

Lipoprotein-associated phospholipase A₂, C-reactive protein, and coronary artery disease in individuals with type I diabetes and macroalbuminuria

Diabetes & Vascular Disease Research
01–09
© The Author(s) 2009
Reprints and permission: <http://www.sagepub.co.uk/journalsPermission.nav>
DOI: 10.1177/1479164109346358
<http://dvr.sagepub.com>



Rachel G. Miller, Tina Costacou and Trevor J. Orchard

Abstract

Given the paucity of data in type I diabetes concerning lipoprotein-associated phospholipase A₂ (Lp-PLA₂), we examined its prospective relationship with coronary artery disease (CAD), as well as the effect of modification by C-reactive protein (CRP) and haptoglobin genotype, in individuals with type I diabetes who are at an increased risk for CAD due to also having macroalbuminuria (n=96). Although Lp-PLA₂ activity was univariately predictive of CAD (HR=1.54 per SD, p=0.009), this relationship was not significant after covariate adjustment (p=0.59). There was a significant interaction between Lp-PLA₂ and CRP (p=0.02), i.e. those with both markers greater than the median level were more likely to have a CAD event than those persons with low levels of both (HR=2.89, p=0.06). When stratified by haptoglobin genotype, Lp-PLA₂ was predictive of CAD in persons with the 2/1 (HR=2.40, p=0.05), but not 2/2 (HR=0.66, p=0.27), genotype. The association between Lp-PLA₂ activity and CAD differs by CRP and haptoglobin genotype in this group of persons with type I diabetes and macroalbuminuria.

Keywords

Lp-PLA₂, type I diabetes, macroalbuminuria, coronary artery disease, C-reactive protein, haptoglobin genotype

Introduction

As atherosclerosis has been increasingly considered an inflammatory process,¹ researchers have turned their focus to evaluating the prognostic value of biomarkers of inflammation. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) has recently been added to this biomarker list. Lp-PLA₂ is an enzyme produced by macrophages in advanced, rupture prone, atherosclerotic plaques. It circulates bound primarily to lipoproteins in the plasma and hydrolyses oxidized low-density lipoprotein (LDL) generating two pro-inflammatory mediators, oxidized free fatty acids and lysophosphatidylcholine. Through this action on LDL particles, it was therefore thought that Lp-PLA₂ may be directly involved in the formation of atherosclerotic lesions.² However, it is now recognized that Lp-PLA₂ induces a broad range of biological responses, is involved in a wide variety of biological actions³ and can thus be attributed a variety of properties, including both pro-inflammatory^{4,5} and anti-inflammatory.^{6–8} Nevertheless, increasing evidence from epidemiological studies in humans suggests that Lp-PLA₂ is independently associated with coronary artery disease (CAD) risk.^{9–17} Thus far, the presence of such an association has not been evaluated among individuals with type 1 diabetes (T1D), despite

persons with T1D being at increased risk of developing cardiovascular disease.^{18–25}

High-sensitivity C-reactive protein (CRP), a marker of systemic inflammation produced in the liver, has also been associated with increased vascular disease risk in both individuals with type 2 diabetes and the general population.^{26–29} Interestingly, in the Atherosclerotic Risk in Communities (ARIC) study, the relationship between Lp-PLA₂ and coronary heart disease was found to be modified by the level of C-reactive protein (CRP) in persons with lower levels of LDL, so that persons with high levels of both markers were at an increased risk for coronary heart disease compared with

University of Pittsburgh, Department of Epidemiology,
Graduate School of Public Health,
Pittsburgh, PA, USA

Corresponding author:

Trevor J Orchard, University of Pittsburgh,
Department of Epidemiology, Graduate School of Public Health,
3512 Fifth Avenue, Pittsburgh, PA 15213, USA.
Email: tjo@pitt.edu

persons with high levels of either marker alone.¹² While CRP has been found to be associated with vascular disease in non-diabetic^{27,28,30-34} and type 2 diabetic^{29, 35, 36} subjects, the relationship between CRP and CAD in T1D is not clear.³⁷⁻³⁹

Recently, several longitudinal studies have provided evidence that a polymorphism of the haptoglobin gene more than doubles cardiovascular disease risk among persons with type 2 diabetes.⁴⁰⁻⁴² We have also shown that the haptoglobin genotype is associated with CAD in the Pittsburgh Epidemiology of Diabetes Complications (EDC) study of T1D, with the 2/2 genotype conferring the greatest risk.⁴³ We assessed the prospective association between Lp-PLA₂ with CAD risk, and examined whether this relationship is modified by CRP and haptoglobin genotype, in a group of individuals with T1D, who are at particularly high risk for CAD due to also having diabetic renal disease.^{24, 44-49} We also examined whether high-density lipoprotein (HDL) or LDL cholesterol concentration modified the prediction of CAD by Lp-PLA₂.

Methods

Study population

The participants were identified from the EDC study, a prospective study of childhood-onset (<18 years old at diagnosis) T1D. All participants were diagnosed with T1D or seen within 1 year of diagnosis at the Children's Hospital of Pittsburgh between 1950 and 1980. A total of 658 individuals met the eligibility criteria and participated in the EDC baseline examination, conducted between 1986 and 1988, and participants were assessed biennially thereafter. Lp-PLA₂ activity was measured on a subgroup of the EDC study cohort with macroalbuminuria (albumin excretion rate ≥ 200 $\mu\text{g}/\text{min}$) at study baseline; the current analyses were performed on these 96 individuals.

Measurement of biomarkers

Lp-PLA₂ activity was assayed using a colorimetric assay (diaDexus, South San Francisco, CA, USA). CRP was measured using a high-sensitivity turbidimetric method (reagents developed by Carolina Liquid Chemicals, Brea, CA, USA). A PureGene kit (Gentra Systems, Minneapolis, MN) was used to isolate high-molecular-weight genomic DNA and haptoglobin was genotyped by the amplification method of Koch *et al.*⁵⁰ Genotypes were assigned visually by comparison with controls of known genotype.

Ascertainment of CAD outcome

CAD was defined as CAD death, MI and/or Q-waves with Minnesota Codes 1.1 or 1.2, stenosis $\geq 50\%$ or revascularization, ischaemic echocardiography (ECG; Minnesota Code 1.3, 4.1-4.3, 5.1-5.3, 7.1), or EDC physician diagnosed angina.

Events were confirmed by medical records and verified by an EDC study physician masked to Lp-PLA₂ measurements.

Clinical measurements

Participants completed questionnaires concerning demographic and medical history information. Weight was measured using a balanced-beam scale with clothing during the clinical examination. Height was measured using the clinic stadiometer, with the Frankfort plane held horizontal. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. Waist circumference was measured twice at the midpoint between the highest point of the iliac crest and the lowest part of the costal margin in the midaxillary line. If the two measurements differed by more than 0.5 cm, a third measurement was performed. The mean of the waist measurements was recorded as waist circumference. Hip girth measurement was performed at the widest point of the glutei, usually at the level of the greater femoral trochanter, and the mean of two measures was recorded as the hip circumference, in the same manner as the waist measurements. The ratio of the waist circumference to the hip circumference (WHR) was used in analyses. For the first 18 months of the study, fasting blood samples were analysed for HbA1c (microcolumn cation exchange; Isolab, Akron, OH, USA). For the remainder of the baseline examinations, automated high-performance liquid chromatography (HPLC; Diamat, BioRad, Hercules, CA, USA) was performed. The two assays were highly correlated ($r=0.95$; Diamat HbA1c = $-0.18 + 1.00$ [Isolab HbA1c]). For analysis purposes, the HbA1c values were converted to a Diabetes Control and Complications Trial (DCCT)-aligned value using a regression equation derived from duplicate assays (DCCT HbA1c = $0.14 + 0.83$ [EDC HbA1c]). Serum total cholesterol and triglycerides were determined enzymatically,^{51,52} HDL cholesterol was determined using a precipitation technique with a modification⁵³ of the Lipid Research Clinics method⁵⁴ and LDL-cholesterol levels were calculated from the measurements of total cholesterol, triglycerides and HDL cholesterol using the Friedewald equation.⁵⁵ All blood samples were taken after at least 8 hours of fasting. Three seated blood pressure readings were taken with a random-zero sphygmomanometer and the mean of the second and third readings was used in analyses, according to the Hypertension Detection and Follow-up Program Protocol.⁵⁶ Hypertension was defined as at least 140/90 mmHg or use of antihypertensive medication. White blood cell count (WBC) was obtained using a counter S-plus IV.

Statistical Analyses

Baseline characteristics were compared between CAD cases and non-cases using Student's *t*-test, Wilcoxon's

two-sample test for non-normally distributed variables, and a chi-squared test for binary variables. T1D duration-adjusted correlations between Lp-PLA₂ and CAD risk factors were assessed using Pearson partial correlations or Spearman partial correlations, when variables were not normally distributed. Cox proportional hazard models were used to estimate the relative risk of CAD associated with a one-SD increase in Lp-PLA₂ activity. Follow-up time was defined as the time from the baseline examination to the date of the first CAD event or, for non-cases, follow-up continued until death, last contact or censoring during 18-year follow-up. Adjusted regression models were built using forward selection. As age and duration of diabetes are highly correlated in this cohort ($r=0.85$), only duration was made available to multivariate models. The proportional hazards assumption was assessed visually by plotting the log cumulative hazard function of CAD by Lp-PLA₂ activity and verified by showing that time-dependent Lp-PLA₂ interaction variables were not statistically significant. An Lp-PLA₂-CRP interaction term was tested with respect to risk of developing CAD and additional proportional hazards models were fit to examine combined categories of the two biomarkers, using indicator variables, in order to further explore the nature of the interaction. The combined categories were created by stratifying baseline Lp-PLA₂ and CRP by their median values and combining as follows:

Low Lp-PLA₂-Low CRP, Low Lp-PLA₂-High CRP, High Lp-PLA₂-Low CRP, High Lp-PLA₂-High CRP. Analyses were repeated after stratifying by haptoglobin genotype in the participants with available genetic material ($n=80$). Persons with the 2/1 genotype were compared with those with the higher risk genotype (2/2). Owing to the low frequency of the 1/1 genotype, those persons ($n=10$) were excluded from the genotype comparison analysis. All analyses were performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC).

Results

A comparison of baseline characteristics between CAD cases and non-cases is shown in Table 1. Lp-PLA₂ activity was significantly higher in persons who went on to develop CAD compared with those who did not ($p=0.02$, Table 1). After adjusting for LDL cholesterol, the difference in Lp-PLA₂ by CAD status was no longer statistically significant ($p=0.12$). Adjustment for HDL also attenuated the difference in Lp-PLA₂ by CAD cases and non-cases, but it remained statistically significant ($p=0.04$). Lp-PLA₂ activity was significantly and positively correlated with total and LDL cholesterol, diastolic blood pressure, WBC and WHR, but not BMI (Table 2). Lp-PLA₂ also had an inverse significant

Table 1. Baseline characteristics by coronary artery disease (CAD) status

	CAD cases (n = 41)	Non-cases (n = 55)	p-value
Diabetes duration (years)	25.5 (6.0)	19.0 (5.4)	<0.0001
Age (years)	32.4 (5.7)	27.5 (5.4)	<0.0001
Sex (% female, n)	39.0% (16)	58.2% (32)	0.06
Lp-PLA₂ activity (nmol/min/mL)	154.6 (32.3)	138.6 (31.8)	0.02
C-Reactive Protein (CRP) (mg/dL)	3.3 (3.5)	2.8 (5.7)	0.67
Haptoglobin 2/2 genotype ^{*,†}	56.4% (22)	51.2% (21)	0.64
White blood cell count (x 10³/mm²)	7.8 (2.2)	6.7 (1.7)	0.006
HbA1c (%)	8.7 (1.2)	9.0 (1.8)	0.29
Body mass index (kg/m²)	24.6 (3.2)	23.2 (2.9)	0.03
Waist-hip ratio	0.85 (0.07)	0.82 (0.08)	0.03
Current smoker [‡]	36.6% (15)	27.3% (15)	0.38
Ever smoker [‡]	46.3% (19)	40.0% (22)	0.53
Systolic blood pressure (mmHg)	123.3 (15.7)	118.8 (15.6)	0.16
Diastolic blood pressure (mmHg)	77.4 (12.4)	72.2 (11.3)	0.01
Hypertension[‡]	58.5% (24)	25.5% (14)	0.001
Blood pressure medication use[‡]	39.0% (16)	20.8% (11)	0.05
Total cholesterol (mg/dl)	217.2 (39.9)	205.4 (47.2)	0.19
HDL cholesterol (mg/dl)	51.4 (11.5)	56.3 (14.1)	0.07
LDL cholesterol (mg/dl)	139.6 (31.5)	123.3 (35.9)	0.03
Triglycerides [‡] (mg/dl)	113 (88–169)	91 (68–135)	0.06
Albumin excretion rate [‡] (µg/min)	881.3 (436.2–1,399.0)	514.6 (357.3–1,297.9)	0.35

Data are means (SD) unless otherwise noted

* $n=80$ participants with available genetic material

[‡]Percentage (n)

[‡]Median (interquartile range)

Table 2. Type 1 diabetes duration-adjusted partial correlations between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity and coronary artery disease risk factors

Risk factor	Partial <i>r</i>	p-value
HbA1c	−0.007	0.95
Total cholesterol	0.48	<0.0001
HDL cholesterol	−0.28	0.01
LDL cholesterol	0.58	<0.0001
Triglycerides ¹	0.19	0.08
Systolic blood pressure	0.19	0.09
Diastolic blood pressure	0.23	0.04
Body mass index	−0.01	0.93
Waist–hip ratio	0.30	0.002
White blood cell count	0.30	0.006
C-reactive protein	−0.02	0.88
Albumin excretion rate ¹	0.11	0.33

Pearson's partial correlation unless indicated

¹Spearman's rank partial correlation

correlation with HDL cholesterol and marginally significant positive correlations with triglycerides and systolic blood pressure (Table 2).

In an unadjusted Cox proportional hazards model, higher Lp-PLA₂ activity was significantly associated with an increased risk of developing CAD (hazard ratio (HR) = 1.54 per one-SD increase in Lp-PLA₂ activity, *p* = 0.009; Table 3). However, after forward selection, in a model adjusting for T1D duration, sex, CRP, LDL cholesterol, systolic blood pressure, blood pressure medication use and WBC, Lp-PLA₂ activity was no longer significantly associated with CAD risk (HR = 1.15, *p* = 0.59). Alternative models were fit including HDL cholesterol both in place of, and in addition to, LDL cholesterol, age in place of T1D duration, and waist circumference in place of WHR, but the results showed little difference from those presented in Table 3 (data not shown). No interactions were found between Lp-PLA₂ activity and either of the lipoproteins. An Lp-PLA₂–CRP interaction term, added to the final model shown in Table 3, was found to be statistically significant (*p* = 0.02).

In an unadjusted model assessing the relationship between combined categories of Lp-PLA₂ activity and CRP with CAD, those in the High Lp-PLA₂ activity–High CRP group had significantly elevated risk of developing CAD (HR = 3.79, *p* = 0.004) compared with the Low–Low group (Table 4). After covariate adjustment, this relationship remained, but was a little attenuated, with the High Lp-PLA₂ activity–High CRP group being nearly three times more likely to develop CAD than the low Lp-PLA₂–Low CRP group (HR = 2.89, *p* = 0.06).

Lp-PLA₂ activity level did not differ by haptoglobin genotype, with a mean of 146.4 (SD 32.1) in the haptoglobin 2/1 genotype and 145.1 (SD 32.6) in the haptoglobin 2/2 genotype (*p* = 0.89). Although an Lp-PLA₂ activity–haptoglobin interaction term was not significant with respect to CAD incidence (*p* = 0.45), after stratifying by haptoglobin genotype, in the 2/1 genotype, the association between Lp-PLA₂ activity and CAD was statistically significant (HR = 1.80, *p* = 0.02) and remained so after adjustment for CRP, T1D duration, LDL-cholesterol, HbA1c and triglycerides (HR = 2.40, *p* = 0.05; Table 5). The increased HR was largely due to the addition of triglycerides into the model. In contrast, there was no apparent association between Lp-PLA₂ activity and CAD in the 2/2 genotype (unadjusted HR = 1.18 *p* = 0.47, fully adjusted HR = 0.66, *p* = 0.27; Table 5). The results remained similar when both genotype models were forced to contain the same covariates for adjustment, namely, T1D duration, sex, LDL-cholesterol, ln(triglycerides) and CRP, covariates that were significant predictors of CAD in an overall model of the participants with genetic data available (Hp 2/1 HR = 1.97 (0.98, 3.95), *p* = 0.05, Hp 2/2 HR = 0.56 (0.23, 1.34), *p* = 0.19). The relationship between CRP and CAD was slightly weaker in the 2/2 genotype (adjusted HR = 1.14, *p* = 0.01) compared with the 2/1 genotypes (adjusted HR = 1.63, *p* = 0.001). A comparison of the incidence of CAD per 100 person-years by stratified groups suggested that having both high Lp-PLA₂ activity and high CRP may lead to an increased risk of CAD in persons with the 2/1 genotype (11.9 events per 100 person-years compared with

Table 3. Hazard ratio of coronary artery disease (CAD) event associated with a one-SD increase in lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity

Model 1 ^a	Model 2 ^b	Model 3 ^c	Model 4 ^d
Hazard Ratio (95% confidence interval), <i>p</i> -value			
1.54 (1.11, 2.12), 0.009	1.60 (1.15, 2.23), 0.005	1.31 (0.91, 1.87), 0.13	1.15 (0.70, 1.87), 0.59

^aModel 1: Lp-PLA₂ Activity (unadjusted)

^bModel 2: Lp-PLA₂ Activity and C-reactive protein (CRP)

^cModel 3: Lp-PLA₂ Activity, CRP, type 1 diabetes (T1D) duration, sex

^dModel 4: Final model after forward selection: Lp-PLA₂ activity, CRP, T1D duration, sex, low-density lipoprotein cholesterol, systolic blood pressure, blood pressure medication use, white blood cells

In addition to the variables included in the final model, the following were also made available to forward selection models: HbA1c, waist–hip ratio, triglycerides, diastolic blood pressure and smoking status

Table 4. Hazard ratios of coronary artery disease (CAD) event associated with combined lipoprotein-associated phospholipase A₂ (Lp-PLA₂) Activity and C-reactive protein (CRP) level

	Model 1 ^a	Model 2 ^b	Model 3 ^c
	Hazard ratio (95% confidence interval), p-value		
Low Lp-PLA ₂ activity–Low CRP	(Reference)	(Reference)	(Reference)
Low Lp-PLA ₂ activity–High CRP	2.28 (0.85, 6.12), 0.10	3.67 (1.35, 9.93), 0.01	2.64 (0.85, 8.21), 0.09
High Lp-PLA ₂ activity–Low CRP	2.06 (0.78, 5.40), 0.14	1.43 (0.53, 3.85), 0.48	1.33 (0.41, 4.31), 0.63
High Lp-PLA ₂ activity–High CRP	3.79 (1.54, 9.34), 0.004	3.44 (1.37, 8.66), 0.009	2.89 (0.98, 8.58), 0.06

Low Lp-PLA₂ activity defined as Lp-PLA₂ activity < median value (147.20 (nmol/min/ml))

High Lp-PLA₂ activity defined as Lp-PLA₂ activity ≥ median value (147.20 (nmol/min/ml))

Low CRP defined as CRP < median value (1.56 (mg/dl))

High CRP defined as CRP ≥ median value (1.56 (mg/dl))

^aModel 1: Unadjusted

^bModel 2: Combined Lp-PLA₂ Activity-CRP level, type 1 diabetes (T1D) duration, sex

^cModel 3: Combined Lp-PLA₂ Activity-CRP level, T1D duration, sex, low-density lipoprotein cholesterol, systolic and diastolic blood pressures, white blood cell count

In addition to the variables included in the final model, the following were also made available to forward selection models: HbA1c, waist-hip ratio, triglycerides, blood pressure medication use and smoking status

Table 5. Hazard ratios of coronary artery disease (CAD) event associated with a one-SD increase in lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity stratified by haptoglobin (Hp) genotype

	Hp 2/1 (n=34, 16 events)	Hp 2/2 (n=36, 19 events)
	Hazard ratio (95% CI), p-value	
Model 1^a	1.80 (1.10, 2.97), 0.02	1.18 (0.75, 1.85), 0.47
Model 2^{b,c}	2.40 (1.02, 5.64), 0.05	0.66 (0.31, 1.39), 0.27

^aModel 1: Lp-PLA₂ activity (unadjusted)

^bModel 2, Hp 2/1: Final model after forward selection, Lp-PLA₂ activity, C-reactive protein, type 1 diabetes duration, sex, HbA1c, low-density lipoprotein (LDL) cholesterol, ln(triglycerides)

^cModel 2, Hp 2/2: Final model after forward selection, Lp-PLA₂ Activity, type 1 diabetes duration, sex, LDL cholesterol

In addition to the variables included in the final model, the following were also made available to forward selection models: waist-hip ratio, blood pressure, blood pressure medication use and smoking status.

the Low Lp-PLA₂ activity–Low CRP group rate of 1.15 events per 100 person-years, p=0.01). This was not seen in those with the 2/2 genotype (Figure 1), where rates were similar across groups (p=0.73). While no analyses were possible by subtype of CAD events due to small sample size, in both Hp 2/1 and 2/2, angina comprised approximately 50% of the CAD events in the high Lp-PLA₂ activity and high CRP category.

Discussion

In this cohort of childhood-onset type 1 diabetic persons with proteinuria, elevated CRP levels are associated with an increased risk of CAD, while Lp-PLA₂ activity is only associated with an increased risk of CAD if CRP is also high. Intriguingly, however, Lp-PLA₂ activity further increases risk in the haptoglobin 2/1 subgroup (but not Hp 2/2), even after adjustment for CRP and other factors. These results suggest that Lp-PLA₂ activity may add to the prediction of CAD in type 1 diabetic persons who are thought to have a lower

genetic predisposition to cardiovascular disease. We are unaware of any previous reports of this relationship in T1D.

The mean level of Lp-PLA₂ activity in the current report, of 145.4 nmol/min/mL (SD 32.9), is higher than the levels reported by most other studies. In reports by Furberg *et al.*,⁵⁷ Kim *et al.*,¹⁷ and Oei *et al.*,¹³ mean Lp-PLA₂ activity was <50 nmol/min/ml. In contrast, however, reports by Allison *et al.*⁵⁸ and Koenig *et al.*¹⁶ reported levels similar to those seen in the current study (mean Lp-PLA₂ activity 145.2 (33.5) and 122.4 (26.2) nmol/min/ml, respectively). One possible reason for these higher levels is that the participants in these studies had either prior coronary heart disease¹⁶ or had previously been referred to a vascular testing center,⁵⁸ whereas most of the participants in the studies with lower levels of Lp-PLA₂ activity were healthy at the time the sample was collected, except for the report by Furberg *et al.*, where approximately 10% had a history of myocardial infarction.⁵⁷ The participants in our study are thus more comparable to those of the Allison *et al.*⁵⁸ and Koenig *et al.*¹⁶ reports, as having both T1D and proteinuria puts them at an increased risk of developing CAD.

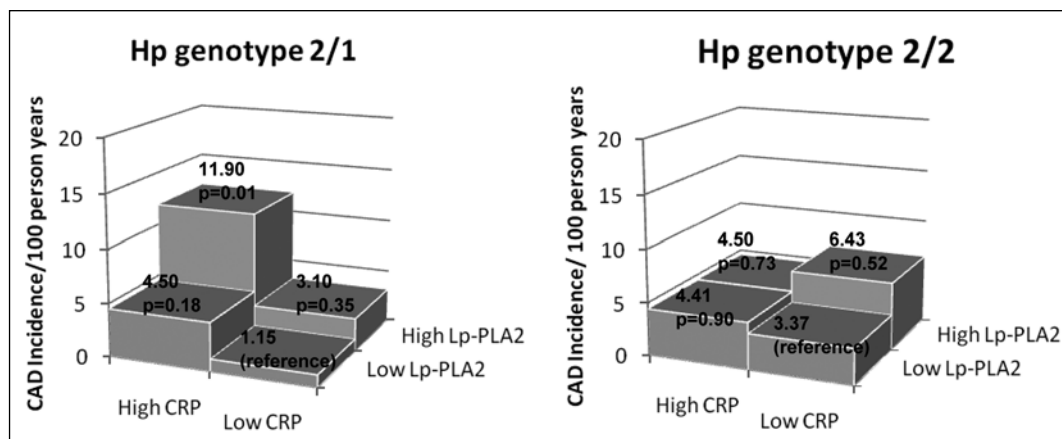


Figure 1. Coronary artery disease (CAD) incidence per 100 person-years of follow-up by lipoprotein-associated phospholipase A2 (Lp-PLA₂) activity and C-reactive protein (CRP) combined category and haptoglobin genotype.

Lp-PLA₂ activity was correlated with traditional cardiovascular disease risk factors, including total, LDL and HDL cholesterol, both of which, to different extents, carry Lp-PLA₂ in the blood. Lp-PLA₂ activity was also correlated with diastolic blood pressure, WBC and WHR. It was not correlated with CRP or measures of proteinuria and kidney function. The lack of correlation with CRP is similar to the findings of the West of Scotland Coronary Prevention Study (WOSCOPS),¹⁰ ARIC,¹² the Rotterdam study,¹³ the report by Brilakis *et al.*¹⁴ and the Ludwigshafen Risk and Cardiovascular Health Study,¹⁵ but is in contrast to Koenig *et al.* who reported a positive correlation between the two markers.¹⁶

A few studies of the general population have examined the Lp-PLA₂-CRP relationship with respect to cardiovascular disease incidence.^{11-13, 59} Most of these studies looked at Lp-PLA₂ mass. In ARIC, a significant three-way interaction between Lp-PLA₂ mass, CRP and LDL was found, so that in those with LDL < 130 mg/dl, high Lp-PLA₂ was associated with coronary heart disease when CRP is also elevated.¹² In the current analysis, no interaction between Lp-PLA₂ activity and LDL cholesterol was observed (data not shown). Similarly, analysis of middle-age men in the Monitoring of Trends and Determinants in Cardiovascular Disease Augsburg (MONICA) survey database also showed that persons with elevations in both Lp-PLA₂ mass and CRP were at an increased risk of coronary heart disease compared with those with an elevation in either marker alone, but did not report directly testing for an interaction.¹¹ In contrast, the Women's Health Study (WHS) did not find an association between Lp-PLA₂ mass and cardiovascular disease, although CRP was significantly associated with disease incidence.⁵⁹ As in our study, the Rotterdam Study examined Lp-PLA₂ activity and reported an independent association between Lp-PLA₂ activity and coronary heart disease, however there was no interaction between Lp-PLA₂ activity and CRP.¹³ We

have also shown that while the level of Lp-PLA₂ did not differ between haptoglobin genotype, Lp-PLA₂ activity was associated with CAD in the 2/1 genotype but not in the 2/2 genotype. Similarly, the incidence of CAD is elevated in those with high Lp-PLA₂ and high CRP in persons with the 2/1 genotype but not the 2/2 genotype. We are unaware of any prior reports suggesting this relationship between haptoglobin and Lp-PLA₂ with respect to CAD.

It is interesting to speculate why Lp-PLA₂ would only predict CAD in the lower risk Hp 2/1 subgroup. It is clearly not simply due to absolute levels being increased only in Hp 2/1 CAD cases, as the mean values for CAD cases are similar in both Hp 2/1 and 2/2 (data not shown). The most likely explanation, beyond chance, is that of a complex interplay with other risk factors and time to CAD event. For example, given one's genetic background (Hp 2/2), one may be predisposed to CAD events, thus the additive effect of Lp-PLA₂ activity is difficult to demonstrate. However, in a lower risk group (Hp 2/1), this added risk (Lp-PLA₂ activity) becomes evident. While in Hp 2/1, those with Lp-PLA₂ activity below the median level seemed to be protected in terms of time to first CAD event (mean follow-up time to event approximately 11.5 years), those with Lp-PLA₂ activity above the median level had a mean time to event that was similar to that seen in Hp 2/2 (mean follow-up time to event approximately 7 years). This difference in time to CAD event demonstrates that Lp-PLA₂ activity yields a comparable risk in Hp 2/1 to that of Hp 2/2 overall. This result is also consistent with events being generally more inflammatory induced in Hp 2/1 and less so in Hp 2/2 where oxidative mechanisms may predominate. However, these results should be viewed with caution, owing to the small sample size (i.e. a total of 70 individuals and 35 CAD events), although the sample size is quite large for long term follow-up of the type of participant being studied, i.e. persons with T1D and renal disease.

Our study has many strengths, including a prospective design with long-term follow-up (through 18 years) to examine the incidence of CAD events which were confirmed by medical records. The major limitation of the study is the small sample size, particularly the small number of study participants with the 1/1 haptoglobin genotype, which does not allow us to run multivariate analysis within that subgroup. In addition, there is a potential for a survivor bias in the high CAD risk 2/2 genotype group, such that the most at-risk individuals are not represented due to death prior to commencement of the study.

In conclusion, this study among individuals with T1D and renal disease has demonstrated an interaction between Lp-PLA₂ activity and CRP with respect to CAD risk. However, this relationship does not seem to exist in individuals with the higher-risk 2/2 haptoglobin genotype. In addition, in persons with the 2/1 haptoglobin genotype, Lp-PLA₂ activity is an independent predictor of CAD incidence, suggesting that it may be useful as a marker of risk in persons expected to have a lower genetic susceptibility to developing CAD.

Acknowledgements

This research was supported by National Institutes of Health Grant DK34818. We thank the EDC staff and all study participants for their contributions. The authors declare that they have no conflicts of interest.

References

- Ross R (1999) Atherosclerosis: an inflammatory disease. *N Engl J Med* 340: 115–126.
- McPhee CH, Moores KE, Boyd HF, *et al.* (1999) Lipoprotein-associated phospholipase A₂, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem J* 338: 479–487.
- Snyder F (1995) Platelet-activating factor and its analogs: metabolic pathways and related intracellular processes. *Biochim Biophys Acta* 1254: 231–249.
- Häkkinen T, Luoma JS, Hiltunen MO, *et al.* (1999) Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 19: 2909–2917.
- Carpenter KL, Dennis IF, Challis IR, *et al.* (2001) Inhibition of lipoprotein-associated phospholipase A2 diminishes the death-inducing effects of oxidised LDL on human monocyte-macrophages. *FEBS Lett* 50: 357–363.
- Tjoelker LW, Wilder C, Eberhardt C, *et al.* (1995) Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature* 374: 549–553.
- Quarck R, De Geest B, Stengel D, *et al.* (2001) Adenovirus-mediated gene transfer of human platelet-activating factor-acetylhydrolase prevents injury-induced neointima formation and reduces spontaneous atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 103: 2495–2500.
- Noto H, Hara M, Karasawa K, *et al.* (2003) Human plasma platelet-activating factor acetylhydrolase binds to all the murine lipoproteins, conferring protection against oxidative stress. *Arterioscler Thromb Vasc Biol* 23: 829–835.
- Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphie CH (2000) Lipoprotein-associated phospholipase A₂, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. *Atherosclerosis* 150: 413–419.
- Packard CJ, O'Reilly DSJ, Caslake MJ, *et al.* (2000) Lipoprotein-associated phospholipase A₂ as an independent predictor of coronary artery disease. *N Engl J Med* 2000; 343: 1148–1154.
- Koenig W, Khuseynova N, Löwel H, Trischler G, Meisinger C (2004) Lipoprotein-associated phospholipase A₂ adds to the risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. *Circulation* 110: 1903–1908.
- Ballantyne CM, Hoogveeën RC, Bang H, *et al.* (2004) Lipoprotein-associated phospholipase A₂, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 109: 837–842.
- Oei HS, van der Meer IM, Hofman A, *et al.* Lipoprotein-associated phospholipase A₂ activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. *Circulation* 111: 570–575.
- Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB (2005) Association of Lipoprotein-associated phospholipase A₂ levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. *Eur Heart J* 26: 137–144.
- Winkler K, Winkelmann BR, Schrnagl H, *et al.* Platelet-activating factor acetylhydrolase activity indicates angiographic coronary artery disease independently of systemic inflammation and other risk factors: the Ludwigshafen Risk and Cardiovascular Health Study. *Circulation* 111: 980–987.
- Koenig W, Twardella D, Brenner H, Rothenbacher D. (2006) Lipoprotein-associated phospholipase A₂ predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. *Arterioscler Thromb Vasc Biol* 26: 1586–1593.
- Kim JY, Hyun YJ, Jang Y, *et al.* (2008) Lipoprotein-associated phospholipase A₂ activity is associated with coronary artery disease and markers of oxidative stress: a case-control study. *Am J Clin Nutr* 88: 360–367.
- Deckert T, Poulsen JE, Larsen M. (1978) Prognosis of diabetics with diabetes onset before the age of thirty-one. II. Factors influencing the prognosis. *Diabetologia* 14: 371–377.

19. Christlieb AR, Warram JH, Krolewski AS, *et al.* (1981) Hypertension: the major risk factor in juvenile-onset insulin-dependent diabetics. *Diabetes* 30: 90–96.
20. Dorman JS, LaPorte RE, Kuller LH, *et al.* (1984) The Pittsburgh Insulin-Dependent Diabetes Mellitus (IDDM) Morbidity and Mortality Study: mortality results. *Diabetes* 33: 271–276.
21. Krolewski AS, Kosinski EJ, Warram JH, *et al.* (1987) Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol* 59: 750–755.
22. Moss SE, Klein R, Klein BE (1991) Cause-specific mortality in a population-based study of diabetes. *Am J Public Health* 81: 1158–1162.
23. Orchard TJ, Olson JC, Erbey JR, *et al.* (2003) Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes. *Diabetes Care* 26: 1374–1379.
24. Soedamah-Muthu SS, Chaturvedi N, Toeller M, *et al.* (2004) The EURODIAB Prospective Complications Study Group: Risk factors for coronary heart disease in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study. *Diabetes Care* 27: 530–537.
25. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ (2006) The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes* 55: 1463–1469.
26. Ridker PM, Hennekens CH, Buring JE, Rifai N (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342: 836–843.
27. Rohde LEP, Hennekens CH, Ridker PM (1999) Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am J Cardiol* 84: 1018–1022.
28. Strandberg TE, Tilvis RS (2000) C-reactive protein, cardiovascular disease, and mortality in a prospective study in the elderly. *Arterioscler Thromb Vasc Biol* 20: 1057–1060.
29. Freeman DJ, Norrie J, Caslake MJ, *et al.* (2002) C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes* 51: 1596–1600.
30. Ridker PM, Glynn RJ, Hennekens CH (1998) C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 97: 2007–2011.
31. Danesh J, Whincup P, Walker M, *et al.* (2000) Low grade inflammation and coronary artery disease: prospective study and updated meta-analyses. *BMJ* 321: 199–204.
32. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 1557–1565.
33. Ridker PM (2003) Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 107: 363–369.
34. Boekholdt SM, Sandhu MS, Day NE, *et al.* (2006) Physical activity, C-reactive protein levels and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Eur J Cardiovasc Prev Rehabil* 13: 970–976.
35. Pu LJ, Lu L, Xu XW, *et al.* (2006) Value of serum glycosylated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol* 5: 27.
36. Hsieh MC, Tien KJ, Chang SJ, *et al.* (2008) High-sensitivity C-reactive protein and silent myocardial ischemia in Chinese with type 2 diabetes mellitus. *Metabolism* 57: 1533–1538.
37. Colhoun HM, Schalkwijk C, Rubens MB, Stehouwer CD (2002) C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification. *Diabetes Care* 25: 1813–1817.
38. Costacou T, Zgibor JC, Evans RW, *et al.* (2005) The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia* 48: 41–48.
39. Jenkins AJ, Rothen M, Klein RL, *et al.* (2008) Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDIC cohort. *J Diabetes Complications* 22: 153–163.
40. Levy AP, Hochberg I, Jablonski K, *et al.* (2002) Strong Heart Study: haptoglobin genotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study. *J Am Coll Cardiol* 40: 1984–1990.
41. Suleiman M, Aronson D, Asleh R, *et al.* (2005) Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. *Diabetes* 54: 2802–2806.
42. Roguin A, Koch W, Kastrati A, Aronson D, Schomig A, Levy AP (2003) Haptoglobin genotype is predictive of major adverse cardiac events in the 1-year period after percutaneous transluminal coronary angioplasty in individuals with diabetes. *Diabetes Care* 26: 2628–2631.
43. Costacou T, Ferrell RE, Orchard TJ (2008) Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. *Diabetes* 57: 1702.
44. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR (1985) The changing natural history of nephropathy in type I diabetes. *Am J Med* 78: 785–794.
45. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T (1987) Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without nephropathy: incidence and risk factors. *Diabetologia* 30: 144–148.
46. Tuomilehto J, Borch-Johnsen K, Mollarius A, *et al.* (1998) Incidence of cardiovascular disease in type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland. *Diabetologia* 41: 784–790.
47. Klein BEK, Klein R, McBride PE, *et al.* (2004) Cardiovascular disease, mortality, and retinal microvascular characteristics

- in type 1 diabetes: Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch Intern Med* 164: 1917–1924.
48. Torffvit O, Lövestam-Adrian M, Agardh E, Agardh C-D (2005) Nephropathy, but not retinopathy, is associated with the development of heart disease in type 1 diabetes: as 12-year observation study of 462 patients. *Diabet Med* 22: 723–729.
 49. Giunti S, Bruno G, Veglio M, *et al.* (2005) Electrocardiographic left ventricular hypertrophy in type 1 diabetes: prevalence and relation to coronary heart disease and the cardiovascular risk factors: the Eurodiab IDDM Complications Study. *Diabetes Care* 28: 2255–2257.
 50. Koch W, Latz W, Eichinger M, *et al.* (2002) Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. *Clin Chem* 48: 1377–1382.
 51. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470–475.
 55. Bucolo G, David H (1973) Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 19: 476–482.
 53. Warnick GR, Albers JJ (1978) Heparin–Mn²⁺ quantitation of high-density-lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples. *Clin Chem* 24: 900–904.
 54. National Institutes of Health, Department of Health (1975) *Lipid Research Clinics Program*. Washington, DC: US Government Printing Office, pp 75–628
 55. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18: 499–502.
 56. Borhani NO, Kass EH, Langford HG, Payne GH, Remington RD, Stamler J (1976) The hypertension detection and follow-up program. *Prev Med* 5: 207–215.
 57. Furberg CD, Nelson JJ, Solomon CS, Cushman M, Swords JN, Psaty BM (2008) Distribution and correlates of lipoprotein-associated phospholipase A2 in an elderly cohort: the Cardiovascular Health Study. *J Am Geriatr Soc* 56: 792–799.
 58. Allison MA, Denenberg JO, Nelson JJ, Natarajan L, Criqui MH (2007) The association between lipoprotein-associated phospholipase A2 and cardiovascular disease and total mortality in vascular medicine patients. *J Vasc Surg* 46: 500–506.
 59. Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM (2001) A prospective evaluation of lipoprotein-associated phospholipase A2 levels and the risk of future cardiovascular events in women. *J Am Coll Cardiol* 38: 1302–1306.