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SHORT COMMUNICATION

Lipoprotein-associated phospholipase A_2 and future risk of subclinical disease and cardiovascular events in individuals with type 2 diabetes: the Cardiovascular Health Study

T. L. Nelson · A. Kamineni · B. Psaty · M. Cushman · N. S. Jenny · J. Hokanson · C. Furberg · K. J. Mukamal

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Abstract
Aims/hypothesis Type 2 diabetes is an established risk factor for cardiovascular disease (CVD). This increased risk may be due in part to the increased levels of inflammatory factors associated with diabetes. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a risk marker for CVD and has pro-inflammatory effects in atherosclerotic plaques. We therefore sought to determine whether Lp-PLA_2 levels partially explain the greater prevalence of subclinical CVD and greater incidence of CVD outcomes associated with type 2 diabetes in the Cardiovascular Health Study.
Methods We conducted a cross-sectional and prospective study of 4,062 men and women without previous CVD from the Cardiovascular Health Study (1989 to 2007). Lp-PLA_2 mass and activity were measured in baseline plasma. Subclinical disease was determined at baseline and incident CVD was ascertained annually. We used logistic regression for cross-sectional analyses and Cox proportional hazards models for incident analyses.
Results At baseline, Lp-PLA_2 mass did not differ significantly by type 2 diabetes status; however, Lp-PLA_2 activity was significantly higher among type 2 diabetic individuals. Baseline subclinical disease was significantly associated with baseline diabetes and this association was similar in models unadjusted or adjusted for Lp-PLA_2 (OR 1.68 [95% CI 1.31–2.15] vs OR 1.67 [95% CI 1.30–2.13]). Baseline

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T. L. Nelson (✉)
Department of Health and Exercise Science,
Colorado State University,
223 Moby,
Fort Collins, CO 80523, USA
e-mail: tnelson@cahs.colostate.edu

A. Kamineni
Group Health Research Institute,
Seattle, WA, USA

B. Psaty
Department of Epidemiology, School of Public Health and Community Medicine, University of Washington,
Seattle, WA, USA

M. Cushman
Department of Medicine, University of Vermont,
Burlington, VT, USA

N. S. Jenny
Department of Pathology,
University of Vermont,
Burlington, VT, USA

J. Hokanson
Department of Epidemiology,
Colorado School of Public Health,
Denver, CO, USA

C. Furberg
Department of Public Health Sciences,
Wake Forest University School of Medicine,
Winston Salem, NC, USA

K. J. Mukamal
Division of General Medicine and Primary Care,
Beth Israel Deaconess Medical Center
and Harvard Medical School,
Boston, MA, USA

type 2 diabetes was also significantly associated with incident CVD events, including fatal CHD, fatal myocardial infarction (MI) and non-fatal MI in multivariable analyses. There were no differences in these estimates after further adjustment for Lp-PLA_2 activity.
Conclusions/interpretation In this older cohort, differences in Lp-PLA_2 activity did not explain any of the excess risk for subclinical disease or CVD outcomes related to diabetes.

Keywords Cardiovascular disease · Cardiovascular Health Study · Diabetes · Lipoprotein-associated phospholipase A_2 · Older adults · Platelet-activating factor acetylhydrolase · Subclinical disease · Type 2 diabetes

Abbreviations

CABG	Coronary artery bypass graft
CHF	Congestive heart failure
CHS	Cardiovascular Health Study
CVD	Cardiovascular disease
Lp-PLA_2	Lipoprotein-associated phospholipase A_2
MI	Myocardial infarction
PTCA	Percutaneous transluminal coronary angioplasty

Introduction

Type 2 diabetes is an established risk factor for cardiovascular disease (CVD). This increased risk may be due in part to the increased levels of inflammatory factors associated with type 2 diabetes. Recently, there has been great interest in the macrophage-derived enzyme lipoprotein-associated phospholipase A_2 (Lp-PLA_2), as it may play a key role in atherosclerotic plaque inflammation [1]. Lp-PLA_2 is also an independent predictor of coronary heart disease and ischaemic stroke in the general population and in clinical populations, including patients with diabetes [2–4]. Although Lp-PLA_2 was originally known as platelet-activating factor (PAF) acetylhydrolase because it degrades PAF and therefore potentially protects against atherosclerosis [5], overwhelming evidence now suggests that this is outweighed by its proatherogenic associations [1].

Previously, Barzilay et al. had shown that glucose disorders are associated with an increased prevalence of total CVD and an increased proportion of clinical diabetes relative to subclinical disease in the Cardiovascular Health Study (CHS) cohort [6]. In addition, Kuller et al. reported that the risk of clinical cardiovascular events was greatest for participants with a history of diabetes compared with those with newly diagnosed diabetes at baseline in the CHS [7]. We therefore hypothesised that Lp-PLA_2 mass and activity would partially explain the greater prevalence of subclinical disease at baseline and the greater incidence of CVD outcomes associated with type 2 diabetes in the CHS cohort.

Methods

Study population Participants were members of the CHS, a population-based cohort study of risk factors for CVD in older adults. The design and recruitment have been described in detail in previous publications [8, 9]. Overall, 5,888 adults aged 65 years and older were randomly sampled from Medicare eligibility lists in four US communities. All participants gave informed consent for participation in the study, and all procedures were conducted under institutionally approved protocols at each centre. Mean age at enrolment was 73 years (range 65–100), with 58% and 16% of participants being women and black, respectively. We excluded 1,517 participants who had CVD at baseline. We also excluded 64 participants for whom diabetes status was missing, and 239 and six participants without Lp-PLA_2 mass and Lp-PLA_2 activity data, respectively. This left 4,062 participants for the present study.

Self-reports of CVD outcomes were validated according to review of medical records [10]. Medication use was confirmed using an inventory at the home interview. Events were ascertained through 30 June 2007. Participants were categorised as having type 2 diabetes mellitus at baseline if they reported use of insulin or oral hypoglycaemic medications, or had a fasting glucose level of ≥7 mmol/l (126 mg/dl) or a random glucose measurement of ≥11 mmol/l (200 mg/dl). Participants who did not have prevalent CVD at baseline were categorised as having subclinical disease if they had any of the conditions listed for subclinical disease in Table 1 (footnotes). Prevalent CVD was defined as having previously had: myocardial infarction (MI), stroke, congestive heart failure (CHF), angina, percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft (CABG), claudication or transient ischaemic attack. Cardiovascular outcomes included fatal CHD, fatal or non-fatal MI, CHF, angina, CABG or PTCA.

GlaxoSmithKline (Research Triangle Park, NC, USA) measured plasma concentrations of Lp-PLA_2 activity using a tritium-labelled form of platelet-activating factor as substrate in a 96-well microplate, as described in detail previously [3]. Samples were tested in duplicate and were retested if the replicate coefficient of variation was greater than 25%. The analytical coefficient of variation was 7.5%. Plasma Lp-PLA_2 mass was measured at the CHS Central Laboratory at the University of Vermont using an enzyme-linked immunosorbent assay kit (second-generation PLAC Test; diaDexus, South San Francisco, CA, USA). All samples were analysed in duplicate and the analytical coefficient of variation was 6.3%.

Table 1 Mean levels or frequencies of CVD risk factors by type 2 diabetes status at the 1989–1990 and 1992–1993 baseline visits

Variable	Diabetes	Non-diabetic	*p* value
n	541	3,521	
Lp-PLA_2 mass (ng/ml)	337.8±116.3	341.5±117.5	0.49
Lp-PLA_2 activity (nmol min^{-1} ml^{-1})	40.1±12.6	38.6±12.7	<0.05
Maximum stenosis (≥25%), *n* (%)	263 (48.9)	1,438 (41.1)	<0.01
Subclinical disease, *n* (%)[a]	363 (68.8)	1,819 (52.7)	<0.001
BMI (kg/m^2)	28.8±4.8	26.3±4.6	<0.0001
HDL-cholesterol (mmol/l)	1.2±0.3	1.5±0.4	<0.0001
LDL-cholesterol (mmol/l)	3.3±1.0	3.4±0.9	0.0910
Triacylglycerol (mmol/l)	1.6 (1.2, 2.3)	1.3 (1.0, 1.7)	<0.001
C-reactive protein (mg/l)	2.5 (1.4, 4.9)	1.7 (0.9, 3.0)	<0.0001

Unless otherwise indicated, values are mean±SD or median (25th percentile, 75th percentile)

p values represent difference in means or proportions between diabetic and non-diabetic participants

[a] Subclinical was defined as not having clinical disease (apart from presence of ECG abnormalities), but having any of the following: ankle–arm index <0.9, internal carotid artery wall thickness >80th percentile, carotid stenosis >25%, major ECG abnormalities (based on Minnesota code) or a Rose questionnaire positive for claudication or angina pectoris in the absence of clinical diagnosis of angina pectoris and claudication [11]

Statistical analysis Univariate differences between diabetic and non-diabetic individuals at baseline were evaluated by χ^2 test for categorical covariates, by *t* tests for normal continuous covariates and by Mann–Whitney non-parametric tests for non-normal continuous covariates. Logistic regression was used to evaluate the cross-sectional association between subclinical disease and type 2 diabetes status. Cox proportional hazards models were used to estimate the relative risk (HR) of CVD outcomes predicted by type 2 diabetes status. Entry time into the analysis corresponded to the participants' study enrolment date, with time-at-risk lasting until the earliest of incident CVD event, death, loss to follow up or the last day of adjudicated follow-up (30 June 2007). Using Cox regression, we also estimated the relative risk of CVD outcomes predicted by Lp-PLA_2 activity and mass, and stratified above or below the 75th percentile by type 2 diabetes status. All multivariable models were evaluated for multiple variables. In general, covariates were retained in the final models if they influenced the risk estimate by 10% or more. We evaluated potential mediation of these associations by Lp-PLA_2 activity by comparing models with and without adjustment for this factor. Analyses were performed using Stata 10.1 (Stata, College Station, TX, USA). All *p* values were two-tailed (α=0.05)

Results

Of the 4,062 participants, 37.4% were men and 84.3% were white. The mean age was 72.3±5.3 years. Table 1 shows the baseline characteristics of CVD risk factors by type 2 diabetes status. Participants with type 2 diabetes had more risk factors, including more subclinical disease, higher Lp-PLA_2 activity, lower HDL-cholesterol, higher triacylglycerol and higher C-reactive protein levels.

Baseline subclinical disease was significantly associated with baseline type 2 diabetes status in multivariable models (OR 1.68 [95% CI 1.31–2.15]). These associations were not significantly altered after adjusting for Lp-PLA_2 activity (OR 1.67 [95% CI 1.30–2.13]). When we stratified by Lp-PLA_2 above or below the 75th percentile, the associations were modestly but not significantly attenuated in those above the 75th percentile for mass and activity (data not shown).

During an average follow up of 12.8 (±4.9) years, type 2 diabetes, in multivariable models, was significantly associated with incident CVD outcomes including fatal CHD and MI, as well as non-fatal MI, CHF, angina and CABG. However, these associations were not altered after adjusting for Lp-PLA_2 activity (Table 2). Moreover, after stratifying by Lp-PLA_2 above or below the 75th percentile, the risk estimates between type 2 diabetes and incident CVD were attenuated in those above the 75th percentile, albeit not significantly (data not shown). Finally, we considered the association between Lp-PLA_2 and incident CVD, stratified by type 2 diabetes status. Similarly to other studies, increased risk of CVD outcomes was associated with higher Lp-PLA_2 activity and mass, but these risks were not modified by type 2 diabetes status (Electronic supplementary material [ESM] Table 1).

Discussion

In this study, Lp-PLA_2 mass or activity did not account for any part of the greater prevalence of subclinical disease or

Table 2 Cox proportional hazards analysis for the association between incident CVD outcomes and type 2 diabetes status

Variables per outcome	HRs not adjusted for Lp-PLA$_2$ activity		HRs adjusted for Lp-PLA$_2$ activity	
	No diabetes	Diabetes	No diabetes	Diabetes
Fatal CHD				
Events (*n*)	378	118		
Person-years	45,594	5,704		
Adjusted HR[a]	1.0	2.55 (2.07–3.14)	1.0	2.54 (2.06–3.13)
Multivariable HR[b]	1.0	2.14 (1.69–2.70)	1.0	2.14 (1.69–2.70)
Fatal MI				
Events (*n*)	51	15		
Person-years	46,203	5,955		
Adjusted HR[a]	1.0	2.51 (1.40–4.50)	1.0	2.51 (1.40–4.49)
Multivariable HR[b]	1.0	2.10 (1.12–3.94)	1.0	2.10 (1.12–3.94)
Non-fatal MI				
Events (*n*)	414	95		
Person-years	44,251	5,575		
Adjusted HR[a]	1.0	1.87 (1.49–2.34)	1.0	1.85 (1.48–2.32)
Multivariable HR[b]	1.0	1.56 (1.21–2.01)	1.0	1.56 (1.21–2.01)
CHF				
Events (*n*)	826	195		
Person-years	43,359	5,194		
Adjusted HR[a]	1.0	2.07 (1.77–2.43)	1.0	2.06 (1.76–2.42)
Multivariable HR[b]	1.0	1.52 (1.28–1.82)	1.0	1.52 (1.28–1.82)
CVD outcome[c]				
Events (*n*)	1,239	265		
Person-years	40,019	4,633		
Adjusted HR[a]	1.0	1.88 (1.64–2.15)	1.0	1.87 (1.64–2.14)
Multivariable HR[b]	1.0	1.53 (1.32–1.77)	1.0	1.53 (1.32–1.77)

[a] HR (95% CI), adjusted for age, sex and race

[b] HR (95% CI), adjusted for age, sex, race (black, non-black), BMI, smoking status (former, never, current), smoking history (lifetime pack-years), alcohol use (never, former, <7 drinks/week, >7 drinks/week), HDL-cholesterol, LDL-cholesterol, use of statins, C-reactive protein, field centre, education (lower than high school, high school or General Education Diploma, higher than high school), physical activity (energy expenditure), creatinine and hypertension

[c] Any of the above (i.e. fatal CHD, fatal MI, non-fatal MI, CHF)

the greater incidence of CVD outcomes associated with type 2 diabetes in the CHS cohort of older adults. As shown previously in this cohort, Lp-PLA$_2$ mass and activity predicted CVD events [2]; however, these associations did not differ by type 2 diabetes status, as shown in the present study.

Importantly, a recently published study by Hatoum et al. found that type 2 diabetic participants with elevated levels of Lp-PLA$_2$ activity were significantly more likely to develop CHD than those without elevated levels [4]. This study included participants in the Health Professionals Follow-up Study and the Nurses' Health Study. Its finding is counter to what we found here, where type 2 diabetic participants in the upper 75th percentile of Lp-PLA$_2$ activity were not more likely to develop any CVD outcome. Perhaps the differences in age between these cohorts (i.e. ~73 years vs ~60 years in the study of Hatoum et al.) explains the difference in findings. An important limitation of the other study [4] is that the investigators did not determine whether the association between Lp-PLA$_2$ and CHD in diabetic participants was different from that in non-diabetic participants. We, in the present study, did not find that Lp-PLA$_2$ altered the association between type 2 diabetes and incident CHD outcomes when compared with non-diabetic participants (see Table 2).

It is possible that Lp-PLA$_2$ is not sufficiently sensitive as a single marker, given all the other factors that contribute to CVD risk in older diabetic individuals. Other limitations include the fact that Lp-PLA$_2$ and type 2 diabetes status were measured at the same time; a later measurement of Lp-PLA$_2$ might, conceivably, have resulted in a stronger association with CVD outcomes. Future studies need to address the temporality of these relationships, as it was not possible to determine whether higher Lp-PLA$_2$ levels preceded or followed the development of type 2 diabetes in this study, which was not designed to test causality. Furthermore, because we studied an older population with type 2 diabetes, those who died earlier in life from diabetes-related complications could not be studied, but may have had higher Lp-PLA$_2$ levels. It is also possible that measurement error in the assays for Lp-PLA$_2$ could have influenced these null findings or that the diabetic participants in this study had adequate amounts of anti-oxidants to counter elevated levels of oxidised LDL. Despite these limitations, this study does have several strengths, including

a large sample size and number of incident CVD events, with 100% follow-up and measurement of numerous confounding factors.

Overall, our results suggest that Lp-PLA_2 does not mediate the association between type 2 diabetes and subclinical CVD and CVD events. Also, type 2 diabetes status does not modify the association between Lp-PLA_2 and CVD outcomes in an older population.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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