Prehypertension-Associated Elevation in Circulating Lysophosphatidlycholines, Lp-PLA$_2$ Activity, and Oxidative Stress

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A Master's Thesis

Submitted to the Department of Science for Aging

and the Graduate School of Yonsei University

in partial of fulfillment of the requirements for the degree of

Master in Science for Aging

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December 2013

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December 2013

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ABSTRACT

Prehypertension-Associated Elevation in Circulating Lysophosphatidlycholines, Lp-PLA$_2$ Activity, and Oxidative Stress

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Objective: Prehypertension is a risk factor for atherosclerosis. We investigated alterations in plasma metabolites that are associated with prehypertension.

Design, Participants, and Methods: A group of 53 individuals was identified who remained within the range of prehypertension [systolic blood pressure (SBP), 130 139 mm Hg; diastolic blood pressure (DBP), 80 94 mm Hg] during repeated measurements in a 3-year period. This group was compared with the control group of 53 normotensive subjects (SBP < 120 mm Hg, and DBP < 80 mm Hg) who were

matched for age and gender. Metabolomic profiles were analyzed with UPLC-LTQ-Orbitrap mass spectrometry.

Results: The prehypertensive group showed higher levels of lysophosphatidylcholines (lysoPCs) containing C14:0, C16:1, C16:0, C18:2, C18:1, C18:0, C20:5, C20:4, C20:3, and C22:6, higher circulating Lp-PLA2 activity, oxidized LDL (ox-LDL), interleukin 6 (IL-6), urinary 8-epi-PGF2α, and higher brachial-ankle pulse wave velocity (ba-PWV),before and after adjusting for BMI, WHR, smoking, alcohol consumption, serum lipid profiles, glucose, and insulin. LysoPC (16:0) was the most important plasma metabolite for evaluating the difference between control and prehypertensive groups, with a variable important in the projection (VIP) value of 17.173, and it showed a positive and independent association with DBP and SBP. In the prehypertensive group, the levels of lysoPC (16:0) positively and significantly correlated with ox-LDL, Lp-PLA2 activity, 8-epi-PGF2α, ba-PWV, and IL-6 before and after adjusting for confounding variables.

Conclusions: Prehypertension-associated elevations in lysoPCs, Lp-PLA2 activity, ox-LDL, urinary 8-epi-PGF2α, IL-6, and ba-PWV could indicate increased oxidative stress from Lp-PLA2-catalyzed PC hydrolysis during increased LDL oxidation, thereby enhancing proinflammation and arterial stiffness.

Key Words: prehypertension; lysoPCs; Lp-PLA_2; ox-LDL; 8-*epi*-$PGF_{2\alpha}$; IL-6; ba-PWV

1. Introduction

Hypertension is a risk factor for atherosclerosis and cardiovascular disease (CVD),[1-4] although the mechanisms by which hypertension is related to atherosclerosis are not clearly established. Several metabolomic studies have been published that investigate the metabolic effects of hypertension.[5-7] These studies reported abnormalities in gender-linked steroid patterns[5] or lipid metabolism.[6,7] Hypertension and its underlying pathophysiology may be present for years before clinical diagnosis, at which time irreversible pathology has already occurred. In 2003, the Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure created a prehypertension category for persons with blood pressures ranging from 120−139 mm Hg (systolic) or from 80−89 mm Hg (diastolic). The committee strongly advocated lifestyle and behavioral modifications for individuals with prehypertension.[8] Prehypertension can precede hypertension and atherosclerosis for decades, and it is a condition that represents early CVD. Therefore, it is necessary to determine the role of prehypertension-associated alterations in circulating metabolic profiles. We performed metabolic profiling in a group of 53 individuals who remained within the range of prehypertension during repeated measurements in a 3-year period, and compared these with the metabolic profiles of age- and gender-matched normotensive controls in the same cohort. We also determined lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity, oxidized LDL (ox-LDL), lipid peroxides, and brachial-ankle pulse wave velocities (ba-PWV).

2. Background

2.1. Prehypertension

Prehypertension is defined as blood pressure (BP) in the range of 120-139 mmHg (in systole) or 80-89 mmHg (in diastole), which was termed as 'normal blood pressure' or 'high-normal blood pressure' in the JNC-6 report (Table 1).[9] BP increases with age, and in Framingham Heart Study, about 90% of those whose BP was normal at age 55 years ultimately developed hypertension in their lifetime(Table 2).[10] The risk of cardiovascular disease (CVD) increased progressively from levels as low as 115/75 mmHg, and each increment of 20/10 mmHg is associated with more than a twofold difference in the stroke death rate and a twofold difference in the death rates from ischemic heart disease (IHD) and from other vascular causes.[11] Therefore, the introduction of the new term prehypertension was appropriate and well-timed.

Table 1 . Blood pressure classification. (Adapted from 9)

JNC-7	JNC-6	Systolic BP (mmHg)		Diastolic BP (mmHg)
Normal	Optimal	<120	and	<80
Prehypertension		120-139	or	80-89
-	Normal	120-129	and	80-84
-	High-normal	130-139	or	85-89
Hypertension:	Hypertension:			
Stage 1	Stage 1	140-159	or	90-99
Stage 2		≥160	or	≥100
-	Stage 2	160-179	or	100-109
-	Stage 3	≥180	or	≥110

Prehypertension focuses on a population who were previously called high-normal BP. The recent data has shown the prevalence of prehypertension and its progression rate to hypertension, its association with CVD risk factors and its relationship with the development of CVD. prehypertension is associated with a cardiovascular risk that lies between normotension and hypertension. A 10 mmHg lower usual SBP or 5 mmHg lower usual would be associated with about 40% lower risk of stroke death and about 30% lower risk of death from IHD or other vascular causes throughout middle to old age.[11]

Table 2. Residual Lifetime Risk of High Blood Pressure According to Baseline Age* (Adapted from 10)

	Risk for High Blood Pressure, % (95% Confidence Interval)			
	Women, Age, y		Men, Age, y	
Time, y	55 (n = 709)	65 (n = 549)	55 (n = 589)	65 (n = 438)
	Stage 1 High Blood Pressure or Higher (≥140/90 mm Hg)			
10	45 (39-51)	58 (53-63)	50 (43-57)	62 (56-67)
15	64 (59-69)	75 (71-79)	70 (65-76)	76 (72-81)
20	77 (73-80)	83 (80-87)	81 (77-85)	84 (80-88)
25	86 (83-89)	. . .	88 (85-91)	. . .
	Stage 2 High Blood Pressure or Higher (≥160/100 mm Hg)			
10	6 (3-9)	26 (22-30)	9 (5-13)	20 (16-24)
15	18 (14-22)	35 (31-40)	20 (15-25)	29 (24-34)
20	30 (26-35)	44 (39-48)	27 (22-32)	40 (34-46)
25	39 (35-44)	. . .	35 (30-41)	. . .
	Stage 2 High Blood Pressure or Higher (≥160/100 mm Hg) or Antihypertensive Medications Use			
10	24 (19-29)	46 (41-50)	20 (15-25)	46 (40-51)
15	45 (40-50)	61 (56-65)	44 (38-49)	59 (53-64)
20	59 (54-63)	70 (66-74)	56 (51-62)	68 (63-73)
25	70 (66-74)	. . .	67 (62-72)	. . .

*For 55-year-old subjects, the risk for developing high blood pressure over 25 years represents their lifetime risk. For 65-year old subjects, the risk for developing high blood pressure over 20 years indicates their lifetime risk. Ellipses indicates not applicable. High blood pressure stages are defined as systolic or diastolic measurements exceeding the specified threshold.

2.2. Lysophosphatidylcholine (lysoPC)

Lysophosphatidylcholine (LysoPC) is thought to be associated with endothelial dysfunction.[12] LysoPC is abundant in the plasma[13] and accumulates in atherosclerotic lesions.[14] The functions of lysoPC to explain possible mechanism of endothelial dysfunction includes downregulation of endothelial nitric oxide synthase mRNA expression,[12,15] an increase in reactive oxygen species and oxidative stress,[16] endothelial cell apoptosis[17] and blocking of endothelial cell migration to sites of endothelial damage.[18,19] The elevated level of lysoPC in oxidatively modified LDL has been shown to be a biochemical factor responsible for the impairment of endothelium dependent relaxation (EDR).[20] In addition, a major lipid constituent formed during oxidation of LDL, inhibits Endothelial cell (EC) movement.[21,22]

2.3. Lp-PLA$_2$

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a marker of inflammation that plays a critical role in atherogenesis; its inhibition may have antiatherogenic effects.[23] Lp-PLA2, also known as platelet activating factor acetylhydrolase (PAF-AH) which may reflect its antiatherogenic activity.[24] The biological actions of PAF and oxidized phospholipids are abolished by hydrolysis of the sn-2 residue. Such hydrolysis is catalyzed by the enzyme, PAF acetylhydrolase (PAF-AH) (Figure 1). In plasma, PAF-AH has been found to be tightly associated with both high- and low-density lipoprotein (LDL). PAF is produced by endothelial cells in response to oxidative injury or various physiological agonists, including thrombin, bradykinin, and histamine, and can induce macrophages to produce superoxide anions.[25]

Lp-PLA2 catalyzes hydrolysis of oxidized phospholipids to generate proinflammatory products implicated in endothelial dysfunction, plaque destabilization, and formation of a necrotic lipid core in advanced atherosclerotic lesions (Figure 2).[26-29] Lp-PLA2 increase in atherosclerotic plaques;[31] when released into circulation, Lp-PLA2 is transported in plasma (80%) associated with low-density lipoprotein (LDL).[32]

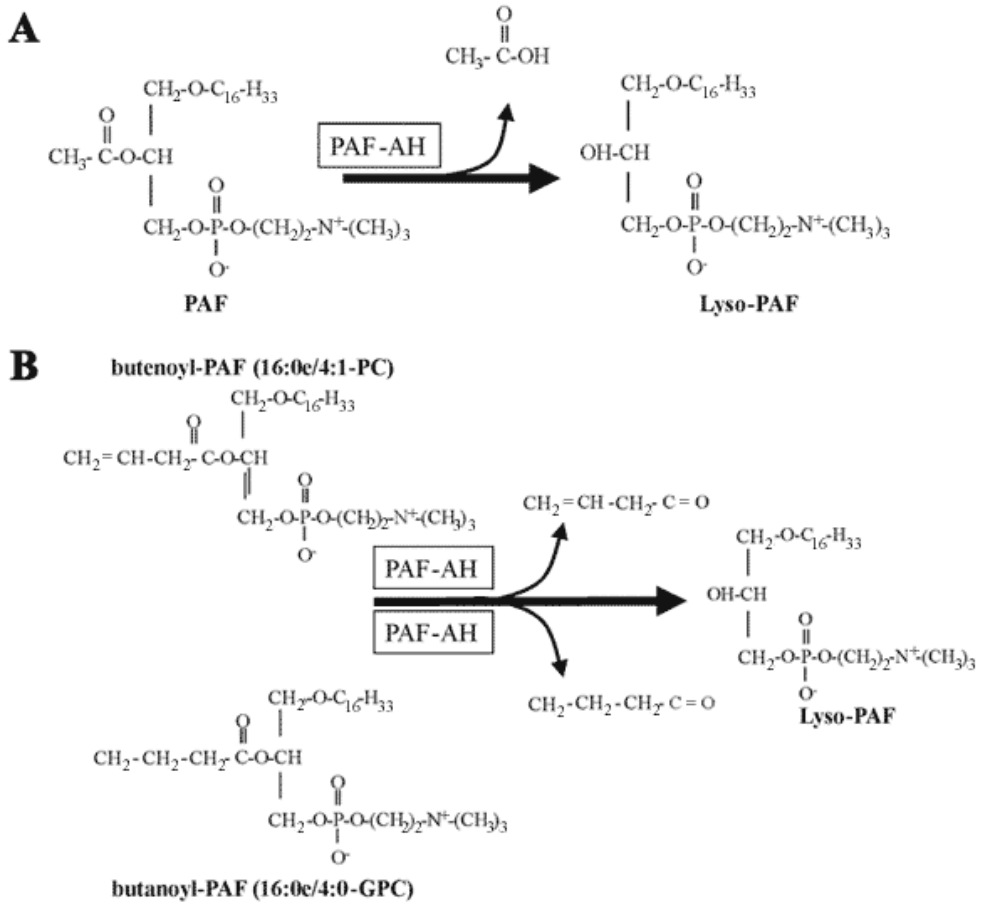
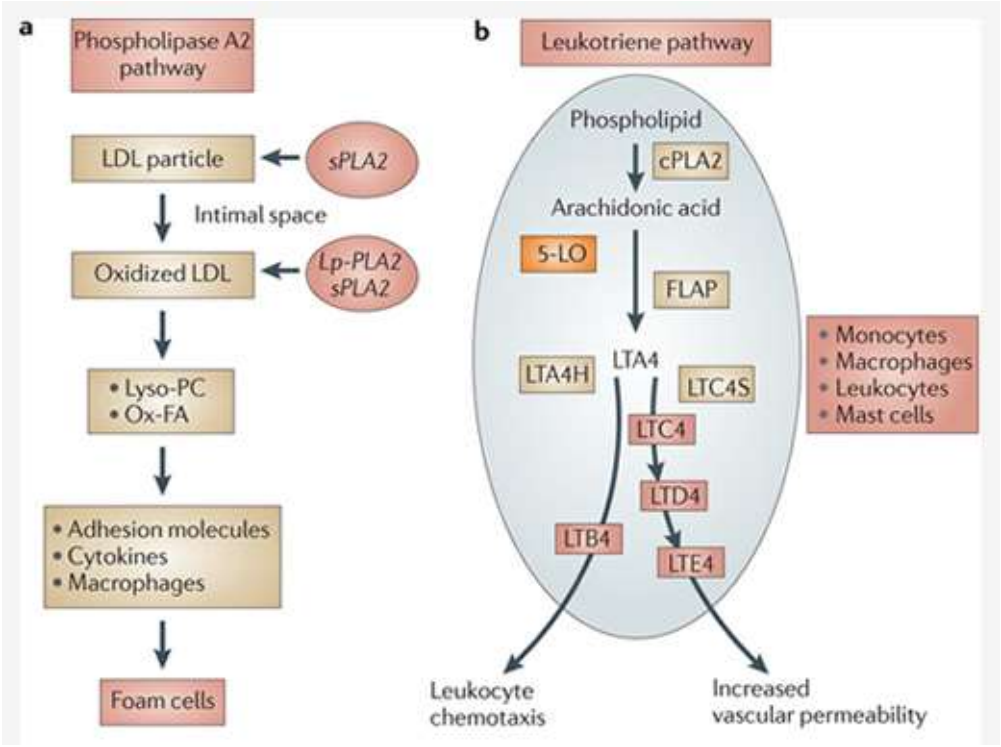


Figure 1. PAF-AH hydrolyzes PAF and oxidized PAF-like lipids to their inactive metabolite Lyso-PAF. (Adapted from 30)



a | Secretory phospholipase A2 (sPLA2) interacts with circulating low-density lipoprotein (LDL) particles and modifies LDL, thereby reducing liver uptake of LDL by the LDL receptor. Both sPLA2 and lipoprotein-associated PLA2 (Lp-PLA2) interact with more oxidized forms of LDL to generate bioactive lipids. **b** | The cysteinyl leukotrienes increase vascular permeability and induce smooth muscle cell proliferation. Leukotriene B4 (LTB4) induces leukocyte chemotaxis into the atherosclerotic plaque. 5-LO, arachidonate 5-lipoxygenase; cPLA2, cytoplasmic PLA2; FLAP, 5-LO-activating protein; LTA4, leukotriene A4; LTA4H, LTA4 hydrolase; LTC4, leukotriene C4, LTC4S; LTC4 synthase; LTD4, leukotriene D4; LTE4, leukotriene E4; Lyso-PC, lysophosphatidylcholine; Ox-FA, oxidized fatty acids.

Figure 2. The phospholipase A2 pathways that result in the generation of foam cells and atherogenic lipids, and the leukotriene pathway with enzymatic steps depicted. (Adapted from 33)

2.4. Oxidized LDL

Oxidized LDL (oxLDL) describes many different chemical, biologic, and immuno-logic modifications in LDL.[34] OxLDL is pro-inflammatory, it can cause endothelial dysfunction and it readily accumulates within the arterial wall. Several factors may influence the susceptibility of LDL to oxidation, including its size and composition, and the presence of endogenous antioxidant compounds.[43] OxLDL particles are taken up by macrophage via scavenger receptor in the subendothelial space, which generate foam cells (Figure 3).[35-39] Such a sequence of steps indicates that oxLDL particles play a promotive role in the development of atherosclerosis.[40] The initial process of atherosclerosis is started endothelial injury, which leads to oxidation and accumulation of LDL in subendothelial space of vessel.[41] Oxidized LDL promotes the chemotaxis of monocyte into the vessel wall by modulating the secretion of numerous cytokines, chemokines and adhesion molecules.[42]

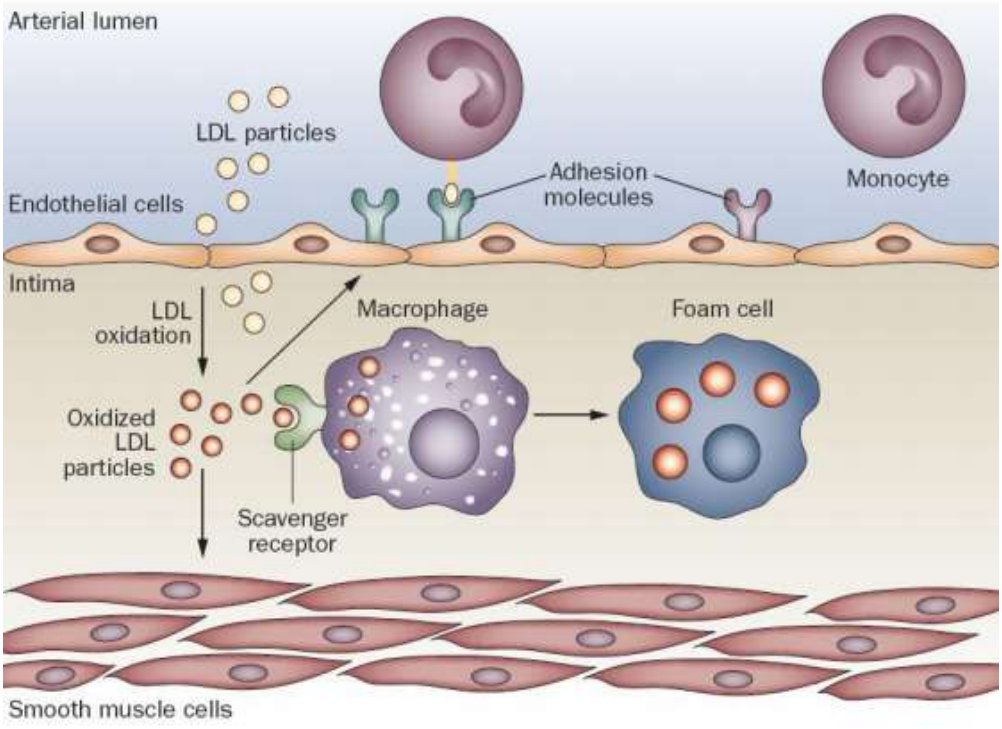


Figure 3. Effects of LDL particles on the vessel wall. (Adapted from 39)

2.5. 8-epi-PGF2α

8-epi-PGF2α is a major component of the F2-isoprostanes family which has been found to modulate platelet aggregation. And 8-epi-PGF2α is a potent renal and pulmonary vasocontrictor in rat kidney and lungs.[43,44] 8-epi PGF2a is a product of oxidative stress that causes potent smooth muscle contraction. Its production increases in conditions associated with oxidative stress such as in diabetes, smoking, and aging.

2.6. Interleukin-6 (IL-6)

IL-6 is secreted by T cells, macrophages and endothelial cells in response to inflammatory stimuli. Unlike other cytokines, IL-6 is unusual in that its major effects take place at sites distinct from its origin and are consequent upon its circulating concentrations. For this reason, it is called the endocrine cytokine.[45] IL-6 is able to increase hepatic de novo fatty acid synthesis[45] and triglyceride synthesis,[46] which may be harmful in atherosclerosis. Furthermore, IL-6 stimulates the central nervous system,[47] leading to activation of the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system, which may result in hypertension. All of these mechanisms may contribute to age-related disorders, a risk that is further enhanced by an age-associated increase in body fat mass. IL-6 can either accelerate or inhibit the inflammatory process. Part of the role of IL-6 in the acute phase response is to upregulate several downstream markers, including CRP (Figure 4). IL-6 levels are also elevated in response to muscle contraction, produced by SMCs in blood vessels and may have a role in lipid catabolism and insulin resistance.[48,49]

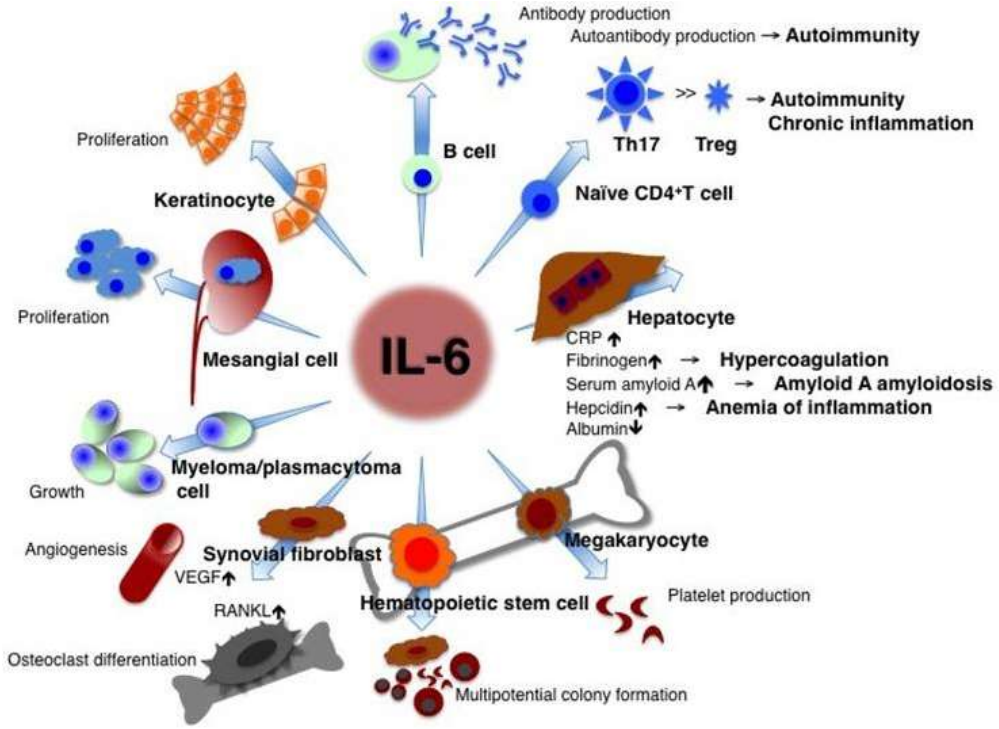


Figure 4. Biological activities of interleukin-6. (Adapted from 50)

2.7. Brachial-Ankle Pulse Wave Velocity (ba-PWV)

PWV is measured by the overlapping of the two waves-the forward wave, which flows from the heart to the periphery, and the reflected wave, which flows from the periphery to the heart.[51] Stiffened arteries return earlier and this wave means imbalance rise in systolic pressure and increases pulse pressure.[52] High PWV is independent determinant of the MetS.[53] Brachial-ankle pulse velocity (baPWV) has recently been used as a simple and suitable marker for examining vascular damages in a large population.[51] High baPWV is also significantly associated with obesity, diabetes mellitus, hypertension, MetS, atherosclerosis and CVD

3. Methods

3.1. Study Subjects

The 3-year prospective cohort study included 600 healthy subjects (30−65 years old) who underwent triennial medical evaluation from January 2008 to December 2011 through the National Health Insurance Corporation Ilsan Hospital in Goyang, Korea. Prehypertension was defined as blood pressure of 130−139 mm Hg (systolic) and 80–94 mm Hg (diastolic). The screening identified 53 nonobese individuals with normal fasting glucose (29 men and 24 women) who remained within the range for prehypertension during repeated measurements in the 3-year period. From the same population, 53 normotensive control individuals matched for age and gender were recruited whose BP remained within the normal range (systolic blood pressure < 120 mm Hg and diastolic blood pressure < 80 mm Hg) during repeated measurements in the 3-year period.

3.2. Anthropometric Parameters, Blood Pressure, Blood Collection, and Dietary Intake Assessments

Body weight and height of unclothed subjects without shoes were measured in the morning to calculate body mass index (BMI, kg/m^2). Waist circumference was measured at the umbilical level with the subjects standing after normal expiration. Blood pressure (BP) was measured in the left arm of seated patients with an automatic BP monitor (TM-2654, A&D, Tokyo, Japan) after a 20-minute rest. After a 12-hour fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to produce plasma or serum, and stored at −70℃ until analysis. The usual dietary intake of the study subjects was assessed using a semi-quantitative food-frequency questionnaire and a 24-hour recall method.[54] Nutrient intake was determined and calculated based on three-day food records using the Computer-Aided Nutritional Analysis Program (CAN-pro 2.0; Korean Nutrition Society, Seoul, Korea).

3.3. Serum Lipid Profile and Free Fatty Acids

Fasting levels of total cholesterol and triglyceride were measured using commercially available kits and a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). ApoB-containing lipoproteins were precipitated with dextran-sulfate magnesium, and HDL-cholesterol concentrations in the supernatants were measured enzymatically. For subjects with serum triglyceride levels < 400 mg/dL, LDL-cholesterol concentrations were estimated indirectly using the Friedwald formula: LDL-cholesterol = Total-cholesterol – [HDL-cholesterol + (Triglycerides/5)]. For subjects with serum triglyceride levels ≥ 400 mg/dL, LDL-cholesterol concentrations were measured indirectly. Free fatty acids were analyzed with a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan).

3.4. Fasting Glucose, Insulin, and Homeostasis-Model Assessment of Insulin Resistance

Fasting glucose levels were measured by the glucose-oxidase method with a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin levels were measured by radioimmunoassay using commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Insulin resistance (IR) was calculated by the homeostasis-model assessment (HOMA) using the following equation: HOMA – IR = [Fasting insulin (μIU/mL) × Fasting glucose (mmol/L)] / 22.5.

3.5. Assessment of Serum High-Sensitivity C-Reactive Protein, Lipoprotein-Associated PLA_2 Activity, Plasma Malondialdehyde, and LDL Particle Size

The concentrations of serum high-sensitivity C-reactive protein (hs-CRP) were measured with an Express Plus TM auto-analyzer (Chiron Diagnostics Co., Walpole, MA, USA) using a commercially available, high-sensitivity CRP-Latex(II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan). The activity of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) was measured using a modification of a previously described, high-throughput radiometric activity assay.[55] Plasma malondialdehyde (MDA) was measured from thiobarbituric acid-reactive substances using the TBARS Assay Kit (Zepto-Metrix Co., Buffalo, NY, USA). LDL particles were isolated by sequential flotation ultracentrifugation; the particle size distribution (1.019–1.063 g/mL) was examined by a pore-gradient lipoprotein system (CBS Scientific, CA, USA) on commercially available, non-denaturing, polyacrylamide slab gels containing a linear gradient of 2–16% acrylamide (Alamo Gels Inc., San Antonio, TX, USA). Latex bead (34 nm) conjugated standards of thyroglobulin (17 nm), apoferritin (12.2 nm), and catalase (10.4 nm) were used to estimate the relative migration rates of each band. Gels were scanned using a GS-800 Calibrated Imaging Densitometer (Bio-Rad, Graz, Austria).

3.6. Plasma Oxidized LDL, Urinary 8-*epi*-Prostaglandin $F_{2\alpha}$, Serum Interleukin-6, and Measurement of Brachial-Ankle Pulse Wave Velocity

Plasma ox-LDL was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The resulting color reaction was determined at 450 nm on a Wallac Victor^2 multilabel counter (Perkin-Elmer Life Sciences, Turku, Finland). Urine was collected in polyethylene bottles containing 1% butylated hydroxytoluene after a 12-hour fast. The bottles were immediately covered with aluminum foil and stored at -70℃ until further analysis. The compound 8-*epi*-$PGF_{2\alpha}$ was measured using an enzyme immunoassay (Bioxytech urinary 8-*epi*-$PGF_{2\alpha}$ Assay kit, OXIS International Inc., Portland, OR). Urinary creatinine levels were determined using the alkaline-picrate (Jaffe) reaction. Serum interleukin (IL)-6 concentrations were measured using Bio-Plex Reagent Kits and a Bio-Plex system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Brachial-ankle pulse wave velocity was measured using an automatic waveform analyzer (Model VP-1000; Nippon Colin Ltd., Komaki, Japan) according to a previously published method.[56] The average ba-PWV from both left and right sides was used for analysis.

3.7. Global (Nontargeted) Metabolic Profiling of Plasma

3.7.1. *Sample Preparation and Analysis*

Prior to analysis, 800 µL of 80% acetonitrile was added to 100 µL of plasma, mixed by vortexing, and centrifuged at 10,000 rpm for 5 minutes at 4℃. The supernatant was dried with N_2, dissolved in 10% methanol, mixed by vortexing, and centrifuged at 10,000 rpm for 5 minutes at 4℃. The supernatant was transferred into a vial.

3.7.2. *Ultra Performance Liquid Chromatography*

The plasma extract samples (7 μL) were injected into an Acquity UPLC-BEH-C18 column (2.1 × 50 mm, 1.7 μm; Waters, Milford, MA) that was coupled in-line with a UPLC-LTQ-Orbitrap XL (Thermo Fisher Scientific, USA). The injected samples were equilibrated with water containing 0.1% formic acid. Samples were eluted with an acetonitrile gradient containing 0.1% formic acid at a flow rate of 0.35 mL/min for 20 minutes. Metabolites were separated by UPLC (Waters, Milford, MA), analyzed, and assigned by LTQ-Orbitrap-XL (Thermo Fisher Scientific, USA). The mass spectrometer was operated in ESI-positive mode. The spray voltage was 5 kV. The flow-rate nitrogen sheath gas and the auxiliary gas were 50 and 5 (arbitrary units). The capillary voltage (V), tube-lens voltage (V), and capillary temperature (℃) were kept constant at 35 V, 80 V, and 370℃. The Orbitrap data were collected in the range of *m*/*z* 50–1,000. For quality control, a mixture of four standard compounds (acetaminophen, sulfadimethoxine, terfenadine, and reserpine) was injected every ten samples. The MS/MS spectra of metabolites were obtained by a collision-energy ramp from 55–65 eV, and conducted with Xcalibur 2.1 and MS Frontier software (Thermo Fisher Scientific, USA).

3.7.3. *Data Processing and Identification of Metabolites*

All MS data including retention times, *m*/*z*, and ion intensities were extracted by SIEVE software (Thermo Fisher Scientific, USA) incorporated into the instrument, and the resulting MS data were assembled into a matrix. SIEVE parameters were set as follows: *m*/*z* range 50–1,000; *m*/*z* width 0.02; retention-time width 2.5; and *m*/*z* tolerance 0.005. Metabolites were searched using the following databases: ChemSpider (www.chemspider.com), Human Metabolome (www.hmdb.ca), Lipid MAPS (www.lipidmaps.org), KEGG (www.genome.jp/kegg), and MassBank (www.massbank.jp). Selected metabolites were confirmed on the basis of retention times and mass spectra of standard samples.

3.8. Statistical Analyses

Statistical analyses were performed using SPSS v. 21.0 (IBM SPSS Statistics 21, Chicago, IL, USA). Skewed variables were logarithmically-transformed for statistical analyses. For descriptive purposes, mean values are presented using untransformed values. Results are expressed as means ± standard error (SE). A two-tailed *P*-value of < 0.05 was considered statistically significant. Differences in clinical variables between the normotensive and prehypertensive groups at the three-year follow-up were tested using Student's independent *t*-tests. General linear model (GLM) tests were applied to compare changes in variables between the two groups by adjusting for confounding factors. Pearson's and partial correlation coefficients were used to examine the relationships between variables over time. Multiple stepwise regression analyses were performed to identify major plasma metabolites of blood pressure. False Discovery Rate (FDR) corrected *q*-values were computed using the R package 'fdrtool'.

Multivariate statistical analysis was performed using SIMCA-P+ software version 12.0 (Umetrics, Umeå, Sweden). Partial least-squares discriminant analysis (PLS-DA) was used as the classification method for modeling the discrimination between normotensive and prehypertensive subjects by visualizing the score plot (*S*-plot) using the first- and second-PLS components. To validate the model, a seven-fold validation was applied to the PLS-DA model, and the reliabilities of the model were rigorously validated by a permutation test (n=200). The goodness of the fit was quantified by R^2Y, whereas the predictive ability was quantified by Q^2Y. Generally,

R^2Y describes how well the data in the training set are mathematically reproduced, and varies between 0 and 1 (a value of 1 indicates a model with a perfect fit). Models with $Q^2Y \geq 0.5$ are considered to have good predictive capabilities.

4. Results

4.1. Clinical Characteristics, Inflammatory and Oxidative-Stress Markers, and Nutrient Intake of Control and Prehypertensive Subjects

The mean SBP/DBP levels in the control and prehypertensive groups were 107/65 and 134/84 mm Hg, respectively (Table 3). At baseline, the mean SBP/DBP level in the control group was 105/65 mm Hg, compared with 134/85 mm Hg in the prehypertensive group. Subjects with prehypertension showed significantly higher BMI, waist-hip ratio (WHR), total- and LDL-cholesterol, and triglyceride than controls. The prehypertension group also had higher lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity, plasma malondialdehyde (MDA), urinary excretion of 8-*epi*-$PGF_{2\alpha}$, ba-PWV, and serum interleukin 6 before and after adjusting for BMI, WHR, smoking, alcohol consumption, serum lipid profiles, glucose, and insulin (Table 3). The estimated total caloric intakes were similar in both the prehypertensive group (2,225 ± 40 kcal/d) and the control group (2,194 ± 33 kcal/d). There were no statistically significant differences between the groups with respect to the proportion of caloric intake from macronutrient intake (data not shown), or the intake ratio of polyunsaturated/monounsaturated/saturated (P/M/S) fats between the control (1:0.80:0.51) and the prehypertensive (1:0.80:0.55) groups. There were no significant differences in the ratio of total energy intake to total energy expenditure between the groups (data not shown).

Table 3. Clinical Characteristics and Inflammatory and Oxidative-Stress Markers

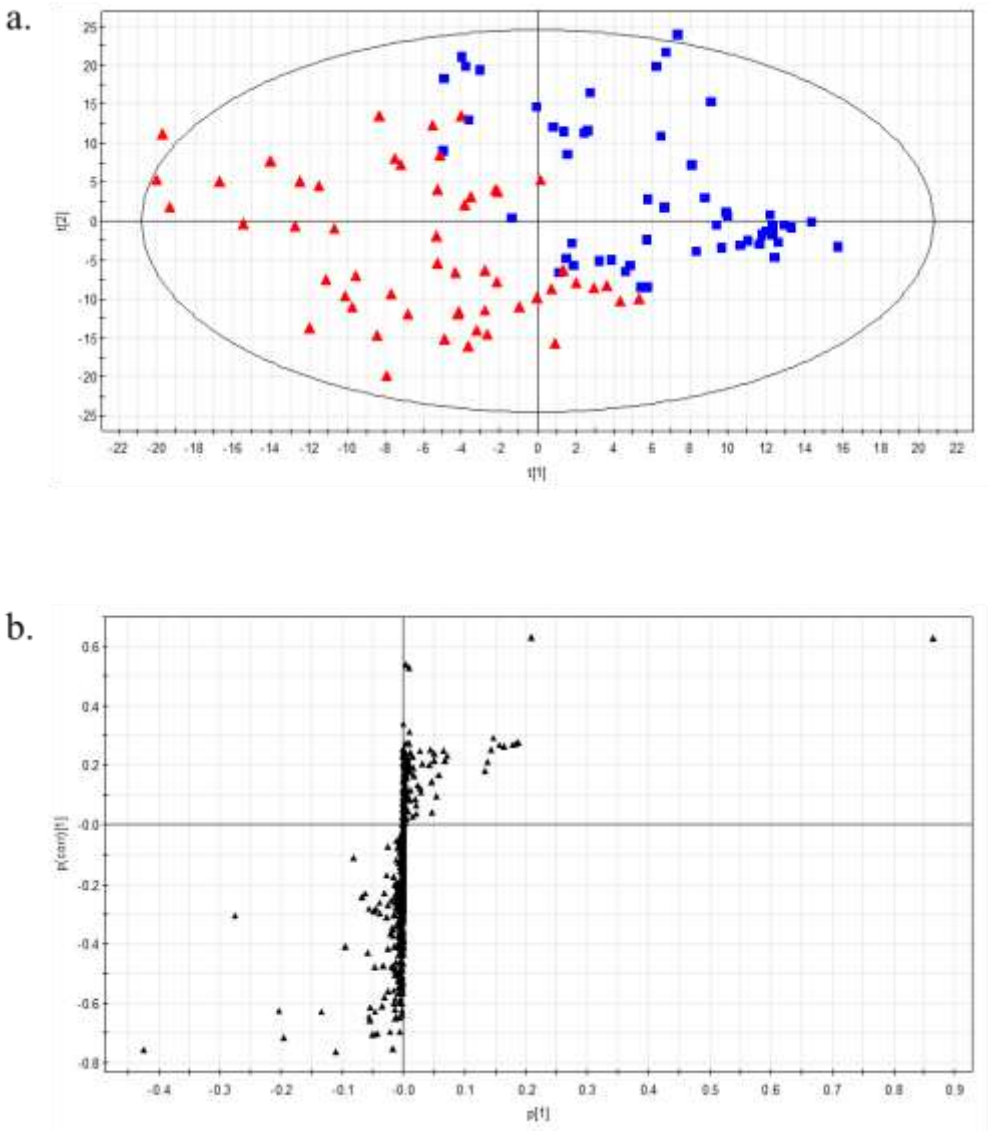
	Normotensive Group (n=53)		Prehypertensive Group (n=53)		P	P'
Age (year)	51.1±0.94		51.1±1.30		0.972	–
Male/female (%)	54.7 / 45.3		54.7 / 45.3		1.000	–
Systolic BP (mm Hg)	107.4	±0.94	134.1	±0.76	<0.001	<0.001
Diastolic BP (mm Hg)	64.6	±0.94	85.0	±0.56	<0.001	<0.001
Body mass index (kg/m^2)	23.2	±0.28	25.3	±0.37	<0.001	–
Waist/hip ratio	0.88	±0.01	0.91	±0.01	0.001	–
Cigarette smoker, *n* (%)	77.4 / 22.6		86.8 / 13.2		0.205	–
Alcohol drinker, *n* (%)	30.2 / 69.8		43.4 / 56.6		0.159	–
Total-cholesterol (mg/dL)$^{\phi}$	184.7	±5.39	208.2	±4.50	<0.001	–
LDL-cholesterol (mg/dL)$^{\phi}$	116.1	±4.60	133.6	±3.79	0.001	–
HDL-cholesterol (mg/dL)$^{\phi}$	51.7	±2.05	48.7	±1.68	0.277	–
Triglyceride (mg/dL)$^{\phi}$	89.6	±10.4	133.1	±12.4	<0.001	–
Glucose (mg/dL)$^{\phi}$	92.0	±1.28	95.9	±1.71	0.156	–
Free fatty acid (Eq/L)$^{\phi}$	468.8	±32.0	539.3	±28.8	0.050	–
Insulin (IU/mL)$^{\phi}$	7.88	±0.46	8.92	±0.54	0.117	–
[1]HOMA-IR$^{\phi}$	1.79	±0.10	2.14	±0.14	0.067	0.783
[2]hs-CRP (mg/dL)$^{\phi}$	1.15	±0.24	1.06	±0.23	0.826	0.170
LDL particle size (nm)$^{\phi}$	24.1	±0.15	23.5	±0.15	0.005	0.589
Lp-PLA$_2$ activity (nmol/mL/min)$^{\phi}$	28.1	±0.81	33.4	±0.98	<0.001	0.010
Malondialdehyde (nmol/mL)$^{\phi}$	9.09	±0.33	11.3	±0.31	<0.001	0.010
Oxidized LDL (U/L)$^{\phi}$	38.3	±1.30	51.6	±2.16	<0.001	0.051
8-*epi*-PGF$_{2\alpha}$ (pg/mg creatinine)$^{\phi}$	1,111.4	±38.8	1,698.2	±97.3	<0.001	<0.001
[3]ba-PWV (cm/sec)$^{\phi}$	2,431.7	±44.5	2,991.3	±58.4	<0.001	<0.001
Serum IL-6 (pg/mL)$^{\phi}$	2.00	±0.12	3.20	±0.14	<0.001	<0.001

Means ± S.E. $^{\phi}$ tested by logarithmic transformation. *P*-values derived from independent *t*-tests. *P'*-values derived from independent *t*-tests after adjusting for BMI, WHR, smoking, alcohol consumption, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, glucose, free fatty acid, and insulin. [1]HOMA-IR=[fasting insulin (μIU/mL) x fasting glucose (mmol/L)]/22.5. [2]hs-CRP = high sensitivity C-reactive protein. [3]ba-PWV=brachial-pulse wave velocity.

4.2. Plasma Metabolic Profiling using UPLC-LTQ-Orbitrap Mass Spectrometry

4.2.1. *Nontargeted Metabolic Pattern Analysis*

The mass spectrometry (MS) data of plasma metabolites obtained from normotensive and prehypertensive subjects were applied to a PLS-DA score plot (Figure 5a). The two-component PLS-DA score plots of the plasma metabolites showed distinct clustering and clear separation for each of the normotensive and prehypertensive groups. Both groups could be clearly differentiated from each other by the primary component t(1) or the secondary component t(2) based on the model with R^2X(cum) and R^2Y(cum) values of 0.232 and 0.661, respectively, which indicates a good fit of the data. The Q^2Y(cum) value of 0.532 provided an estimate of the predictive ability of the model. The PLS-DA model was validated using a permutation test, which indicated an R^2Y intercept value of 0.131 and a Q^2Y intercept value of −0.294. To identify the metabolites that contributed to the differentiation between normotensive and prehypertensive groups, *S*-plots of p(1) and p(corr)(1) were generated using centroid scaling (Figure 5b). The *S*-plot revealed that the metabolites with higher or lower p(corr) values served as the more relevant metabolites for discriminating between the two groups.



Figures 5. Partial least-squares discriminant analysis models.

(A) Score plots classifying subjects as normotensive (filled square) or prehypertensive (filled triangle). (B) Score plots for covariance [p] and reliability correlation [p(corr)].

4.2.2. *Identification of Plasma Metabolites*

The metabolites (variables) that play important roles in the differentiation between normotensive and prehypertensive groups were selected according to the variable important in the projection (VIP) parameter (VIP values > 1.0 indicate a high relevance to the differences between the sample groups). Among 932 metabolites in plasma, 52 metabolites were selected based on VIP values > 1.0; of these, 20 metabolites were identified and 32 were unknown. The results are shown in Table 4. Among the 20 identified plasma metabolites, the normalized peak intensities of 3 amino acids (leucine, phenylalanine, and tryptophan) were significantly higher in prehypertensive subjects than in control subjects; however, these differences disappeared after adjusting for BMI, WHR, smoking, alcohol consumption, serum lipid profiles, glucose, and insulin. Also, these 3 amino acids showed 0.1025, 0.1208, and 0.1000 of *q*-value, respectively. Ten lysophosphatidylcholines (lysoPCs) containing C14:0, C16:1, C16:0, C18:2, C18:1, C18:0, C20:5, C20:4, C20:3, and C22:6 were significantly higher in prehypertensive subjects than in control subjects (*q*=5.41E-08, 5.05E-14, 6.28E-16, 1.27E-0.5, 4.46E-10, 1.70E-05, 6.81E-05, 6.52E-11, 2.35E-09, and 3.23E-09, respectively) before and after adjusting for all confounding variables including Lp-PLA_2 activity and ox-LDL.

Table 4. Identification of Plasma Metabolites of Normotensive and Prehypertensive Subjects

Identity	Formula [M + H]+	Exact Mass (M + H)	Mass Error (mDa)	Normalized Peak Intensities				Fold Change[a]	*P*	*P′*	*P″*	VIP
				Normotensive Group (*n*=53)		Prehypertensive Group (*n*=53)						
L-Leucine	$C_6H_{13}NO_2$	132.1025	–1.3	3,676,275	±105,146	3,988,998	±96,549	1.09	0.031	0.055	0.248	2.045
L-Phenylalanine	$C_9H_{11}NO_2$	166.0868	–1.4	2,247,548	±54,466	2,415,093	±58,200	1.07	0.038	0.157	0.404	1.079
L-Tryptophan	$C_{11}H_{12}N_2O_2$	205.0977	–1.3	1,572,767	±57,695	1,760,331	±62,565	1.12	0.030	0.100	0.187	1.225
Palmitic amide	$C_{16}H_{33}NO$	256.2640	–2.1	711,550	±97,369	703,228	±82,904	0.99	0.948	0.853	0.543	1.156
Oleamide	$C_{18}H_{35}NO$	282.2797	–2.2	4,551,082	±418,609	4,714,739	±407,173	1.04	0.780	0.760	0.621	4.818
LysoPC (14:0)	$C_{22}H_{46}NO_7P$	468.3090	–3.6	261,210	±14,207	413,695	±18,650	1.58	<0.001	<0.001	<0.001	1.028
LysoPC (16:1)	$C_{24}H_{48}NO_7P$	494.3247	–3.3	551,486	±21,985	911,147	±30,487	1.65	<0.001	<0.001	<0.001	2.471
LysoPC (16:0)	$C_{24}H_{50}NO_7P$	496.3403	–3.6	9,219,069	±155,236	11,815,774	±183,606	1.28	<0.001	<0.001	<0.001	17.499
LysoPC (18:2)	$C_{26}H_{50}NO_7P$	520.3403	–4.1	3,670,567	±113,737	4,603,096	±140,602	1.25	<0.001	<0.001	<0.001	6.010
LysoPC (18:1)	$C_{26}H_{52}NO_7P$	522.3560	–4.6	3,256,978	±88,919	4,299,302	±103,385	1.32	<0.001	<0.001	<0.001	6.867
LysoPC (18:0)	$C_{26}H_{54}NO_7P$	524.3716	–4.5	4,354,018	±129,401	5,339,069	±144,196	1.23	<0.001	<0.001	0.001	5.852
LysoPC (20:5)	$C_{28}H_{48}NO_7P$	542.3247	–3.9	437,396	±18,626	597,653	±28,416	1.37	<0.001	<0.001	0.001	1.088

LysoPC (20:4)	$C_{28}H_{50}NO_7P$	544.3403	–4.0	941,761	±23,633	1,285,336	±35,484	1.36	<0.001	<0.001	<0.001	2.341
LysoPC (20:3)	$C_{28}H_{52}NO_7P$	546.3560	–4.3	388,869	±19,137	592,630	±20,649	1.52	<0.001	<0.001	<0.001	1.396
LysoPC (22:6)	$C_{30}H_{50}NO_7P$	568.3403	–4.1	537,886	±21,797	807,587	±30,720	1.50	<0.001	<0.001	<0.001	1.808
PC (16:0/18:2)	$C_{42}H_{80}NO_8P$	758.5700	–5.6	4,847,743	±352,770	4,938,788	±384,917	1.02	0.862	0.713	0.602	3.297
PC (16:1/20:4)	$C_{44}H_{78}NO_8P$	780.5543	–5.0	837,185	±114,131	835,170	±157,967	1.00	0.992	0.964	0.719	1.534
PC (18:2/18:2)	$C_{44}H_{80}NO_8P$	782.5700	–5.1	1,875,687	±156,964	2,156,458	±157,026	1.15	0.209	0.410	0.528	2.191
PC (18:0/18:2)	$C_{44}H_{84}NO_8P$	786.6013	–5.8	1,200,418	±89,200	1,347,258	±99,846	1.12	0.275	0.473	0.639	1.791
Lactosylceramide (d18:1/12:0)	$C_{42}H_{79}NO_{13}$	806.5630	2.6	1,832,933	±253,443	1,436,077	±185,650	0.78	0.209	0.269	0.548	2.824

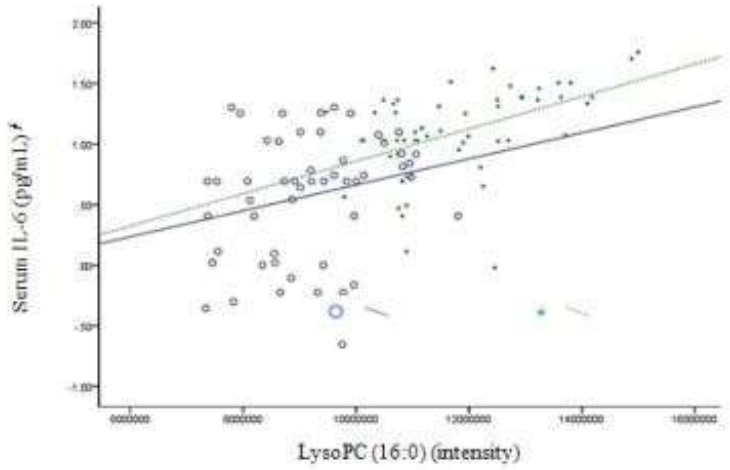
Means ± S.E. [♯] tested by logarithmic transformation. [a]Calculated by the mean intensity of each metabolite from the prehypertensive group divided by the mean intensity of each metabolite from the normotensive group. *P*-values derived from independent *t*-tests. *P′*-values derived from independent *t*-tests after adjusting for BMI, WHR, smoking, alcohol consumption, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, glucose, free fatty acid, and insulin. *P″*-values derived from independent *t*-tests after adjusting for BMI, WHR, smoking, alcohol consumption, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, glucose, free fatty acid, insulin, Lp-PLA_2, and ox-LDL.

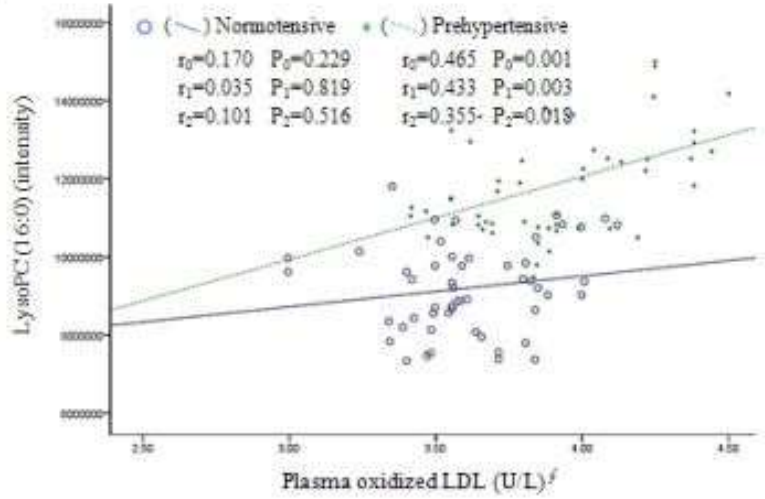
4.3. Blood Pressure Correlates with Clinical and Biochemical Parameters and Major Plasma Metabolites

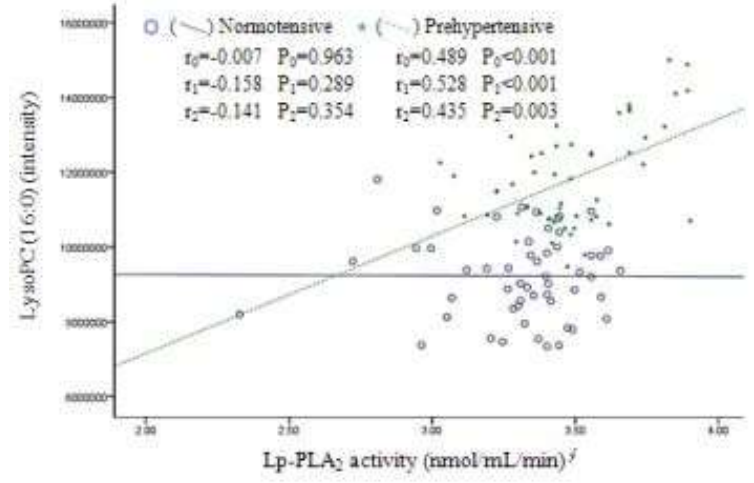
In all subjects (*n*=106), SBP and DBP positively correlated with BMI, WHR, total- and LDL-cholesterol, triglyceride, HOMA-IR index, Lp-PLA_2 activity, ox-LDL, MDA, 8-*epi*-$PGF_{2\alpha}$, ba-PWV, IL-6, and lysoPCs (14:0, 16:1, 16:0, 18:2, 18:1, 18:0, 20:5, 20:4, 20:3, and 22:6), and negatively correlated with LDL particle size (all *P* values < 0.01). Systolic blood pressure also positively correlated with tryptophan (*P* = 0.015). Based on these results, we performed a multiple-regression analysis to determine independent predictors of SBP and DBP. Age, gender, BMI, WHR, total cholesterol, HOMA-IR index, Lp-PLA_2 activity, leucine, phenylalanine, tryptophan, and lysoPCs (14:0, 16:1, 16:0, 18:2, 18:1, 18:0, 20:5, 20:4, 20:3, and 22:6) were tested. LysoPC (16:0) emerged as an independent predictor of SBP (standardized β = 0.399, *P* = 0.046), as did BMI (standardized β = 0.225, *P* = 0.025). LysoPC (16:0) also was an independent predictor of DBP (standardized β = 0.453, *P* = 0.025).

4.4. LysoPC (16:0) Correlates with ox-LDL, Lp-PLA_2 Activity, 8-*epi*-$PGF_{2\alpha}$, IL-6, and Other LysoPCs

In prehypertensive subjects, the levels of lysoPC (16:0) positively and significantly correlated with ox-LDL, Lp-PLA_2 activity, 8-*epi*-$PGF_{2\alpha}$, and IL-6 before and after adjusting for BMI, WHR, smoking, alcohol consumption, and LDL cholesterol (Figure 6). However, these associations were not found in normotensive subjects. In both prehypertensive and control subjects, the levels of lysoPC (16:0) positively correlated with other lysoPCs (14:0, 16:1, 18:2, 18:1, 18:0, 20:5, 20:4, 20:3, and 22:6) and ba-PWV (all P values < 0.014). Serum IL-6 positively correlated with urinary 8-*epi*-$PGF_{2\alpha}$ (r = 0.262, P = 0.007) and plasma MDA (r = 0.449, P < 0.001).


Serum IL-6 (pg/mL)
LysoPC (16:0) (intensity)


Normotensive
Prehypertensive
r0=0.170 P0=0.229
r1=0.035 P1=0.819
r2=0.101 P2=0.516
r0=0.465 P0=0.001
r1=0.433 P1=0.003
r2=0.355 P2=0.019
LysoPC (16:0) (intensity)
Plasma oxidized LDL (U/L)


Normotensive
Prehypertensive
r0=-0.007 P0=0.963
r1=-0.158 P1=0.289
r2=-0.141 P2=0.354
r0=0.489 P0<0.001
r1=0.528 P1<0.001
r2=0.435 P2=0.003
LysoPC (16:0) (intensity)
Lp-PLA2 activity (nmol/mL/min)

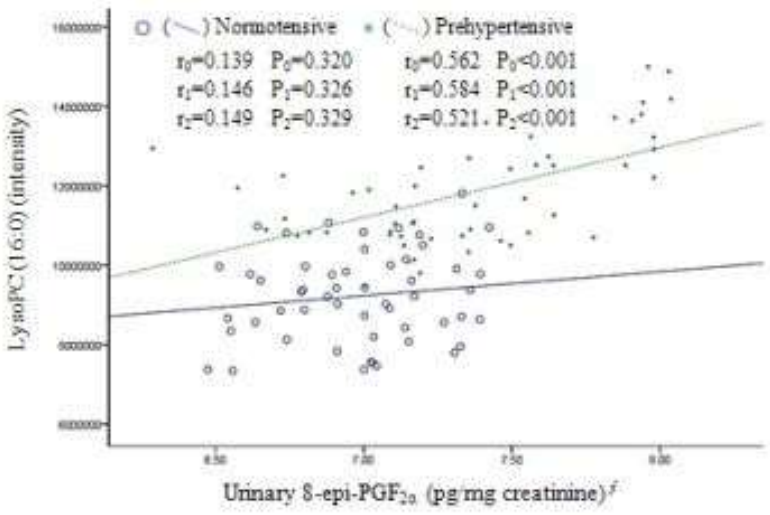


Figure 6. Relationship of lysoPC (16:0) with plasma oxidized LDL, Lp-PLA$_2$ activity, urinary 8-*epi*-PGF$_{2\alpha}$, and serum IL-6 in normotensive and prehypertensive subjects. Notes: f, tested by log-transformed; r_0, tested by Pearson correlation analysis (unadjusted); r_1, tested by partial correlation analysis (after adjusting for age, gender, BMI, WHR, smoking, and alcohol consumption); r_2, tested by partial correlation analysis (after adjusting for age, gender, BMI, WHR, smoking, alcohol consumption, and LDL-cholesterol).

5. Discussion

This study identified prehypertension-associated alterations in lysoPC levels and amino acid metabolism. Significant differences in the metabolic profiles were found between prehypertensive and normotensive individuals, including lysoPCs (14:0, 16:1, 16:0, 18:2, 18:1, 18:0, 20:5, 20:4, 20:3, and 22:6) and amino acids (leucine, phenylalanine, and tryptophan). Among the 10 lysoPCs, the majority contained long-chain acyl groups (i.e., C≥16).[57] This result is in agreement with previous studies on the effects of long-chain lysoPC (C≥16) in vasodilation impairment,[58] and the identification of higher levels of lysoPCs with long-chain acyl groups in the plasma of spontaneously hypertensive rats.[6] This study identified lysoPC (16:0) (VIP value of 17.173) as the most important plasma metabolite for evaluating the difference between prehypertensive and normotensive individuals. LysoPC (16:0) was positively and independently associated with DBP and SBP. This observation could support previous work on the role of increased lysoPC (16:0) in arterial stiffness.[59]

LysoPC constitutes only 1–5% of the total PC content of non-ox-LDL; however, as much as 40–50% of the PC contained within LDL is converted to lysoPC during LDL oxidation.[60] A saturated or monounsaturated fatty acid predominates in the *sn*-1 position of the phospholipid.[61] The generation of free radicals as a result of oxidative stress can activate PLA_2, which catalyzes the hydrolysis of the ester bond at the *sn*-2 position of phospholipids. Stafforini et al.[61] showed that the secreted form of Lp-PLA_2 released F_2-isoprostanes (the end-products of lipid oxidation) from the *sn*-2 position of PC with high affinity. Kono et al.[62] reported that intracellular type II Lp-

PLA_2, which shares homology with the plasma enzyme Lp-PLA_2, was involved in the metabolism of esterified 8-*iso*-$PGF_{2\alpha}$. We also measured prehypertension-associated elevations in plasma Lp-PLA_2 activity, plasma ox-LDL, lysoPCs, and urinary 8-*epi*-$PGF_{2\alpha}$. These results are in agreement with a previous report of higher mean levels of Lp-PLA_2 and 8-*epi*-$PGF_{2\alpha}$ in subjects with high ox-LDL than those in subjects with low ox-LDL,[63] and a positive association between plasma ox-LDL and Lp-PLA_2 activity in metabolic syndrome.[64]

In prehypertensive individuals, a strongly positive correlation of lysoPC (16:0) with ox-LDL, Lp-PLA_2 activity, and 8-*epi*-$PGF_{2\alpha}$ (a sensitive marker for oxidative stress[65,66]) could indicate increased production of oxidative stress from Lp-PLA_2-catalyzed PC hydrolysis during increased LDL oxidation. This result is consistent with a previous report of a correlation between increased Lp-PLA_2 activity and increased levels of lysoPC, ox-LDL, and cytokine in hypercholesterolemic minipigs.[67] A close correlation between the circulating lysoPC (16:0) levels and urinary 8-*epi*-$PGF_{2\alpha}$ also was observed in middle-aged nonobese men.[59] However, this association was not found in normotensive subjects, possibly due to low levels of LDL cholesterol, ox-LDL, and Lp-PLA_2 activity.

LysoPC, a major atherogenic component of oxLDL, was elevated in the plasma of atherosclerotic patients,[68] and was an important biomarker of coronary artery disease (CAD).[69] Lp-PLA_2 levels were observed to correlate with three lysoPCs (16:0, 18:0, and 18:1) in carotid plaques before and after adjusting for age, gender, hypertension, CAD, and statin usage.[55] A close association between circulating

lysoPC (16:0) and the ba-PWV index of central arterial stiffness,[70,71] and serum IL-6, was reported in human subjects.[59] Production of lysoPC (16:0) can stimulate the expression of adhesion molecules and the release of cytokines in endothelial cells.[71] IL-6 was induced by lysoPC (16:0) treatment in human umbilical vein endothelial cells. LysoPC (16:0) induced higher release of arachidonic acid mediated via cytosolic PLA_2 from human coronary artery smooth muscle cells, compared to that for treatment with lysoPC (14:0) or unsaturated lysoPC.[72] Consistent with these reports, the current study showed significant increases in ba-PWV, serum IL-6 levels, and lipid peroxides including 8-*epi*-$PGF_{2\alpha}$ and MDA in the prehypertensive group, and positive correlations among lysoPC (16:0), IL-6, 8-*epi*-$PGF_{2\alpha}$, and MDA.

LysoPCs represent 5–20% of the total plasma phospholipids, and are formed by the action of lecithin cholesterol acyltransferase (LCAT) in plasma.[73] Human LCAT releases lysoPC 20:4 and 22:6 from the *sn*-1 position of PC.[74] Up to 80% of the lysoPC in plasma is found in the non-lipoprotein fraction, in which albumin is considered as the main lipid-binding protein.[73,75] Unsaturated lysoPCs are associated primarily with albumin rather than lipoproteins. We observed a positive relationship between the levels of lysoPC (16:0) and those of other lysoPCs (14:0, 16:1, 18:2, 18:1, 18:0, 20:5, 20:4, 20:3, and 22:6), which could reflect an alternative source of lysoPC (16:0) production in addition to ox-LDL. LysoPC is formed by PLA_2-induced hydrolysis or oxidation of PC in LDL and cell membranes.[68] LysoPCs (14:0, 16:0, 18:0, and 18:1) and branched-chain or aromatic amino acids were elevated in overweight/obese, insulin-resistant subjects compared with the levels in lean control

subjects.[76-79] In the current study, differences in the levels of leucine, phenylalanine, and tryptophan between control and prehypertensive subjects disappeared after adjusting for BMI, WHR, smoking, alcohol consumption, serum lipid profiles, glucose, and insulin. However, differences in the levels of lysoPCs in the two groups remain highly significant. This result suggests an important role of lysoPCs in prehypertension, independently from confounding variables.

Our results have similar limitations as all cross-sectional and observational studies. We evaluated associations rather than prospective prediction; thus, the causal relationships among the identified metabolites and the exact mechanisms of the prehypertensive changes are unknown. A large number of markers were detected using UPLC-LTQ-Orbitrap MS, but most remain unidentified. Unlike the large databases for GC-MS, the endogenous biomolecule databases for LC-MS-based metabolomics research have not yet been constructed.[80] Our study used UPLC-LTQ-Orbitrap MS-based metabolomics and multivariate data analyses to identify a cluster of prehypertension-associated changes in plasma metabolites for lysoPCs containing C14:0, C16:1, C16:0, C18:2, C18:1, C18:0, C20:5, C20:4, C20:3, and C22:6, which were significant before and after adjusting for BMI, WHR, smoking, alcohol consumption, serum lipid profiles, glucose, and insulin. LysoPC (16:0) had a VIP value of 17.173 and showed positive and independent association with DBP and SBP. We also found prehypertension-associated elevations in Lp-PLA_2 activity, ox-LDL, urinary 8-*epi*-$PGF_{2\alpha}$, IL-6, and ba-PWV. These results could indicate increased production of lysoPCs and oxidative stress from Lp-PLA_2-catalyzed PC hydrolysis

during increased LDL oxidation in prehypertension. The potential activation of a proinflammatory phenotype could result in arterial stiffness related to prehypertension, a risk factor for atherosclerosis.[81]

In summary, the results of our metabolomics analysis of plasma from normotensive versus prehypertensive subjects offer novel insights into metabolic alterations occurring during the prehypertensive period preceding the manifestation of hypertension or atherosclerosis. These results could provide valuable candidates for new intervention targets.

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국문요약

경계성 고혈압과 lysoPCs, Lp-PLA_2 활성, 산화스트레스 증가와의 연관성

경계성 고혈압은 죽상동맥경화증의 위험요소로 알려져 있다. 본 연구에서는 경계성 고혈압과 연관성 있는 혈장 대사물질의 변화에 대해 연구하였다.

본 연구를 위하여 3년 동안 반복적으로 혈압을 측정하였으며, 경계성 고혈압 범위에 [SBP 130-139 mm mg; DBP 80-94 mm mg] 해당 되는 53명의 그룹과 나이와 성별이 동일한 53명의 정상 혈압[SBP < 120 mm mg; DBP < 80 mm mg] 그룹으로 나누어 비교하였다. 대사 프로필은 UPLC-LTQ-Orbitrap MS로 분석하였다.

경계성 고혈압 그룹은 체질량 지수, 허리·엉덩이둘레 비율, 흡연, 음주 소비, 혈청 지질 프로필, 혈당, 인슐린을 보정한 전과 후, C14:0, C16:1, C16:0, C18:2, C18:1, C18:0, C20:5, C20:4, C20:3, and C22:6를 포함하는 lysophosphatidylcholines (lysoPCs)의 수치 및 Lp-PLA2 활성, oxidized LDL (ox-LDL), interleukin 6 (IL-6), urinary 8-epi-PGF2α, brachial-ankle pulse wave velocity (ba-PWV) 수치가 정상보다 높았다.

LysoPC (16:0)은 경계성 고혈압 그룹과 정상 그룹 사이의 차이점을 평가하기에 가장 중요한 혈청 대사물질이며, 두 군간에 영향을 미치는 변수값(VIP)이 17.173으로 DBP·SBP와 관련하여 독립적이며, 양의 상관관계를 보인다. 경계성 고혈압 그룹에서 교란인자를 보정하기 전과 후, lysoPC (16:0)는 ox-LDL, Lp-PLA_2 활성, 8-*epi*-$PGF_{2\alpha}$, ba-PWV, and IL-6와 유의적인 양의 상관성을 보였다.

결론적으로, 경계성 고혈압과 관련된 lysoPCs, Lp-PLA_2 활성, ox-LDL, urinary 8-epi-PGF2α, IL-6, and ba-PWV의 증가는 증가된 산화LDL의 Lp-PLA2-catalyzed PC hydrolysis로부터 산화스트레스를 증가시켜 염증반응과 혈관경직도를 증가시킬 수 있음을 제시한다.

핵심되는 말 : 경계성 고혈압; lysoPCs; Lp-PLA2; ox-LDL; 8-epi-PGF2α; IL-6; ba-PWV