (mg/dl)*	189.4	174.5	169.0	185.2	193.3	203.1
LDL cholesterol (mg/dl)	110.3	122.6	127.8	140.5	141.4	155.0
HDL cholesterol (mg/dl)	43.3	59.1	41.0	51.0	37.9	46.2

*Triglycerides based on 406 men and 527 women with fasted blood samples.

term using −2 log likelihood tests. If there was absence of heterogeneity of results between the NHS and HPFS as assessed by the *Q*-statistic, we pooled results by weighing each estimate by the inverse of its variance (STATA; StataCorp, College Station, TX). All other analyses were performed using SAS statistical software (SAS Institute, Cary, NC).

RESULTS

During 8,010 person-years of follow-up in the men, 178 cases of CHD occurred, including 64 cases of nonfatal MI and fatal CHD and 114 CABG/PTCA. In the women, 146 cases of CHD occurred during 9,643 person-years of follow-up, including 73 cases of nonfatal MI and fatal CHD and 73 cases of CABG/PTCA. Among both men and women, smoking and less aspirin use were related to higher levels of Lp-PLA$_2$ activity. Additionally, among women only, history of hypertension and family history of heart disease were positively associated, and postmenopausal hormone use was inversely associated with Lp-PLA$_2$ activity (Table 1). In addition, shorter duration of diabetes was associated with higher Lp-PLA$_2$ activity. In both men and women, Lp-PLA$_2$ activity was positively associated with LDL and intercellular adhesion molecule and inversely associated with HDL (supplementary Table 1, available in the online appendix).

We calculated the relative risk associated with tertiles of Lp-PLA$_2$ activity for specific CHD end points and for total CHD (Table 2). With no differences in risk estimates between sexes, we pooled the results from the two studies ($Q = 0.12$, $P = 0.73$ for total CHD; $Q = 0.15$, $P = 0.70$ for nonfatal MI and fatal CHD; and $Q = 0.85$, $P = 0.36$ for CABG/PTCA); sex-specific results are presented in supplementary Tables 2 and 3. The age-adjusted RR between the extreme tertiles of Lp-PLA$_2$ activity was 1.93 (95% CI 1.25–2.97; P for trend = 0.001) for nonfatal MI and fatal CHD, 1.61 (1.12–2.30; P for trend = 0.009) for CABG/PTCA, and 1.76 (1.34–2.32; $P = 0.001$) for total CHD. After adjusting for lifestyle and clinical risk factors other than LDL, these relative risks were only modestly attenuated. However, after additional adjustment for LDL, the relative risk was strongly attenuated for CABG/PTCA (RR 1.12; 95% CI 0.74–1.70), but not so for nonfatal MI and fatal CHD (RR 1.75; 1.05–2.92). These results were slightly stronger for fatal CHD (RR 2.01; 0.90–4.50; P for trend <0.001 for fully adjusted extreme tertiles) than nonfatal MI (RR 1.62; 0.81–3.22; P for trend = 0.002 for fully adjusted extreme tertiles), but there was no statistically significant difference between these two end points ($Q = 0.16$; $P = 0.69$). Lp-PLA$_2$ activity added significantly to the area under the receiver-operating characteristic curve for MI in men ($P = 0.02$), but did not significantly add to predictive ability for any other end point in men or women. Additional adjustment for hypertension medications did not alter these results.

We next examined whether Lp-PLA$_2$ activity is more strongly related to cardiovascular events occurring closer to the date of Lp-PLA$_2$ measurement. Over the first 6 years of follow-up, there were 138 total cases of CHD among the men and women, and over the next 6 years in the HPFS and 8 years in the NHS, there were 186 total cases of CHD. Lp-PLA$_2$ activity was more strongly related to incident CHD during the first 6 years of follow-up (RR 2.00, 95% CI 1.16–3.44 for extreme tertiles) but less so after 6 years of follow-up (1.23, 0.81–1.89) (Table 3), irrespective of type of CHD (data not shown).

Because the risk of CHD associated with Lp-PLA$_2$ may vary by levels of other lifestyle and biological markers, we tested for interactions in the combined datasets and found no significant or meaningful interactions with LDL, HDL, C-reactive protein, hormone replacement therapy use, or duration of diabetes (data not shown).

TABLE 2
Relative risk (95% CIs) of incident CHD by tertiles of Lp-PLA_2 activity among 1,517 male and female diabetic subjects from the HPFS and NHS

	Tertiles of Lp-PLA_2			
	1	2	3	*P* for trend
Nonfatal MI and fatal CHD	32	42	63	
Age adjusted	1.0	1.25 (0.79–1.99)	1.93 (1.25–2.97)	0.001
Multivariate adjusted*	1.0	1.22 (0.75–1.99)	1.74 (1.10–2.76)	0.009
Multivariate adjusted + LDL	1.0	1.23 (0.75–2.01)	1.75 (1.05–2.92)	0.001
CABG/PTCA	49	58	80	
Age adjusted	1.0	1.13 (0.77–1.65)	1.61 (1.12–2.30)	0.009
Multivariate adjusted*	1.0	1.11 (0.75–1.63)	1.50 (1.02–2.22)	0.040
Multivariate adjusted + LDL	1.0	0.94 (0.63–1.41)	1.12 (0.74–1.70)	0.571
Total CHD	81	100	143	
Age adjusted	1.0	1.18 (0.88–1.58)	1.76 (1.34–2.32)	<0.001
Multivariate adjusted*	1.0	1.15 (0.85–1.55)	1.62 (1.21–2.17)	<0.001
Multivariate adjusted + LDL	1.0	1.05 (0.77–1.43)	1.39 (1.01–1.90)	0.034

Results were pooled between men and women using inverse variance weights. n = 1,517. *Multivariate relative risks adjusted for age, fasting status, smoking, alcohol intake, physical activity, duration of diabetes, aspirin use, cholesterol-lowering medication use, family history of MI, history of hypertension, BMI, HDL, A1C, C-reactive protein, intercellular adhesion molecule, insulin use, waist circumference, estimated glomerular filtration rate, and hormone replacement therapy use (women only).

DISCUSSION

In the present study, higher levels of Lp-PLA_2 activity were associated with increased risk of incident coronary heart disease among diabetic men and women. This is the first prospective study specifically to examine Lp-PLA_2 and incident CHD among diabetic subjects, and the relative risks found in this study are similar to those from studies that have examined general community and clinical populations.

We found that Lp-PLA_2 activity was more strongly associated with nonfatal MI and fatal CHD than with CABG/PTCA. It is possible that CABG/PTCA is a weaker clinical end point, since reasons to clinically intervene may be more subjective and less likely to be conducted in the sickest patients. In the only other study explicitly to investigate several different CHD outcomes, Oei et al. (9) found virtually no difference in relative risk by outcome. We also found that Lp-PLA_2 activity was more strongly related to cardiovascular events that occurred within the first 6 years of follow-up. Our study is the first to examine the relation between Lp-PLA_2 and incident CHD stratified by time to event in a nonclinical population. Although Lp-PLA_2 concentration has been associated with coronary artery calcification (16,17), Lp-PLA_2 activity has consistently demonstrated lack of association with coronary artery calcification (17,18). This, in combination with our observations that both later occurrence of CHD and CABG/PTCA were more weakly associated with Lp-PLA_2, could indicate that Lp-PLA_2 activity is related to unstable or rupture-prone lesions rather than to atherosclerosis alone. This is supported by recent evidence from a randomized trial that demonstrated that use of an Lp-PLA_2 activity inhibitor decreased plaque severity over 12 weeks (19).

In a recent investigation in the population-based Atherosclerosis Risk in Communities (ARIC) study, the authors found that of 19 novel biomarkers, Lp-PLA_2 concentration was the only marker to add significantly to the area under the receiver operating curves, although this magnitude was relatively small (20). This is of considerable importance for diabetic populations because of their increased risk of CHD. In the present study, Lp-PLA_2 activity added significantly to risk prediction for MI among men, but not for other end points in men or women. It is possible that our study is underpowered. Future studies with larger populations should examine the predictive ability of Lp-

TABLE 3
Relative risk (95% CIs) of early versus late incident non-fatal MI, fatal CHD, and CABG/PCTA by tertiles of Lp-PLA_2 activity among 1,517 male and female diabetic subjects from the HPFS and NHS

	Tertiles of Lp-PLA_2			
	1	2	3	*P* for trend
Total CHD within 6 years (n = 1,517)	28	43	67	
Age adjusted	1.0	1.70 (1.04–2.77)	2.41 (1.51–3.84)	<0.001
Multivariate adjusted*	1.0	1.72 (1.03–2.86)	2.34 (1.42–3.86)	<0.001
Multivariate adjusted + LDL	1.0	1.57 (0.93–2.65)	2.00 (1.16–3.44)	0.012
Total CHD after 6 years (n = 1,309)†	54	54	78	
Age adjusted	1.0	1.10 (0.75–1.62)	1.65 (1.15–2.37)	0.005
Multivariate adjusted*	1.0	1.10 (0.73–1.63)	1.51 (1.02–2.23)	0.039
Multivariate adjusted + LDL	1.0	0.98 (0.65–1.47)	1.23 (0.81–1.89)	0.323

Results were pooled between men and women using inverse variance weights. *Multivariate relative risks adjusted for age, fasting status, smoking, alcohol intake, physical activity, duration of diabetes, aspirin use, cholesterol-lowering medication use, family history of MI, history of hypertension, BMI, HDL, A1C, C-reactive protein, intercellular adhesion molecule, insulin use, waist circumference, estimated glomerular filtration rate, and hormone replacement therapy use (women only). †Follow-up began 6 years after blood draw. Participants who sustained a nonfatal MI or fatal CHD or were lost to follow-up before year 6 were not considered at risk for a cardiovascular event after 6 years.

PLA$_2$ because markers such as Lp-PLA$_2$ that can add to risk prediction can help uncover important etiologic pathways for cardiovascular disease.

In this study, although Lp-PLA$_2$ activity was associated with incident CHD, Lp-PLA$_2$ concentration was not. It is possible that the diabetes-associated disruption of metabolic parameters also affects Lp-PLA$_2$ concentrations and renders these values less informative. This will need to be confirmed in other populations. It is unclear which parameterization of Lp-PLA$_2$ will ultimately be most relevant for prediction and etiology, and future studies with both measurements should tease out these distinct pathways.

Lp-PLA$_2$ is hypothesized to contribute to the development of atherosclerosis through the propagation of inflammatory processes in the arterial intima (21). Several characteristics of Lp-PLA$_2$ suggest that this enzyme could be specifically relevant in people with diabetes. First, diabetic subjects have increased circulating levels of small dense LDL (2), potentially providing an efficient vehicle for delivery of Lp-PLA$_2$ to the intima, since Lp-PLA$_2$ has apparent binding preference for small dense LDL (22). Second, free radicals from oxidation of excess glucose may increase the oxidation of lipoproteins (23) and thereby increase levels of the substrate for Lp-PLA$_2$. Third, diabetes is characterized by increased macrophage infiltration of the arterial wall (2). Lp-PLA$_2$ is secreted by macrophages, although the actual level of Lp-PLA$_2$ production within the artery wall is unclear (21). Finally, in addition to diabetic and insulin-resistant conditions providing a milieu that would foster increased Lp-PLA$_2$ concentration and activity, Lp-PLA$_2$ could potentially perpetuate insulin resistance through its generation of free fatty acids and increased chemotaxis, both of which could lead to persistent chronic inflammation (21,24,25).

This study has several limitations. First, although we have a relatively large number of men and women with diabetes in this study, we have less power to detect relationships when stratifying by type of CHD. Second, Lp-PLA$_2$ was only measured at baseline, which may lead to modest misclassification over time. However, in a pilot study from the Health Professionals Follow-Up Study, men provided two samples 1 year apart, and Lp-PLA$_2$ activity was shown to be highly reproducible (intraclass $r = 0.87$).

In conclusion, among male and female participants with type 2 diabetes, Lp-PLA$_2$ activity was an independent risk factor for coronary heart disease, particularly for nonfatal MI and fatal CHD. This is the first prospective study to document this relationship among diabetic subjects who were free of CVD at baseline. Although the risk for CHD potentially conferred by Lp-PLA$_2$ among diabetic subjects appears to be consistent with those in the general population, further research is needed to explore whether reduction in Lp-PLA$_2$ reduces risk of CHD in diabetic subjects.

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