Lipoprotein-Associated Phospholipase A_2 Activity is Cross-Sectionally Associated with Coronary Artery Disease and Markers of Oxidative Stress

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ABSTRACT

Lipoprotein-Associated Phospholipase A_2 Activity is Cross-Sectionally Associated with Coronary Artery Disease and Markers of Oxidative Stress

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Background: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a lipoprotein-bound enzyme that is possibly involved in inflammation and atherosclerosis. This study investigates the association between Lp-PLA_2 and coronary artery disease (CAD) in relationship to oxidative stress markers.

Methods and Results: We conducted a case-control study. Lp-PLA_2 activity, lipoproteins, high sensitive C-reactive protein (hs-CRP) and oxidative stress were determined cross-sectionally in 799 patients with angiographically confirmed CAD and in 925 healthy control subjects.

Lp-PLA$_2$ activities were higher in CAD patients than controls (32.9±0.46nmol/ml/min vs 29.7±0.42; P<0.001). In addition, values in the second, third and top quartile of Lp-PLA$_2$ activity were associated with an increased odds ratio (OR) for CAD when compared with values in the bottom quartile. OR for Lp-PLA$_2$ (top quartile) was 2.14 (95% CI, 1.61-2.84) in the sex-, age- and BMI-adjusted model and 2.50 (95% CI, 1.81-3.44) after additional adjustment for blood pressure, smoking and drinking status and total-, LDL-, HDL-cholesterol. Furthermore, Lp-PLA$_2$ activity was positively correlated with urinary excretion levels of PGF$_{2\alpha}$ (r=0.253, P<0.001) and lymphocyte DNA damage [tail DNA (r=0.136, P<0.001), tail length (r=0.116, P=0.002) and tail moment(r=0.129, P<0.001)] and plasma MDA levels (r=0.089, P<0.001) and negatively correlated with HDL-cholesterol (r=-0.069, P=0.004) in all subjects including controls and case patients.

Conclusion: This result show the association of elevated activities of Lp-PLA$_2$ with CAD risk. This could be compatible with a positive correlation between Lp-PLA$_2$ and oxidative stress markers.

Key words: Lp-PLA$_2$ activity, oxidative stress, coronary artery disease, lipid peroxidation, DNA damage

1. INTRODUCTION

Several studies link lipoprotein-associated phospholipase A_2 (Lp-PLA_2) to atherogenesis and increased risk of coronary artery disease (CAD) (1-5); however, the role of Lp-PLA_2 in atherosclerotic diseases remains to be established. Lp-PLA_2 may represent a potent antioxidative, anti-inflammatory and anti-atherogenic enzyme because it degrades platelet-activating factor, a potent lipid mediator, and proinflammatory oxidized phospholipids (6). Conversely, Lp-PLA_2 may also generate bioactive oxidized free fatty acids (7) and lysophosphatidylcholine (8). In addition, plasma Lp-PLA_2 activity catalyzes the release of F_2-isoprostanes form esterified phospholipids (9).

Recent studies have indicated F_2-isoprostanes, end product of lipid peroxidation, as probably the most valid in vivo marker of oxidative stress (10-11). Cardiovascular risk is believed to be at least in part related to increased systemic oxidative stress. Oxidative damage is known to be involved in the pathogenesis of atherosclerosis and CAD (12-13), in which it is closely associated with the inflammatory response and bioactive lipid formation. To determine the oxidation status of the person, a set of oxidative markers is recommended, including at least one marker for the lipid peroxidation, the protein oxidation, and the total antioxidative status and ideally also one for

DNA damages (14).

This cross-sectional study set out to determine the relationship of Lp-PLA_2 activity with angiographically proven CAD. In addition, we wanted to explore the correlation between Lp-PLA_2 and a variety of oxidative stress markers to further shed light on the pathogenetic role of this emerging CAD risk marker.

2. BACKGROUND

2.1 Characteristics of Lp-PLA$_2$

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) is a member of the phospholipase A$_2$ superfamily, a family of enzymes that hydrolyze phospholipids. (15) First cloned in 1995, Lp-PLA$_2$ is a 45-kDa protein with 441 amino acids that is distinct from other members of the phospholipase A$_2$ family in that it is calcium-independent.(16) The secreted isoform was first identified on the basis of its ability to degrade platelet-activating factor(PAF), hence it is also known as PAF-acetylhydrolase(PAF-AH)(17). In contrast to other phospholipase A$_2$ enzymes, Lp-PLA$_2$ acts preferentially on water-soluble polar phospholipids with oxidatively truncated *sn*-2 chains, lacking enzymatic activity on naturally occurring long-chain fatty acids in phospholipids found in cellular membranes.(18) The major features of the structure, catalytic properties and plasma transport of Lp-PLA$_2$ are summarized in Table 1. (19)

Plasma Lp-PLA$_2$ is enzymatically active, and its highly restricted substrate specificity is essential to prevent the continuous hydrolysis of the phospholipids of lipoproteins and cell membranes(20).

With the exception of PAF, PAF-AH can also effectively hydrolyze oxidized

Table 1. Major features of the structure, catalytic properties and plasma transport of PAF-AH

(1) 45.4-kDa monomeric protein.

(2) Contains N-linked heterogenous sugar chain(s), 9 kDa, involving sialic acid.

(3) The cDNA encodes a 441-amino acid protein containing a secretion signal sequence(Met-1-Ala-17)

(4) The catalytic site contains the Gly-His-Ser-Phe-Gly consensus sequence characteristic of lipases and esterases.

(5) Ser-273, Asp-296 and His-351 are essential for catalytic activity consistent with an α/β hydrolase conformation.

(6) Expresses Ca2+ − independent PLA_2 activity towards PAF and oxidized phospholipids.

(7) Expresses lipase, transacetylase and PLA1 activities.

(8) Enzyme sources: cells of hematopoietic origin (monocytes-macrophages, hepatic kupffer cell, mast cells, platelet).

(9) The gene is located at chromosomal region 6p12-21.1, and comprises 12 exons.

(10) Enzyme expression is primarily regulated by the differentiation state of the cell and by proinflammatory mediators.

(11) Plasma transport; 80-85% LDL(primarily small-dense LDL); 15-20% HDL.

(12) PAF-AH binds directly to –COOH terminal of LDL-Apo B100 (PAF-AH residues: Tyr 205, Tryp 115, Leu 116)

phospholipids produced by peroxidation of phosphatidylcholines containing an *sn*-2 polyunsaturated fatty acyl residue(21-23). (Fig 1.) Such oxidized phospholipids are formed during the oxidative modification of LDL and play key roles in several aspects of atherogenesis(24). Early studies had shown that PAF-AH is an interfacial enzyme(17) however, more recent work has revealed that PAF-AH access its substrates only form the aqueous phase, thus this enzyme may hydrolyze other lipid esters that are partially soluble in the aqueous phase(18). Indeed, with the exception of its PLA_2 activity, PAF-AH equally can hydrolyze short-chain diacylglycerols, triacylglycerols, and

acetylated alkanols, and also displays a PLA_1, activity. Consequently PAF-AF possesses broad substrate specificity toward lipid esters containing short acyl chains. Since oxidative damage occurs not only in phospholipids but also in various types of compounds containing unsaturated bonds, this enzyme may play an important anti-oxidative scavenger role(25). In addition to the lipase and esterase activities of plasma PAF-AH, this enzyme also exhibits a transacetylase activity. Indeed semipurified PAF-AH from human plasma was able to transfer the acetate group from PAF to 1-acyl-sn-glycero-3-phosphocholine (lyso-PC) (26,27).

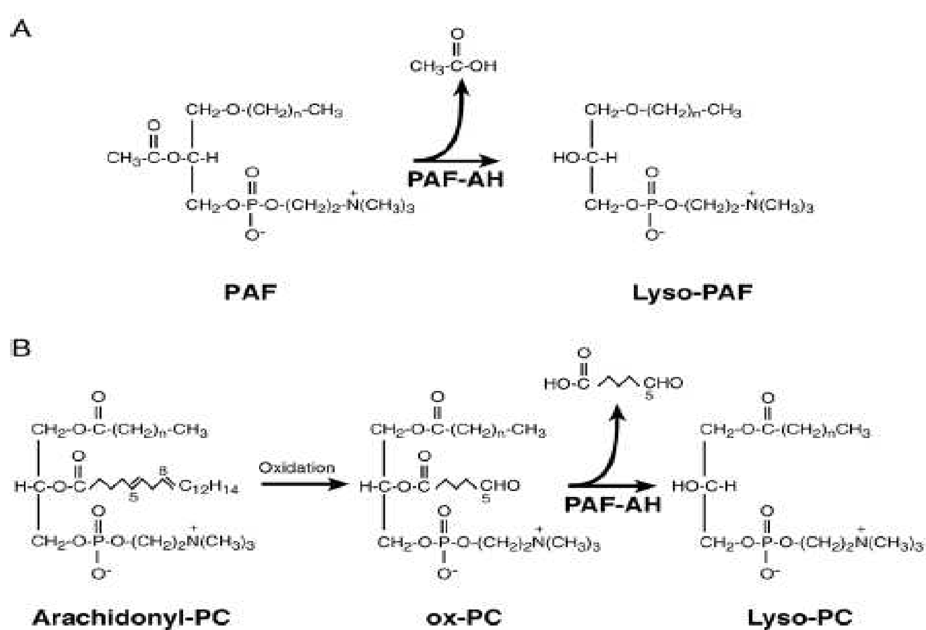

Figure 1. Degradation of PAF into lyso-PAF(A) and oxidized PC into lyso-PC(B) by PAF-AH;n-14 to 16 (adapted from ref.27)

2.2 Lp-PLA$_2$ in atherosclerosis

2.2.1. Pro-inflammatory role of Lp-PLA$_2$.

Increasing evidence suggests that Lp-PLA$_2$ plays a critical role in the development of atherosclerosis and its clinical sequelae. Lp-PLA$_2$ is up-regulated in atherosclerotic plaques(28) and strongly expressed in macrophage within the fibrous cap of rupture prone lesion(29); when released into circulation, Lp-PLA$_2$ is transported in plasma predominantly(80%) associated with low-density lipoprotein(LDL)(1). The key to the role of Lp-PLA$_2$ in atherogenesis is its hydrolysis of oxidized LDL(oxLDL), which is generated when LDL becomes oxidized in the milieu of the artery wall(1,30-31). The hydrolysis of OxLDL by Lp-PLA$_2$ produces the proinflammatory, atherogenic by-products lysophosphatidylcholine(LysoPC) and oxidized fatty acids(OxFA)(7)(Fig 2). LysoPC plays a critical role in atherogenesis. It acts as a chemoattractant for monocytes, impairs endothelial function, causes cell death by disrupting plasma membranes, and induces apoptosis in smooth muscle cells and macrophages(7,32-33).

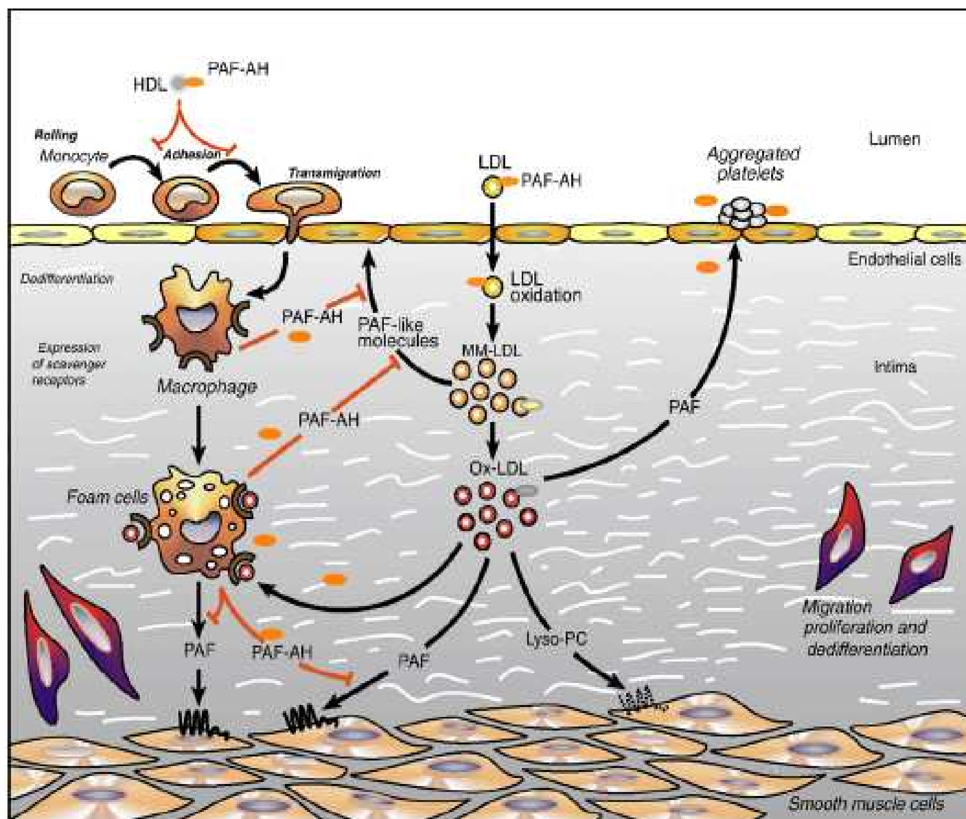

Figure 2. Cartoon depicting the role of PAF-AH in atherosclerotic plaque formation. Abbreviations: MM-LDL, minimally-oxidized LDL; Ox-LDL, oxidized LDL. (adapted from ref 27)

2.2.2. Anti-inflammatory role of Lp-PLA_2

Lp-PLA_2 is also known as the platelet activating factor acetylhydrolase(PAF-AH) which may reflect its antiatherogenic activity: to catalyze the degradation of PAF and oxidized phospholipids; in HDL cholesterol in particular, Lp-PLA_2 has been suggested as protective factor against the accumulation of oxidation products thereby preventing further LDL oxidation(16,34-35). Indeed, data from two prospective epidemiologic studies assessing the association of Lp-PLA_2 with cardiovascular endpoints yielded inconsistent results(2,36).

In mouse models, transgenic apolipoprotein(apoE)-deficient, apoAI-overexpressing mice have increased plasma PAF-AH activity(37); adenovirus-mediated gene transfer of PAF-AH resulted in increased PAF-AH activity, decreased phospholipids oxidation, and reduced recruitment of macrophages to lesion-prone sites in the aortic root of apoE-deficient mice(37). Furthermore, in another study, adenovirus-mediated gene transfer of human PAF-AH prevented injury-induced neointima formation and reduced spontaneous atherosclerosis in apoE-deficient mice(35). These data suggested that in species such as mice, in which Lp-PLA_2 of PAF-AH is predominantly associated with HDL, it may protect against atherosclerosis.

3. SUBJECTS AND METHODS

3.1. Subjects

A total of 1,724 unrelated Korean subjects (1,520 males and 204 females) aged 31 to 83 years were included in this study. They were distributed into two subgroups: (1) A control group of 925 individuals (805 males and 120 females) without history or clinical evidence of CAD were eligible who were either recruited from the Health Service Center during the course of routine check-up visits or by advertisements briefly describing the study design; (2) a group of 799 individuals (715 males and 84 females) with CAD recruited from the outpatient clinic at Yonsei University Severance Hospital, Seoul, Korea and National Health Insurance Corporation Ilsan Hospital, Goyang-si, Korea. The inclusion criteria of CAD patients were: (a) angiographic evidence with ≥50% occlusion of one or more major coronary arteries or previous myocardial infarction (MI) confirmed according to the World Health Organization (WHO) criteria for symptoms, enzyme elevation, or electrocardiographic changes, (b) absence of nonatherogenic occlusion, such as osteal stenosis and spasm, and (c) no history or diagnosis of diabetes mellitus or any diagnosis of thyroid or pituitary disease. The inclusion criteria for control group included no history or diagnosis of atherosclerosis, vascular

disease, chronic heart failure and arrhythmias, diabetes mellitus, cancer and no pathological electrocardiogram patterns.

Exclusion criteria included: 1) abnormal liver or renal function (serum aminotransferase activity $> 40IU/l$, serum creatinine levels $> 1.2mg/dl$, respectively); 2) cancer (clinically or by anamnesis); 3) extreme weight loss/gain over the previous 6 months; 4) thyroid or pituitary disease; 5) infection determined by medical questionnaire examination and complete blood count; 6) acute or chronic inflammatory disease.

Before participation, the purpose of the study was carefully explained to all participants, and their informed consent was obtained. The study protocol complied with the Guidelines for Genome/Genetic Research issued by the Korean government and was approved by the Institutional Review Board of Yonsei University and the study was carried out in accordance with the Helsinki Declaration.

3.2. Methods

3.2.1. Anthropometric parameters, blood pressure measurements and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Waist and hip circumferences were measured with paper tape horizontally at the umbilicus in the standing position after normal expiration. Blood pressure was read from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 min of rest. The average of three measurements was recorded for each subject.

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

3.2.2. Serum lipid profile

Blood fasting serum concentrations of total cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Auto analyzer (Hitachi Ltd. Tokyo, Japan). After using dextran sulfate magnesium to precipitate serum chylomicron, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol from the supernatant was measured by an enzymatic method. LDL cholesterol was indirectly estimated in subjects with serum triglyceride concentrations <4.52 mol/l (400 mg/ml) using the Friedewald formula. In subjects with serum triglyceride concentrations ≥4.52 mol/l, LDL cholesterol was measured by an enzymatic method on a Hitachi 7150 Autoanalyzer directly. Serum apolipoprotein A-I and B were determined by turbidometry at 340 nm using a specific anti-serum (Roche, Switzerland).

3.2.3. Lp-PLA_2 activity and plasma oxidized LDL levels

The activity of Lp-PLA_2, which is also known as platelet activating factor acetylhydrolase (PAF-AH), was measured using a previously described modified method (38). Plasma oxidized LDL (ox-LDL) was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden) and the resultant color reaction was read at 450 nm with a $Victor^2$ (Perkin Elmer Life Sciences, Turku, Finland).

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

Urine was collected in polyethylene bottles containing 1% butylated hydroxytoluene after 12 hours of fasting. The tubes were immediately covered with aluminum foil and stored at –70℃ until analysis. 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) was measured using an enzyme immunoassay (BIOXYTECH urinary 8-epi-$PGF_{2\alpha}$™ Assay kit, OXIS International Inc., OR, USA). The resulting color reaction from the enzyme immunoassay was read at 650 nm using a $Victor^2$ (Perkin Elmer Life Sciences, Turka, Finland). Urinary creatinine was determined by the alkaline picrated (Jeffe) reaction. Urinary 8-

epi-$PGF_{2\alpha}$ concentrations were expressed as picomole per millimole (pmol/mmol) of creatinine. Plasma malondialdehyde (MDA) was assayed according to the fluorometric method described by Buckingum (39).

3.2.5. Serum CRP levels

Serum high-sensitive CRP levels were measured with an Express^{+} autoanalyzer (Chiron Diagnostics Co., Walpole, MA, U.S.A.) using a commercially-available, high-sensitivity CRP-Latex (II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan) that allowed detection of CRP levels as low as 0.001 mg/dL and as high as 32 mg/dL.

3.2.6. Alkaline comet assay for DNA damage

For the comet assay, 120 μL whole blood was mixed with 900 μL phosphate-buffered saline and poured gently over 150 μL lymphocyte separation solution (Histopaque-1077; Sigma-Aldrich Korea Ltd, Yong-In, Korea). After centrifugation at 1450 rpm and 4 °C for 4 min, lymphocytes were removed by pipette and transferred to another tube. DNA damage was analyzed as described by Green et al (40).

3.2.7. Statistical analysis

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, USA). Differences in biomarkers between CAD patients and healthy controls were analyzed by Student's t-test and general linear model to adjust age, BMI, blood pressure, smoking and alcohol consumption which showed differences between case and control subjects. A χ^2 test was used to test whether there was a difference in cigarette smoking, alcohol consumption, and medication therapy.

Lp-PLA_2 plasma levels were divided into quartiles according to the distribution in the control group and multivariable logistic regression analysis was applied to estimate the independent association of elevated Lp-PLA_2 with the presence of CAD while simultaneously controlling for confounding factors. Multiple logistic regression models including CAD as a dependent variables and the Lp-PLA_2 concentration as an independent variable were used to assess the association of increase in serum Lp-PLA_2 levels and CAD after subdivision of the sample into quartiles of Lp-PLA_2 levels based on the distribution of control subjects. Odds ratio (ORs) and 95% confidence intervals (CIs) were estimated from the logistic regression model controlling

for sex, age and BMI and additionally controlling for blood pressure, cigarette smoking, alcohol consumption, and lipid profiles.

Pearson's correlation test was used to examine the relation of Lp-PLA_2 concentrations and markers of oxidative stress. Each variable was examined for normal distribution patterns. Significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are expressed as mean±S.E. A 2-tailed value of $P<0.05$ was considered statistically significant.

4. RESULTS

4.1 Characteristics of patients and controls

General characteristics of 799 patients with CAD and 925 control subjects are shown in Table 2. CAD patients were slightly older, heavier and larger WHR than controls, whereas there was no significant difference between both groups in sex distribution. Current smoking and drinking alcohol was more common in the control group than in cases. Lipid-lowering agents (50% vs 0.9%, patients and controls, respectively), β-blockers (27% vs 0%), ACE inhibitors (20% vs 0.1%), calcium antagonists (27% vs 0.5%), and aspirin (69% vs 1.1%) were used more frequently in patients. Likewise, systolic and diastolic blood pressure, and concentrations of total-, LDL- and HDL-cholesterol were lower in CAD patients compared to controls. CAD patients showed higher levels of triglyceride. Oxidative stress and inflammation were evaluated by oxidized LDL, isoprostanes, MDA, lymphocyte DNA damage and CRP, respectively. CRP was more abundant in patients. CAD patients showed higher levels of $PGF_{2\alpha}$, lymphocyte DNA damage and MDA and lower oxidized LDL concentrations than controls. Lp-PLA_2 activity was higher in CAD patients than controls (Table 2).

Table 2. Anthropometric and biochemical parameters of healthy controls and CAD patients

	Healthy controls (n=925)	CAD patients (n=799)
Age (yrs)	55.2 ± 0.29	56.1 ± 0.32
Female (%)	13	11
Body Mass Index (kg/m^2)	24.6 ± 0.08	25.1 ± 0.10
Waist hip ratio	0.91 ± 0.00	0.92 ± 0.00
Cigarette smoker, n (%)	256 (28)	178 (22)
Alcohol drinker, n (%)	620 (67)	432 (54)
Antidyslipidemic therapy, n (%)	8 (0.9)	399 (50)*
Antihypertensive therapy, n (%)	44 (4.8)	548 (69)*
Antiplatelet therapy, n (%)	11 (1.2)	567 (71)*
Systolic BP (mmHg)	126 ± 0.5	124 ± 0.6
Diastolic BP (mmHg)	80 ± 0.4	78 ± 0.3
Total cholesterol (mg/dL)	196 ± 1.2	171 ± 1.4*
Triglyceride (mg/dL)∮	132 ± 2.2	141 ± 2.4*
HDL-cholesterol (mg/dL)	51.0 ± 0.45	43.6 ± 0.40*
LDL-cholesterol (mg/dL)	117.4 ± 1.05	97.8 ± 1.32*
hs-CRP (mg/dL)∮	1.24 ± 0.07	2.15 ± 0.15*
$PGF_{2\alpha}$ (pg/mg creatinine)∮	1124 ± 20	1333 ± 29*
Lymphocyte DNA damage		
Tail DNA (%)	13.0 ± 0.26	13.1 ± 0.28
Tail length (μm)	49.1 ± 0.91	73.1 ± 2.35*
Tail moment	7.84 ± 0.26	13.2 ± 0.70*
MDA (nmol/mL)∮	9.16 ± 0.12	11.8 ± 0.23*
Oxidized LDL (U/L)∮	57.6 ± 0.95	48.8 ± 1.07*
Lp-PLA_2 (nmol/ml/min)	29.7 ± 0.42	32.9 ± 0.46*

Mean±S.E., ∮log-transformed

*P<0.001 compared with controls adjusted for age, BMI, blood pressure, smoking and alcohol consumption.

4.2. Correlation between Lp-PLA_2 activities, lipoproteins, CRP and markers of oxidative stress

In controls, Lp-PLA_2 activity was positively correlated with urinary excretion levels of $PGF_{2\alpha}$ and lymphoctye DNA damage (tail DNA, tail length and tail moment) (Table 3). In case patients, Lp-PLA_2 activity was positively correlated with urinary excretion levels of $PGF_{2\alpha}$ and lymphoctye tail DNA. In case, Lp-PLA_2 was weakly but significantly correlated with LDL-cholesterol. Lp-PLA_2 activity was not significantly correlated with age, BMI, total cholesterol, triglyceride, HDL-cholesterol, oxidized LDL, MDA and CRP in both the case and control groups. In all subjects including controls and case patients, Lp-PLA_2 activity was positively correlated with urinary excretion levels of $PGF_{2\alpha}$ and lymphocyte DNA damage (tail DNA, tail length and tail moment) and plasma MDA levels and negatively correlated with HDL-cholesterol (Table 3).

Table 3. Pearson correlation coefficients(r) between lipid profiles, CRP and oxidative stress markers and Lp-PLA$_2$ activities

	All individuals (n=1724)		Controls (n=925)		Cases (n=799)	
	r	p	r	p	r	p
Age (yrs)	0.012	0.617	0.025	0.450	-0.015	0.667
Body Mass Index (kg/m^2)	0.032	0.188	0.014	0.662	0.027	0.439
Total cholesterol (mg/dL)	0.001	0.973	0.035	0.282	0.048	0.174
Triglyceride (mg/dL)$^∮$	0.028	0.248	0.011	0.735	0.026	0.476
HDL-cholesterol (mg/dL)	-0.069	0.004	-0.015	0.647	-0.062	0.081
LDL-cholesterol (mg/dL)	0.029	0.230	0.062	0.066	0.076	0.035
hs-CRP (mg/dL)$^∮$	0.031	0.218	-0.014	0.692	0.025	0.492
PGF$_{2α}$ (pg/mg creatinine)$^∮$	0.253	<0.001	0.277	<0.001	0.202	<0.001
Lymphocyte DNA damage						
tail DNA (%)	0.136	<0.001	0.190	<0.001	0.100	0.047
tail length (μm)	0.116	0.002	0.165	<0.001	0.046	0.360
tail moment	0.129	<0.001	0.213	<0.001	0.068	0.174
MDA[1] (nmol/mL)$^∮$	0.089	0.001	0.061	0.092	0.061	0.094
Oxidized LDL (U/L)$^∮$	-0.052	0.073	-0.046	0.226	-0.018	0.690

Mean±S.E., $^∮$log-transform

4.3. Association between Lp-PLA_2 and CAD

Table 4 shows the association between the three upper quartiles of Lp-PLA_2 activity in comparison to the bottom quartile and CAD. The OR for the presence of CAD after adjustment for sex, age and BMI was 2.14 (95% CI, 1.61-2.84) when the top quartile of the Lp-PLA_2 distribution was compared to the bottom quartile (Model 1). After additional adjustment for blood pressure, smoking and drinking status and total-, LDL-, HDL-cholesterol, the OR increased to 2.50 (95% CI, 1.81-3.44) (Model 4)

Table4. Odds ratios (OR) of CAD associated with Lp-PLA$_2$ activities after various adjustments

Lp-PLA$_2$ (nmol/ml/min)	Quartile 1 (<20.4)	Quartile 2 (≥20.4-25.4)	P value	Quartile 3 (≥25.4-41.0)	P value	Quartile 4 (≥41.0)	P value	P for trend
Model 1	1.0	1.54 (1.15-2.06)	0.004	1.78 (1.34-2.38)	<0.001	2.14 (1.61-2.84)	<0.001	<0.001
Model 2	1.0	1.49 (1.10-2.01)	0.009	1.78 (1.33-2.38)	<0.001	2.15 (1.61-2.88)	<0.001	<0.001
Model 3	1.0	1.61 (1.17-2.23)	0.004	1.81 (1.32-2.48)	<0.001	2.32 (1.69-3.17)	<0.001	<0.001
Model 4	1.0	1.72 (1.24-2.40)	0.001	1.90 (1.37-2.64)	<0.001	2.50 (1.81-3.44)	<0.001	<0.001

Data are ORs (95% CI) for association of CAD with Lp-PLa$_2$ activities. Quartiles are based on data from control subjects.
Model 1: adjusted for sex, age, BMI
Model 2: adjusted for sex, age, BMI, systolic BP and diastolic BP, smoking and drinking status
Model 3: adjusted for sex, age, BMI, systolic BP and diastolic BP, smoking and drinking status, total-C, HDL-C
Model 4: adjusted for sex, age, BMI, systolic BP and diastolic BP, smoking and drinking status, total-C, HDL-C, LDL-C

5. DISCUSSION

In this case-control study, Lp-PLA_2 activities were higher in patients with CAD compared to healthy controls and elevated activities of Lp-PLA_2 were associated with the risk of CAD, which persisted after the adjustment of sex, age, BMI, blood pressure, smoking and drinking status and lipid profiles. This result is consistent with previous findings of an association between enhanced Lp-PLA_2 activities and the CAD risk (5,41-42). In addition, the Val279Phe variant in the Lp-PLA_2 gene indicating the loss of enzyme activity has been reported to be associated with a decreased risk of cardiovascular disease in Korean men (43).

Lp-PLA_2 may generate bioactive oxidized free fatty acids (7) and lysophosphatidylcholine (8). In addition, Lp-PLA_2 activity may liberate arachidonic acid, a precursor of eicosanoids including prostaglandins and leukotrienes (44). A family of prostaglandin F_2-isomers also called F_2-isoprostanes is the result of a radical peroxidation of arachidonic acid (14). The 8-epi-$PGF_{2\alpha}$, a sensitive and independent risk marker of coronary heart disease (45-47), is a member of a series of F_2-isoprostanes, being presumably released into biological fluids through a phospholipase-mediated pathway and consequently excreted by urine. In fact, Stafforini et al. (9) have found that

both the secreted form of Lp-PLA_2 and intracellular type II Lp-PLA_2 released F_2-isoprostanes from the *sn*-2 position of phosphatidylcholine with high affinity. Furthermore, intracellular type II Lp-PLA_2, which share homology to the plasma enzyme Lp-PLA_2, has been reported to be involved in the metabolism of esterified 8-iso-$PGF_{2\alpha}$ (48). The present study showed that Lp-PLA_2 activity was positively correlated with urinary excretion levels of $PGF_{2\alpha}$ in both controls and CAD patients.

The previous observation that plasma Lp-PLA_2 release atherogenic F_2 isoprostanes from esterified phospholipids indicates that this enzyme may modulate oxidant stress in vivo (9). Semiquantitative analysis for isoprostane, end product of lipid peroxidation, 8-epi-$PGF_{2\alpha}$ has been shown to serve as one of the most valid markers to assess oxidative stress (45-46,49-51). Oxidative stress has been suggested to be the major causative mechanism for DNA damage and increased levels of DNA damage in patients with CAD have been reported recently (52-54). In the present study, there was a positive relationship of lymphocyte DNA alterations with Lp-PLA_2 activity, by using the comet assay, a sensitive and reliable marker of oxidative stress and DNA damage (55). These data may suggest that individuals who are high in Lp-PLA_2 activity presumably have higher levels of lipid peroxidation and oxidative stress, thus these subjects are at higher risk of CAD compared with

those with normal Lp-PLA_2 activity. Therefore, a positive correlation between Lp-PLA_2 activity and 8-epi-$PGF_{2\alpha}$ as well as a positive correlation to lymphocyte DNA damage in this study would support the conclusion that this enzyme is proatherogenic (56). These positive correlations between Lp-PLA_2 activity, 8-epi-$PGF_{2\alpha}$ and lymphocyte damage persisted after the adjustment of sex, age, BMI and smoking and drinking status which are known to affect levels of $PGF_{2\alpha}$ (14,57-58) and DNA damage (14,59-62) (data not shown).

MDA, another lipid peroxide, is widely used as an index of oxidative damage for its ability to interact with lipoprotein(14) and increased MDA levels in CAD patients have been found(63). However, plasma MDA determination was reported to be a less reliable assay than lymphocyte DNA damage for detecting the severity of vascular lesions in CAD patients(64). In this study, a weak correlation between MDA and Lp-PLA_2 activity may indicate differences in specificity and sensitivity in lipid peroxides. The specificity of MDA is low, thus MDA is known to be a general indicator of oxidative stress rather than a specific marker of lipid peroxidation(14,65-66).

Oxidized-LDL, another parameter of lipid peroxidation, was not correlated with Lp-PLA_2 activity in this study. This might be partly explained by only slightly association between Lp-PLA_2 and LDL-cholesterol in Korean populations (43), who have relatively low LDL-cholesterol (115mg/dL for

middle-aged Koreans) and high incidence of the Val279Phe variant in the Lp-PLA_2 gene. However, this result might relate to the previous findings that Lp-PLA_2 does not affect the lipid peroxidation of LDL (33,67-68).

In case patients, we found decreased levels of total cholesterol, LDL cholesterol, and oxidized-LDL, reflecting the wide-spread use of lipid-lowering therapy in CAD patients (66%). Although statins reduce Lp-PLA_2 activity (69), CRP levels (70) and oxidative stress (71), Lp-PLA_2 activity, CRP and urinary excretion of 8-iso- $PGF_{2\alpha}$ were higher in CAD patients of this case-control study. In addition, interestingly, CRP was positively correlated with urinary excretion level of $PGF_{2\alpha}$ ($r=0.134$, $P<0.001$) in the absence of correlation between Lp-PLA_2 and CRP in controls of this study. This observation may indicate that inflammation works hand in hand with oxidative stress. Schwedhelm et al. (45) also found the association between CRP and urinary excretion of 8-iso-$PGF_{2\alpha,}$ which was not confounded by either lipid-lowering therapy or by total cholesterol, LDL cholesterol, or triglyceride levels. However, this present study was designed as a case-control study and therefore could not explain the reason of such effects.

The limitation of this study, as always in case-control studies in which exposure and outcome are collected at one point in time, is difficult to assess the time sequence of the described associations. Despite this limitation, this

result show the association of elevated activities of Lp-PLA_2 with CAD risk and thus lend further support the hypothesis that Lp-PLA_2 may be considered as a novel risk marker for CAD. This could be compatible with a positive correlation between Lp-PLA_2 and oxidative stress markers.

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국문요약

관상동맥질환과 산화스트레스 표지 인자로서의 Lp-PLA_2 activity에 대한 단면연구

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) 는 지단백에 결합되어있는 효소로서 염증반응과 죽상동맥경화에 관여한다고 알려져 있다. 산화된 저밀도 지단백 콜레스테롤이 가수분해되면서 생긴 염증성 물질인 lysophosphatidyl choline과 free fatty acid가 죽상동맥경화에 관여하는데, 만성적인 염증단계뿐만 아니라 심장혈관질환 및 인슐린 비의존성 당뇨병과의 관련성에 대한 연구 결과들이 보고되고 있다. 본 연구에서는 산화 스트레스 지표와의 관계를 중심으로 Lp-PLA_2와 관상동맥질환의 연관성에 대해 연구하였다.

관상동맥질환(coronary artery disease)를 가지고 있는 799명의 성인남녀와 건강한 성인남녀 925명을 대상으로 Lp-PLA_2 활성, 지단백농도, hs-CRP, 산화스트레스를 측정하였다. 관상동맥질환자에서 대조군에 비해 높은 Lp-PLA_2 농도를 나타냈으며(32.9±0.46nmol/ml/min vs 29.7±0.42; $P<0.001$), Lp-PLA_2 활성을 기준으로 네 그룹으로 나누었을 때 가장 활성이 높은 순서대로 세 개의 군에서 가장 낮은 한 군에 비해 관상동맥질환발생위험(odds ratio)이 높았다. 성별과 연령, BMI를 보정했을 때, Lp-PLA_2 활성이 가장 높은 top quartile에서

관상동맥질환에 대한 OR가 2.14(95% CI, 1.61−2.84)이었으며 혈압, 흡연유무 및 음주 상태, 총콜레스테롤, LDL−, HDL−콜레스테롤을 추가적으로 보정했을 때, OR가 2.5(95% CI, 1.81−3.44)이었다. 또한 Lp−PLA$_2$ 활성은 urinary 8−epi−PGF$_{2\alpha}$(r=0.253, $P<0.001$)와 임파구 DNA 손상[tail DNA (r=0.136, $P<0.001$), tail length (r=0.116, P=0.002) and tail moment(r=0.129, $P<0.001$)] 및 혈장 MDA 농도(r=0.089, $P<0.001$)와 양의 상관관계를 보였으며 HDL−콜레스테롤(r=−0.069, P=0.004)과는 음의 상관관계를 나타내었다.

이러한 연구 결과는 증가된 Lp−PLA$_2$ 활성과 관상동맥질환 위험도의 연관성을 보여주며, 이것은 또한 Lp−PLA$_2$와 산화스트레스 인자가 양의 상관관계를 보이는 것과 양립하는 결과이다.

핵심 되는 말 : Lp−PLA$_2$ 활성, 산화스트레스 관상동맥질환, 지질과산화, DNA 손상