Lipoprotein-associated phospholipase A_2 activity is associated with coronary artery disease and markers of oxidative stress: a case-control study[1–3]

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ABSTRACT

Background: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a lipoprotein-bound enzyme that can release atherogenic isoprostanes from esterified phospholipids and that may be involved in inflammation and atherosclerosis.

Objective: This study investigates the association between Lp-PLA_2 activity and coronary artery disease (CAD) in relation to oxidative stress markers, in particular urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$).

Design: We conducted a case-control study in which the cross-sectional relation between Lp-PLA_2 activity, lipoproteins, and oxidative stress markers was determined in 799 patients with angiographically confirmed CAD and 925 healthy controls.

Results: Lp-PLA_2 activity was significantly ($P < 0.001$) higher in CAD cases than in controls (32.9 ± 0.46 and 29.7 ± 0.42 $nmol \cdot mL^{-1} \cdot min^{-1}$, respectively). Both elevated Lp-PLA_2 activity and urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$ were associated with greater CAD risk (P for trend < 0.001). Odds ratios for the upper quartiles of Lp-PLA_2 activity and 8-epi-$PGF_{2\alpha}$ excretion were 2.47 (95% CI: 1.79, 3.40) and 2.19 (1.52, 3.15), respectively, after adjustment for sex, age, BMI, blood pressure, smoking and alcohol consumption status, and LDL and HDL cholesterol. When we examined the additive effect of both markers for CAD risk, the relation between 8-epi-$PGF_{2\alpha}$ and CAD was weakened above the second quartile of Lp-PLA_2 activity. Moreover, Lp-PLA_2 activity was positively correlated with urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$ in controls ($r = 0.277$, $P < 0.001$) and cases ($r = 0.202$, $P < 0.001$) and with the tail moment of lymphocyte DNA ($r = 0.213$, $P < 0.001$) in controls.

Conclusion: This study shows an association of elevated Lp-PLA_2 activity with CAD risk in relation to oxidant stress and thus supports a proatherogenic role of Lp-PLA_2. *Am J Clin Nutr* 2008;88:630–7.

INTRODUCTION

The biological role of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is controversial: whereas initial reports indicate an antiatherogenic effect (1), there is growing evidence of a role for Lp-PLA_2 as a proinflammatory molecule and an independent risk factor for coronary artery disease (CAD) (2–6). Lp-PLA_2 is a lipoprotein-bound enzyme that catalyzes the hydrolysis of oxidized phospholipids, generating bioactive oxidized free fatty acids and lysophosphatidylcholine, which are potent proinflammatory and proatherogenic products (7). In addition, plasma Lp-PLA_2 is responsible for the release of F_2-isoprostanes, the end-products of lipid peroxidation, into the bloodstream (8).

Some studies have indicated that F_2-isoprostanes are probably the most valid in vivo markers of oxidative stress (9, 10). There is evidence that cardiovascular risk is related, at least in part, to greater systemic oxidative stress, and oxidative damage is known to be involved in the pathogenesis of atherosclerosis and CAD (11, 12), in which it is closely associated with the inflammatory response and bioactive lipid formation. To determine the oxidation status of a person, a set of oxidative markers is recommended, including ≥1 marker each for lipid peroxidation, protein oxidation, and total antioxidative status and, ideally, 1 marker for DNA damage (13).

This case-control study was designed to determine the relation between Lp-PLA_2 activity and angiographically proven CAD. In addition, we wanted to explore the relation between Lp-PLA_2 and a variety of oxidative stress markers, in particular urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), to shed further light on the pathogenic role of this emerging biomarker for CAD risk.

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SUBJECTS AND METHODS

Subjects

This study involved 1724 unrelated Korean subjects (1520 men and 204 women) aged 31–83 y. Subjects were divided into 2 subgroups: a control group of 925 persons (805 men and 120 women) with no history or clinical evidence of CAD, who were recruited either from the Health Service Center during the course of routine check-up visits or by advertisements briefly describing the study design, and a case group of 799 patients (715 men and 84 women) with CAD, who were recruited from the Cardiovascular Center at Yonsei University Severance Hospital (Seoul, Korea) or National Health Insurance Corporation Ilsan Hospital (Goyang-si, Korea).

The inclusion criteria for controls were no history or diagnosis of atherosclerosis, vascular disease, chronic heart failure and arrhythmias, diabetes mellitus, or cancer and no pathologic electrocardiogram patterns. The inclusion criteria for CAD cases were angiographic evidence of ≥50% occlusion of ≥1 major coronary artery or previously confirmed myocardial infarction according to the World Health Organization criteria for symptoms, enzyme elevation, or electrocardiographic changes; absence of nonatherogenic occlusion, such as osteal stenosis and spasm; and no history or diagnosis of diabetes mellitus or any diagnosis of thyroid or pituitary disease. Exclusion criteria included abnormal liver or renal function (ie, serum aminotransferase activity > 40 IU/L and serum creatinine concentrations > 1.2 mg/dL); cancer (diagnosed clinically or by anamnesis); extreme weight loss or gain over the previous 6 mo; thyroid or pituitary disease; infection determined by medical questionnaire and complete blood count; and acute or chronic inflammatory disease.

The purpose of the study was carefully explained to all participants, and their written informed consent was obtained. The study protocol was approved by the Institutional Review Board of Yonsei University, and the study was carried out in accordance with the Helsinki Declaration.

Survey method, anthropometric measurements, and blood collection

All subjects completed a standardized questionnaire administered by a specially trained interviewer to provide information on lifestyle factors, current medication use, and medical history. For CAD patients, information was also taken from the hospital medical records. Briefly, subjects were categorized into current smokers, ex-smokers, and never smokers and current alcohol consumers, ex-consumers, or nonconsumers. Data on the frequency of use of all medications, in particular the antihypertensive, hypoglycemic, antidyslipidemic, and antiplatelet drugs, were collected.

Body weight and height were measured in the morning, while the participants were unclothed and not wearing shoes. Body mass index (BMI; in kg/m^2) was calculated. Waist and hip circumferences were measured with the use of a paper tape placed horizontally at the umbilicus while the participants were in a standing position after normal expiration. Blood pressure was measured from the left arm of seated participants with the use of an automatic blood pressure monitor (TM-2654; A&D, Tokyo, Japan) after 20 min of rest. The average of 3 measurements was recorded for each subject.

Venous blood specimens were collected in EDTA-treated or plain tubes after a 12-h fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1–3 h), where they were stored at −70 °C until they were analyzed.

Serum lipid profile and C-reactive protein concentraion

Fasting serum concentrations of total cholesterol and triglycerides were measured by using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan). After precipitation of serum chylomicron conducted with dextran sulfate magnesium, the concentrations of LDL and HDL cholesterol in the supernatant fluid were measured by using an enzymatic method. LDL cholesterol was indirectly estimated in participants with serum triglyceride concentrations < 4.52 mol/L (400 mg/mL) by using the Friedewald formula. In participants with serum triglyceride concentrations ≥ 4.52 mol/L, LDL cholesterol was directly measured by using an enzymatic method on a Hitachi 7150 Autoanalyzer. Serum apolipoprotein A-I and B concentrations were measured by using turbidometry at 340 nm with a specific antiserum (Roche, Basel, Switzerland). Serum high-sensitivity C-reactive protein (hs-CRP) concentrations were measured with an Express plus autoanalyzer (Chiron Diagnostics Co, Walpole, MA) by using a commercially available hs-CRP Latex (II) X2 kit (Seiken Laboratories Ltd, Tokyo, Japan) that allowed detection of CRP concentrations in the range of 0.001 to 32 mg/dL.

Lipoprotein-associated phospholipase A_2 activity and plasma oxidized LDL concentrations

Lp-PLA_2 activity, also known as platelet-activating factor (PAF)–acetylhydrolase, was measured by using a modification of a previously described high-throughput radiometric activity assay (4). Briefly, a micelle substrate was prepared with unlabeled PAF and [^{3}H]PAF (100 μCi/mL, 21.5 Ci/mmol; NET 910; PerkinElmer, Waltham, MA) in 10 mmol phosphate-buffered saline (PBS)/L (pH 7.4), containing 2.7 mmol EDTA/L (PBS-EDTA). The reaction mixture, containing 20 μL human plasma and 140 μL PBS-EDTA, was incubated at 37 °C for 15 min. The reaction was initiated by the addition of 40 μL micelle substrate (0.05 μCi, final concentration 80 μmol PAF/L) to measure the initial Lp-PLA_2 activity. The reaction was stopped by mixing by vortex with 600 μL CHCl:MeOH (2:1), and the $CHCl_3$ and aqueous layers were separated by centrifugation at 1500 rpm for 3 min at 4 °C. The aqueous layer (300 μL) was removed and mixed by vortex with 300 μL CHCl. The aqueous layer (100 μL) was again removed and added to 3 mL scintillation cocktail (Lumagel; Lumac Co, Groningen, Netherlands) in a scintillation vial. The [^{3}H] acetate counts were determined by using a liquid scintillation counter (1450 Microbeta Trilux; Qallac Oy, Turku, Finland). The raw counts were corrected for background by using an enzyme-noncontaining blank and were expressed as nmol degraded PAF · min^{-1} · mL^{-1} plasma. The intraassay and interassay CVs were 11.27% and 8.71%, respectively.

Plasma oxidized LDL (ox-LDL) was measured by using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The resultant color reaction was read at 450 nm with a Wallac Victor^2 multilabel counter (Perkin Elmer Life Sciences, Turku, Finland).

Lipid peroxidation: urinary 8-epi-prostaglandin $F_{2\alpha}$and plasma malondialdehyde

Urine was collected in polyethylene bottles containing 1% butylated hydroxytoluene after a 12-h fast. The tubes were immediately covered with aluminum foil and stored at −70 °C until they were analyzed. We measured 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) by using an enzyme immunoassay (BIOXYTECH urinary 8-epi-$PGF_{2\alpha}$ assay kit; OXIS International Inc, Portland, OR) and the resulting color reaction was read at 650 nm by using a Wallac Victor^2 multilabel counter. Urinary creatinine was measured by using the alkaline picrated (Jaffe) reaction. Urinary 8-epi-$PGF_{2\alpha}$ concentrations were expressed as pmol/mmol creatinine. Plasma malondialdehyde (MDA) was assayed by using the fluorometric method described by Buckingham (14).

Alkaline comet assay for DNA damage

For the comet assay, 120 μL whole blood was mixed with 900 μL PBS, and the mixture was poured gently over 150 μL lymphocyte separation solution (Histopaque-1077; Sigma-Aldrich Korea Ltd, Yong-In, Korea). After a 4-min centrifugation at 1450 rpm and 4 °C, lymphocytes were removed with a pipette and transferred to another tube. DNA damage was analyzed as described by Green et al (15).

Statistical analysis

Statistical analyses were performed with SPSS for WINDOWS software (version 12.0; SPSS Inc, Chicago, IL). Differences in biomarkers between CAD cases and controls were analyzed by using Student's *t* test. A chi-square test was used to test whether there was a difference in sex distribution, cigarette smoking, alcohol consumption, and medication therapy between CAD cases and controls. Pearson and partial correlation coefficients were used to examine the relation of Lp-PLA_2 activity and markers of oxidative stress. In addition, Bonferroni correction for multiple testing was used with $\alpha = 0.005$ after the 11 different laboratory variables tested were taken into account.

Plasma Lp-PLA_2 activity or urinary 8-epi-$PGF_{2\alpha}$ concentrations were divided into quartiles according to their distribution in the control group. Next, multiple logistic regression analysis including CAD as a dependent variable and Lp-PLA_2 activity or urinary excretion concentration of 8-epi-$PGF_{2\alpha}$ as an independent variable was applied to estimate the independent association of elevated Lp-PLA_2 activity or urinary 8-epi-$PGF_{2\alpha}$ excretion concentration with the presence of CAD; simultaneous control for confounding factors was exerted. Results were presented as odds ratio (ORs) and 95% CIs estimated from the logistic regression model with initial control for sex, age, and BMI and subsequent further control for blood pressure, cigarette smoking, alcohol consumption, lipid profiles, and statin therapy. Finally, the Lp-PLA_2 activity × urinary excretion concentration of 8-epi-$PGF_{2\alpha}$ interaction for CAD risk was evaluated by using multiple logistic regression analysis. Each OR was estimated with comparison of the reference group in the lowest quartiles for both variables after adjustment for sex, age, BMI, blood pressure, and smoking and alcohol consumption status.

We examined whether each variable presented a normal distribution before statistical testing, and then we performed logarithmic transformation on the skewed variable (ie, triglycerides, 8-epi-$PGF_{2\alpha}$, CRP, malondialdehyde, and ox-LDL). For descriptive purposes, mean values are presented by using untransformed and unadjusted values. Results are expressed as mean ± SEs, and a 2-tailed value of $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of cases and controls

General characteristics of 799 CAD cases and 925 controls are shown in **Table 1**. Cases were significantly older and heavier and had a larger waist-to-hip ratio than did controls, whereas there was no significant difference between the groups in sex distribution. Current smoking and alcohol consumption were more common in the controls than in the cases. Statins, antihypertensives, and antiplatelet agents were used more frequently in cases than in controls (47.8% and 0.9%, 69% and 4.8%, and 71% and 1.2%, respectively). Consequently, systolic and diastolic blood pressure and concentrations of total, LDL, and HDL cholesterol were lower in cases than in controls, although cases had higher concentrations of triglyceride. Oxidative stress and inflammation were evaluated by measurement of urinary 8-epi-$PGF_{2\alpha}$, plasma MDA, lymphocyte DNA damage, ox-LDL, and serum hs-CRP. Compared with controls, cases had significantly higher concentrations of urinary 8-epi-$PGF_{2\alpha}$, plasma MDA, and serum hs-CRP; significantly greater lymphocyte DNA damage; and significantly lower concentrations of ox-LDL. Lp-PLA_2 activity was significantly higher in cases than in controls (32.9 ± 0.46 and 29.7 ± 0.42 nmol · mL^{-1} · min^{-1}, respectively; $P < 0.001$) (Table 1).

Correlation between lipoprotein-associated phospholipase A_2 activity, lipoproteins, high-sensitivity C-reactive protein, and markers of oxidative stress

In controls, Lp-PLA_2 activity was positively correlated with urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$ and lymphoctye DNA damage (tail DNA, tail length, and tail moment) after Bonferroni correction for multiple testing ($\alpha = 0.005$) (**Table 2**). These associations remained significant after adjustment for age and medication use. In cases, Lp-PLA_2 activity was positively correlated with urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$ after Bonferroni correction; however, Lp-PLA_2 activity was not significantly correlated with age; BMI; total, HDL, or LDL cholesterol; triglyceride; MDA; ox-LDL; or CRP in either cases or controls (Table 2).

Association of lipoprotein-associated phospholipase A_2 activity and 8-epi-prostaglandin F2α concentration with coronary artery disease

Both Lp-PLA_2 activity and urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$ showed a concentration-dependent association with CAD in the logistic regression model after control for the confounding factors in stages (**Table 3**). Lp-PLA_2 activity in the highest quartile (≥41.0 nmol · mL^{-1} · min^{-1}) was associated with a 2.14-fold risk of CAD (95% CI: 1.61, 2.84) compared with the lowest quartile of Lp-PLA_2 activity (<20.4 nmol · mL^{-1} · min^{-1}) in a model adjusted for sex, age, and BMI (model 1). After additional adjustment for blood pressure, smoking and alcohol consumption status, and LDL and HDL cholesterol, the OR increased to 2.47 (95% CI: 1.79, 3.40) (model 4). Further adjustment for statin therapy (model 5) attenuated the risk associated

TABLE 1
Anthropometric and biochemical characteristics of controls and coronary artery disease (CAD) cases[1]

	CAD cases (n = 799)	Controls (n = 925)	P[2]
Age (y)	56.1 ± 0.3[3]	55.2 ± 0.3	0.036
Female [n (%)]	84 (11)	120 (13)	NS
BMI (kg/m^2)	25.1 ± 0.1	24.6 ± 0.1	<0.001
Waist-to-hip ratio	0.9 ± 0.0	0.9 ± 0.0	<0.001
Current smoker [n (%)]	178 (22)	256 (28)	0.009
Current drinker [n (%)]	432 (54)	620 (67)	<0.001
Statin therapy [n (%)]	382 (47.8)	8 (0.9)	<0.001
Antihypertensive therapy [n (%)]	548 (69)	44 (4.8)	<0.001
Antiplatelet therapy [n (%)]	567 (71)	11 (1.2)	<0.001
Systolic BP (mm Hg)	124.1 ± 0.6	125.8 ± 0.5	0.034
Diastolic BP (mm Hg)	77.7 ± 0.3	79.8 ± 0.4	<0.001
Total cholesterol (mg/dL)	171.2 ± 1.4	196.1 ± 1.2	<0.001
HDL cholesterol (mg/dL)	43.6 ± 0.4	51.0 ± 0.5	<0.001
LDL cholesterol (mg/dL)	97.8 ± 1.3	117.4 ± 1.1	<0.001
Triglyceride (mg/dL)[4]	141.2 ± 2.4	132.2 ± 2.2	<0.001
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)[4]	1332.9 ± 29.4	1123.6 ± 20.1	<0.001
Malondialdehyde (nmol/mL)[4]	11.8 ± 0.2	9.2 ± 0.1	<0.001
Lymphocyte DNA damage			
Tail DNA (%)	13.1 ± 0.3	13.0 ± 0.3	NS
Tail length (μm)	73.1 ± 2.4	49.1 ± 0.9	<0.001
Tail moment[5]	13.2 ± 0.7	7.8 ± 0.3	<0.001
Oxidized LDL (U/L)[4]	48.8 ± 1.1	57.6 ± 1.0	<0.001
hs-CRP (mg/dL)[4]	2.2 ± 0.2	1.2 ± 0.1	<0.001
Lp-PLA_2 activity (nmol · mL^{-1} · min^{-1})	32.9 ± 0.5	29.7 ± 0.4	<0.001

[1] BP, blood pressure; 8-epi-$PGF_{2\alpha}$, 8-epi-prostaglandin $F_{2\alpha}$; hs-CRP, high-sensitivity C-reactive protein; Lp-PLA_2, lipoprotein-associated phospholipase A_2. Data are reported by using untransformed values.
[2] Tested by Student's t test or chi-square test (2-sided).
[3] $\bar{x}$ ± SE (all such values).
[4] Tested by logarithmic transformation.
[5] Tail moment = % of DNA in the tail × tail length (μm).

with high Lp-PLA_2 activity, although the risk remained significantly elevated, with an OR of 1.92 (95% CI: 1.32, 2.79), in the highest quartile compared with the lowest quartile.

The urinary excretion concentration of 8-epi-$PGF_{2\alpha}$ was associated with a higher OR for CAD in the third (≥1012.9–1381.8 pg/mg creatinine) and top (≥1381.8 pg/mg creatinine) quartiles, as compared with the lowest quartile (<744.6 pg/mg creatinine): the OR for 8-epi-$PGF_{2\alpha}$ in the highest quartile was 2.19 (95% CI: 1.59, 3.0) after adjustment for sex, age, and BMI model (model 1) and 2.55 (95% CI: 1.60, 4.06) after further adjustment for blood pressure, smoking and alcohol consumption status, lipids, and statin therapy (model 5). However, when we also adjusted for Lp-PLA_2 activity (model 6), the OR for the highest quartile was reduced to 1.80 (95% CI: 1.01, 2.94), and 8-epi-$PGF_{2\alpha}$ concentrations in the highest quartile were significantly associated with a greater CAD risk only when compared with values in the lowest quartile.

Because the joint effect of the quartile of Lp-PLA_2 activity and the quartile of urinary 8-epi-$PGF_{2\alpha}$ concentration was significant ($P < 0.001$ for χ^2 test with 15 df), the interactive effect of both markers with respect to CAD risk was evaluated (**Figure 1**). Compared with participants in the lowest categories of both variables, participants in almost all of the other categories had significantly greater risk of CAD; those in the lowest quartile of Lp-PLA_2 activity and the second and third quartiles of 8-epi-$PGF_{2\alpha}$ concentrations did not have greater risk. The combination of elevated Lp-PLA_2 activity and elevated 8-epi-$PGF_{2\alpha}$ concentrations was generally more significant than was the elevation of either one separately. Overall, the increase in CAD risk with increasing Lp-PLA_2 activity was sustained with respect to increases in 8-epi-$PGF_{2\alpha}$ concentrations; however, the relation between 8-epi-$PGF_{2\alpha}$ and CAD risk was weakened above the second quartile of Lp-PLA_2 activity.

DISCUSSION

To our knowledge, the present study is the first to focus on the relation between Lp-PLA_2 activity and markers of oxidative stress and, in particular, on the association between urinary excretion of 8-epi-$PGF_{2\alpha}$ and CAD. In this case-control study, Lp-PLA_2 activity and urinary 8-epi-$PGF_{2\alpha}$ concentration were higher in patients with CAD than in healthy controls, and both elevated Lp-PLA_2 activity and urinary excretion concentration of 8-epi-$PGF_{2\alpha}$ were significantly associated with the risk of CAD, even after control for potential confounders including HDL cholesterol, LDL cholesterol, and statin therapy. It is interesting that, when the combined effect of 8-epi-$PGF_{2\alpha}$ concentration and Lp-PLA_2 activity on CAD risk was examined, the association between 8-epi-$PGF_{2\alpha}$ concentration and CAD risk was weakened by greater Lp-PLA_2 activity. In addition, when CAD risk for the upper quartiles of 8-epi-$PGF_{2\alpha}$ was compared with that for the lowest quartile, additional adjustment for Lp-PLA_2 activity attenuated the risk associated with elevated 8-epi-$PGF_{2\alpha}$. Our data show the confirmatory association of elevated

TABLE 2
Correlation coefficients between lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity and lipid profiles, C-reactive protein, and oxidative stress markers in coronary artery disease (CAD) cases and controls[1]

	CAD cases (n = 799)				Controls (n = 925)			
	r	P	R^2	P^2	r	P	R^2	P^2
Age (y)	−0.015	0.667			0.025	0.450		
BMI (kg/m^2)	0.027	0.439	0.032	0.429	0.014	0.662	0.010	0.789
Total cholesterol (mg/dL)	0.048	0.174	0.071	0.081	0.035	0.282	0.034	0.344
HDL cholesterol (mg/dL)	−0.062	0.081	−0.050	0.226	−0.015	0.647	−0.008	0.827
LDL cholesterol (mg/dL)	0.076	0.035	0.084	0.042	0.062	0.066	0.042	0.241
Triglyceride (mg/dL)[3]	0.026	0.476	0.075	0.069	0.011	0.735	−0.001	0.983
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)[3]	0.202	<0.001[4]	0.192	<0.001[4]	0.277	<0.001[4]	0.291	<0.001[4]
Malondialdehyde (nmol/mL)[3]	0.061	0.094	0.022	0.601	0.061	0.092	0.034	0.388
Lymphocyte DNA damage								
Tail DNA (%)	0.100	0.047	0.096	0.058	0.190	<0.001[4]	0.199	<0.001[4]
Tail length (μm)	0.046	0.360	0.045	0.381	0.165	<0.001[4]	0.161	0.005
Tail moment[5]	0.068	0.174	0.068	0.177	0.213	<0.001[4]	0.220	<0.001[4]
Oxidized LDL (U/L)[3]	−0.018	0.690	−0.040	0.490	−0.046	0.226	0.063	0.137
hs-CRP (mg/dL)[3]	0.025	0.492	0.042	0.305	−0.014	0.692	0.008	0.819

[1] 8-epi-$PGF_{2\alpha}$, 8-epi-prostaglandin $F_{2\alpha}$; hs-CRP, high-sensitivity C-reactive protein.
[2] Controlled for age and medication use (antihypertensive, statin, and antiplatelet therapy).
[3] Tested by logarithmic transformation.
[4] Statistically significant correlation after Bonferroni correction.
[5] Tail moment = % of DNA in the tail × tail length (μm).

Lp-PLA_2 activity with CAD risk, which is consistent with previous reports (2–5, 16, 17), and which sheds further light on a proatherogenic role of Lp-PLA_2 in relation to oxidant stress.

Lp-PLA_2 is known to catalyze hydrolysis of the *sn*-2 position of glycerophospholipids to liberate arachidonic acid, a precursor of eicosanoids such as prostaglandins and leukotrienes (18). Radical peroxidation of arachidonic acid results in a family of prostaglandin F_2-isomers called F_2-isoprostanes (13). One such F_2-isoprostane is 8-epi-$PGF_{2\alpha}$, a sensitive and independent risk marker for coronary heart disease (19–21) that is probably released into biological fluids through a phospholipase-mediated pathway and consequently excreted in urine. Indeed, Stafforini et al (8) showed that the secreted form of Lp-PLA_2 released F_2-isoprostanes from the *sn*-2 position of phosphatidylcholine with high affinity, and Kono et al (22) recently reported that intracellular type II Lp-PLA_2, which shares homology with the plasma enzyme Lp-PLA_2, is involved in the metabolism of esterified 8-iso-$PGF_{2\alpha}$. Moreover, plasma samples from Lp-PLA_2–deficient subjects do not release F2-isoprostanes from esterified precursors ex vivo (8). Our finding of a positive correlation between Lp-PLA_2 activity and urinary excretion of 8-epi-$PGF_{2\alpha}$ in both

TABLE 3
Odds ratios (OR) of coronary artery disease associated with lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity and urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) excretion concentrations after various adjustments[1]

	Cases	Controls	Model 1[2]	Model 2[3]	Model 3[4]	Model 4[5]	Model 5[6]	Model 6[7]
	n	n						
Lp-PLA_2 activity (nmol · mL^{-1} · min^{-1})								
Quartile 1 (<20.4)	124	231	1.0	1.0	1.0	1.0	1.0	—
Quartile 2 (≥20.4–25.4)	191	231	1.54 (1.15, 2.06)	1.49 (1.10, 2.01)	1.59 (1.17, 2.18)	1.75 (1.26, 2.45)	1.18 (0.79, 1.77)	—
Quartile 3 (≥25.4–41.0)	221	232	1.78 (1.34, 2.38)	1.78 (1.33, 2.38)	1.67 (1.23, 2.27)	1.93 (1.39, 2.68)	1.76 (1.21, 2.55)	—
Quartile 4 (≥41.0)	263	231	2.14 (1.61, 2.84)	2.15 (1.61, 2.88)	2.12 (1.57, 2.86)	2.47 (1.79, 3.40)	1.92 (1.32, 2.79)	—
P for trend			<0.001	<0.001	<0.001	<0.001	0.001	
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)								
Quartile 1 (<744.6)	160	231	1.0	1.0	1.0	1.0	1.0	1.0
Quartile 2 (≥744.6–1012.9)	136	232	0.89 (0.63, 1.25)	0.83 (0.58, 1.18)	0.85 (0.59, 1.22)	0.80 (0.54, 1.18)	0.76 (0.44, 1.29)	0.66 (0.38, 1.13)
Quartile 3 (≥1012.9–1381.8)	202	231	1.39 (1.00, 1.93)	1.43 (1.02, 2.01)	1.48 (1.05, 2.09)	1.39 (0.96, 2.01)	1.80 (1.11, 2.89)	1.45 (0.89, 2.37)
Quartile 4 (≥1381.8)	299	231	2.19 (1.59, 3.00)	2.20 (1.58, 3.06)	2.35 (1.67, 3.31)	2.19 (1.52, 3.15)	2.55 (1.60, 4.06)	1.80 (1.01, 2.94)
P for trend			<0.001	<0.001	<0.001	<0.001	<0.001	0.001

[1] Data are ORs (95% CI) for the association between the 3 upper quartiles of Lp-PLA_2 activity or urinary 8-epi-$PGF_{2\alpha}$ concentrations in comparison with the bottom quartile and coronary artery disease, tested by multivariable logistic regression analysis. Quartiles are based on data from control subjects.
[2] Adjusted for sex, age, and BMI.
[3] Adjusted for sex, age, BMI, systolic and diastolic blood pressure, and smoking and alcohol consumption status.
[4] Adjusted for sex, age, BMI, systolic and diastolic blood pressure, smoking and alcohol consumption status, and HDL cholesterol.
[5] Adjusted for sex, age, BMI, systolic and diastolic blood pressure, smoking and alcohol consumption status, and HDL and LDL cholesterol.
[6] Adjusted for sex, age, BMI, systolic and diastolic blood pressure, smoking and alcohol consumption status, HDL and LDL cholesterol, and statin therapy.
[7] Adjusted for sex, age, BMI, systolic and diastolic blood pressure, smoking and alcohol consumption status, HDL and LDL cholesterol, statin therapy, and Lp-PLA_2 activity.

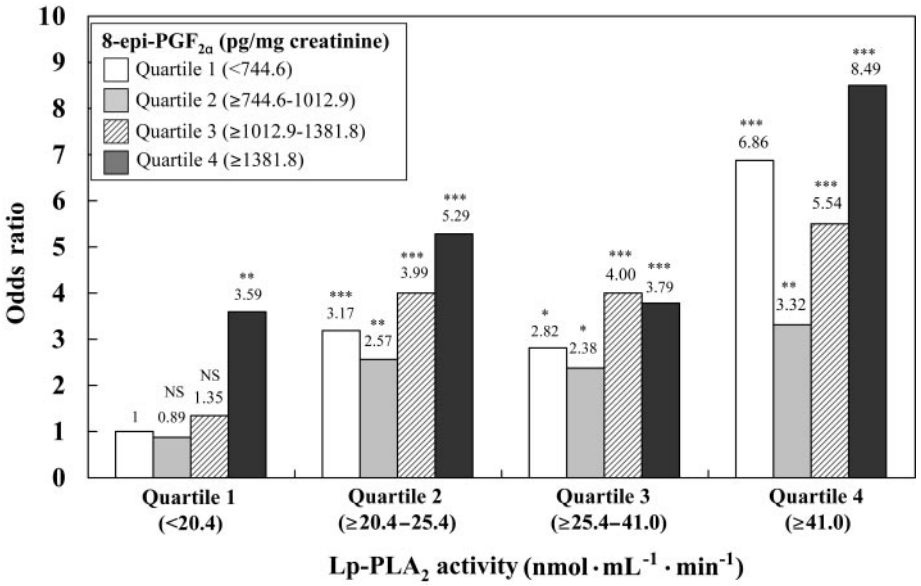

FIGURE 1. Association of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity and urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) concentrations with coronary artery disease. Odds ratios were adjusted for sex, age, BMI, systolic and diastolic blood pressure, and smoking and alcohol consumption status. Lp-PLA_2 activity × 8-epi-$PGF_{2\alpha}$ concentrations interactions were significant for coronary artery disease risk: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Compared with persons in the lowest quartile of Lp-PLA_2 activity (<20.4 nmol · mL^{-1} · min^{-1}) and of 8-epi-$PGF_{2\alpha}$ (<744.6 pg/mg creatinine), persons in most of the other categories had a significant association with a greater risk of coronary artery disease risk; those in the lowest quartile of Lp-PLA_2 activity and the second and third quartiles of 8-epi-$PGF_{2\alpha}$ did not have a significantly greater risk.

controls and cases adds evidence to support the possibility that this enzyme may modulate oxidant stress.

When the interactive effect between Lp-PLA_2 activity and urinary excretion of 8-epi-$PGF_{2\alpha}$ on CAD risk was evaluated, persons with Lp-PLA_2 activity in the highest quartile (≥41.0 nmol · mL^{-1} · min^{-1}) and 8-epi-$PGF_{2\alpha}$ concentrations in the highest quartile (≥1381.8 pg/mg creatinine) had a CAD risk >8 times greater than that in persons with Lp-PLA_2 activity (<20.4 nmol · mL^{-1} · min^{-1}) and 8-epi-$PGF_{2\alpha}$ (<744.6 pg/mg creatinine) in the lowest quartile ($P < 0.001$; 95% CI: 4.50, 16.0). Although this result has the limitation of reflecting only the relative risk in the present population, it may suggest that elevated Lp-PLA_2 activity and urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$, along with other factors that promote inflammation and oxidant stress, may drive the more susceptible status to atherosclerosis in persons. In addition, we show that the elevation of CAD risk by an increase in the urinary excretion of 8-epi-$PGF_{2\alpha}$ was attenuated by greater Lp-PLA_2 activity, which suggests that Lp-PLA_2 may play an active role in atherogenesis, rather than simply being a risk marker. However, because the present study was designed as a case-control study, we are not currently able to explain the reason for such effects.

In the present study, Lp-PLA_2 activity positively correlated with 8-epi-$PGF_{2\alpha}$ and lymphocyte DNA damage in controls. These correlations persisted after Bonferroni correction for multiple testing ($\alpha = 0.005$), even after adjustment for sex, age, BMI, smoking and alcohol consumption status, and medication therapy, all of which are known to affect concentrations of $PGF_{2\alpha}$ (13, 23, 24) and degrees of DNA damage (13, 25–28) (data not shown). Semiquantitative analysis has shown that the isoprostane 8-epi-$PGF_{2\alpha}$ is one of the most reliable markers of oxidative stress (19, 20, 29–31). Oxidative stress has been suggested as the major cause of DNA damage, and greater degrees of DNA damage in patients with CAD have been reported (32–34). In the present study, we measured lymphocyte DNA damage by using the comet assay, a sensitive and reliable determinant of oxidative stress and DNA damage (35). The positive correlation observed between Lp-PLA_2 activity and both 8-epi-$PGF_{2\alpha}$ and lymphocyte DNA damage suggests that persons with high Lp-PLA_2 activity may have higher levels of lipid peroxidation and oxidative stress and may therefore have a greater risk of CAD than do persons with normal Lp-PLA_2 activity. Such observations support the conclusion that Lp-PLA_2 is proatherogenic (36).

Ox-LDL, another variable of lipid peroxidation, did not correlate with Lp-PLA_2 activity in the present study. This lack of correlation may be partly explained by the low association between Lp-PLA_2 and LDL cholesterol in the Korean population (37), which has relatively low concentrations of LDL cholesterol (115 mg/dL for middle-aged Koreans) and a high incidence of the Val279Phe variant in the *Lp-PLA_2* gene, which indicates loss of enzyme activity. The Val279Phe variant of *Lp-PLA_2* has been reported to be associated with a lower risk of cardiovascular disease in Korean men (37). However, this result may relate to previous findings that Lp-PLA_2 does not affect the lipid peroxidation of LDL (38–40). In fact, this possibility is plausible, because the main role of Lp-PLA_2 in atherogenesis is the hydrolysis of ox-LDL, which is generated when LDL becomes oxidized in the milieu of the artery wall (41).

MDA, another lipid peroxide, is widely used as an index of oxidative damage because of its ability to interact with lipoprotein (13); however, plasma MDA measurement was reported to be a less reliable assay than lymphocyte DNA damage for detecting the severity of vascular lesions in CAD patients (42). In the present study, the weak correlation observed between MDA and Lp-PLA_2 activity may reflect differences in specificity and sensitivity for different lipid peroxides. Because the specificity of the MDA assay is low, MDA is regarded as a general indicator

of oxidative stress rather than as a specific marker of lipid peroxidation (13, 43, 44).

We observed lower concentrations of total cholesterol, LDL cholesterol, and ox-LDL in CAD patients, which reflected their widespread use of statin therapy. Although statins can reduce Lp-PLA_2 activity (45), CRP concentrations (46), and oxidative stress (47), we found that Lp-PLA_2 activity, CRP, and urinary excretion of 8-epi-$PGF_{2\alpha}$ were higher in CAD cases than in controls. Moreover, in these controls, CRP was positively correlated with urinary excretion concentrations of $PGF_{2\alpha}$ ($r = 0.134, P < 0.001$) in the absence of correlation between Lp-PLA_2 and CRP. This observation may indicate that inflammation is closely associated with oxidative stress. Schwedhelm et al (19) also found an association between CRP and urinary excretion concentrations of 8-epi-$PGF_{2\alpha}$, which was not confounded by lipid-lowering therapy or by total cholesterol, LDL-cholesterol, or triglyceride concentrations.

One limitation of the present study, as for all such case-control studies in which exposure and outcome are collected at one time-point, is the inability to assess the temporal sequence of the described associations. Despite this limitation, our results show an association of elevated Lp-PLA_2 activity with a higher incidence of CAD in relation to greater oxidant stress and thus support the hypothesis that Lp-PLA_2 represents a novel, viable marker for CAD risk.

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