Circulating Lp-PLA₂ activity correlates with oxidative stress and cytokines in overweight/obese postmenopausal women not using hormone replacement therapy

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Abstract Controversy remains regarding whether there is an association between circulating lipoprotein-associated phospholipase A₂ (Lp-PLA₂), cytokines, and oxidative stress in healthy postmenopausal women. We investigated the influence of age on Lp-PLA₂ activity in postmenopausal women not using hormone therapy and the relationship of Lp-PLA₂ enzyme activity to serum cytokine levels and oxidative stress indices. Normal weight ($n=1284$) and overweight/obese ($n=707$)

postmenopausal women not using hormone therapy were categorized into five age groups: 50–54, 55–59, 60–64, 65–69, and 70–89 years. Overweight-obese women showed higher plasma Lp-PLA₂ activity, urinary 8-epi-prostaglandin F_{2α} (8-epi-PGF_{2α}), serum interleukin (IL)-6, and smaller LDL particles than normal-weight women after adjusting for age, years postmenopause, smoking, drinking, blood pressure, glucose, insulin, lipid profiles, BMI, and waist circumference. Overweight/obese women 70–89 years old showed higher Lp-PLA₂ activity than those aged 50–54 years, whereas no significant difference in Lp-PLA₂ activity existed across normal-weight female age groups. Overweight/obese women aged ≥65 years showed higher Lp-PLA₂, oxidized LDL (ox-LDL), IL-6, and 8-epi-PGF_{2α} than age-matched normal-weight controls. Overweight/obese women aged ≥70 years had higher ox-LDL levels than those aged 50–59, and overweight/obese women aged 65–89 showed higher IL-6 and 8-epi-PGF_{2α}. There were strong positive correlations between Lp-PLA₂ and ox-LDL ($r=0.385$, $P<0.001$), Lp-PLA₂ and IL-6 ($r=0.293$, $P<0.001$), and ox-LDL and IL-6 ($r=0.303$, $P<0.001$) in overweight/obese women; however, these relationships were weak in normal-weight women. These results suggest that aging and obesity-related oxidative and inflammatory mediators are associated with Lp-PLA₂ activity in overweight/obese postmenopausal women not using hormone therapy.

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Introduction

Many studies have associated lipoprotein-associated phospholipase A₂ (Lp-PLA₂) with circulating triglyceride, LDL cholesterol, and HDL cholesterol levels, body mass index (BMI), age, sex, postmenopausal hormone use, and smoking (Oei et al. 2005; Brilakis et al. 2005; Hatoum et al. 2010; Detopoulou et al. 2009; Koenig et al. 2004; Persson et al. 2007; Iribarren et al. 2005; Ballantyne et al. 2005; Yang et al. 2006; Khuseynova et al. 2004). Lp-PLA₂ catalyzes oxidized LDL (ox-LDL) hydrolysis, which produces inflammatory mediators, lysophosphatidylcholine (LysoPC), and oxidized fatty acid (MacPhee et al. 1999). LysoPC induces inflammatory cytokine expression, including interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF- α) (Carlquist et al. 2007). In addition, the major part of LDL-associated Lp-PLA₂ is bound to the atherogenic small dense LDL (sdLDL) particles, and serum Lp-PLA₂ enzyme activity is a marker of circulating sdLDL particles (Tsimihodimos et al. 2002; Rizzo and Berneis 2006; Rizzo et al. 2009; Gazi et al. 2005). There has been a growing interest in Lp-PLA₂ because of its key roles in lipid metabolism and initiating inflammation (Mohler et al. 2008).

Elevated plasma Lp-PLA₂ activity is reportedly associated with increased circulating IL-6 and ox-LDL in metabolic syndrome women, and this association is affected by menopause status (Chae et al. 2011). Indeed, Lp-PLA₂ is positively associated with an increased ischemic stroke incidence among hormone replacement therapy (HRT) nonusers in postmenopausal women, in a fashion that is independent of traditional cardiovascular risk factors (Hatoum et al. 2010; Detopoulou et al. 2009; Koenig et al. 2004; Persson et al. 2007; Iribarren et al. 2005; Ballantyne et al. 2005; Yang et al. 2006; Khuseynova et al. 2004; Wassertheil-Smoller et al. 2008). However, others have failed to observe a correlation between Lp-PLA₂ activity and inflammation. For example, Furberg et al. (2008) observed only a weak correlation between Lp-PLA₂ activity and circulating IL-6 levels in an elderly cohort aged ≥ 65 years. Therefore, to clearly define the association between Lp-PLA₂ activity and cytokines and oxidative stress in postmenopausal overweight/obese women, we evaluated potential differences in Lp-PLA₂ activity between non-HRT users in postmenopausal women with normal weight versus HRT nonusers with overweight/obesity and the relationships of Lp-PLA₂ activity to cytokines and oxidative stress indices in these two groups of women.

Methods

Study subjects

Overweight/obese female subjects (50 to 89 years old; $N=1991$) were recruited between January 2009 and December 2013 through the National Health Insurance Corporation (NHIC), Ilsan Hospital, in Goyang, Korea. We defined the normal group as BMI <25 kg/m² and the overweight/obese group as BMI 25 to <30 kg/m². Exclusion criteria were prior diagnoses of either type 2 diabetes, cardiovascular disease, or psychiatric problems, and any current medication use. To compare the effect of different ages and overweight/obesity on Lp-PLA₂ activity, oxidative stress, and cytokines, the 1991 postmenopausal women were divided into 10 subgroups based on age and weight: 50–54 years (normal weight: $n=318$; overweight/obese: $n=174$), 55–59 years (normal weight: 174; overweight/obese: 111), 60–64 years (normal weight: 280; overweight/obese: 129), 65–69 years (normal weight: 252; overweight/obese: 129), and 70–89 years (normal weight: 260; overweight/obese: 164). The purpose of the study was carefully explained to all participants, and written informed consent was obtained prior to their participation. The Institutional Review Board of the NHIC-sponsored Ilsan Hospital and Yonsei University provided ethical approval of the study protocol, which was performed in accordance with the tenets of the Declaration of Helsinki.

Anthropometry and blood pressure analysis

Body weight and height of unclothed subjects without shoes were measured in the morning for calculating BMI (kg/m²). 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 (FT-200S, Jawon Medical, Gyeongsan, Republic of Korea) after a 20-min rest. After a 12-h fasting period, venous blood specimens were collected in EDTA-treated and plain tubes and centrifuged to yield plasma or serum, respectively, which were stored at -70 °C until analysis.

Serum lipid profile and free fatty acids

Fasting total cholesterol and triglyceride levels were analyzed using a Hitachi 7600 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). ApoB-containing lipoproteins were precipitated with dextran-magnesium sulfate, and HDL-

cholesterol concentrations in patient serum samples 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 directly. Free fatty acids (FFA) were analyzed by enzymatic assay using the acylCoA synthetase-acylCoA oxidase (ACS-ACOD) method, with a Hitachi 7600 Autoanalyzer.

Measurement of fasting glucose and insulin and homeostasis model assessment of insulin resistance

Fasting glucose levels were analyzed by the hexokinase method using a Hitachi 7600 Autoanalyzer. Insulin levels were measured by an immunoradiometric assay kit from DIAsource ImmunoAssays S.A. (Louvain, Belgium). Insulin resistance (IR) was calculated by the homeostasis model assessment (HOMA) using the following equation: $HOMA-IR = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mmol/L)}] / 22.5$.

Assessment of serum high-sensitivity C-reactive protein, Lp-PLA₂ activity, plasma malondialdehyde, LDL particle size, ox-LDL, and urinary 8-epi-prostaglandin F_{2α}

Serum high-sensitivity C-reactive protein (hs-CRP) was measured with an ADVIA® 2400 Clinical Chemistry System (Siemens Ltd., Tarrytown, NY) using a commercially available, high-sensitivity CRP-Latex(II) X2 kit (Denka-Seiken Co., Ltd., Tokyo, Japan). The activity of Lp-PLA₂ was measured using a modification of a previously described high-throughput radiometric activity assay (Wilensky et al. 2008). Plasma malondialdehyde was measured from thiobarbituric acid-reactive substances (TBARS) using the TBARS Assay Kit (ZeptoMetrix Co., Buffalo, NY). LDL particles were isolated by sequential flotation ultracentrifugation, and particle size distribution (1.019–1.063 g/mL) was examined by a pore gradient lipoprotein system (CBS Scientific Company Inc., San Diego, CA) on commercially available, nondenaturing, polyacrylamide gels containing a linear gradient of 2–16 % acrylamide (CBS Scientific Company Inc.). Latex bead (30 nm) conjugated standards for thyroglobulin (17 nm), ferritin (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 Laboratories, Inc., Hercules, CA). Plasma ox-LDL was measured using an enzyme immunoassay (Mercodia AB, Uppsala, Sweden), and the resulting color reaction was determined at 450 nm on a Wallac Victor² multilabel counter (Perkin-Elmer Life Sciences, Boston, MA). Urine was collected in polyethylene bottles containing 1 % butylated hydroxytoluene after a 12-h fast, and bottles were immediately covered with aluminum foil and stored at –20 °C until further analysis. Levels of 8-epi-PGF_{2α} were measured using a Urinary Isoprostane ELISA kit (Oxford Biomedical Research Inc., Rochester Hills, MI). Urinary creatinine levels were determined by the Creatinine Jaffe Method.

Serum IL-6, IL-1β, and TNF-α measurement

Serum IL-6, IL-1β, and TNF-α concentrations were measured using the Bio-Plex Reagent Kit and a Bio-Plex (Bio-Rad Laboratories, Inc.) according to manufacturer instructions.

Statistical analysis

Statistical analyses were performed using SPSS v. 21.0 software (IBM/SPSS Corp. Chicago, IL). 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 indicative of statistically significant differences between comparator groups. Differences in clinical characteristics between the normal and overweight/obese group were tested using Student's independent *t* tests. General linear model tests were applied to variables to adjust for potential confounding factors. Pearson's correlation coefficients were used to examine the relationships among variables. A heat map was created to visually evaluate correlations among variables in study subjects.

Results

Clinical characteristics of study participants

Compared to normal-weight postmenopausal women (*n*=1284), those with overweight/obesity (*n*=707) had significantly more years since menopause, higher BMI,

and waist circumference, included a higher percentage of alcohol drinkers, and had higher blood pressure. Overweight/obese postmenopausal women also had higher circulating glucose, insulin, and triglyceride levels, lower HDL cholesterol, and smaller LDL particle size (Table 1). Overweight/obese postmenopausal women also showed higher plasma malondialdehyde, ox-LDL, and Lp-PLA₂ activity, elevated urinary 8-epi-PGF_{2α}, and increased serum IL-6 and hs-CRP versus their normal-weight counterparts. After adjusting for age, years since menopause, smoking, drinking, blood pressure, fasting glucose, insulin, and serum lipid profiles, overweight/obese postmenopausal women showed larger waist circumference, higher plasma ox-LDL

levels and Lp-PLA₂ activity, urinary 8-epi-PGF_{2α}, serum IL-6 and hs-CRP, and smaller LDL particle size. After further adjusting for BMI and waist circumference, elevated plasma Lp-PLA₂ activity, urinary 8-epi-PGF_{2α}, and serum IL-6 levels and smaller LDL particle size still remained significant in postmenopausal women with overweight/obesity versus normal-weight postmenopausal females.

Plasma Lp-PLA₂ activity and ox-LDL, urinary 8-epi-PGF_{2α}, and serum IL-6 according to BMI and age

We examined samples from normal-weight and overweight/obese postmenopausal women from 50 to

Table 1 Clinical characteristics of postmenopausal women study subjects

	Normal (<i>n</i> =1284)	Overweight/obese (<i>n</i> =707)	<i>P</i>	<i>P</i> ₁	<i>P</i> ₂
Age (years)	61.3±0.20	61.7±0.29	0.271	–	–
Years since menopause	13.2±0.28	14.5±0.38	0.008	–	–
Body mass index (kg/m ²)	22.6±0.05	26.7±0.06	<0.001	<0.001	–
Waist circumference (cm)	83.7±0.18	92.2±0.25	<0.001	<0.001	–
Cigarette smoker, <i>n</i> (%)	131 (10.3)	66 (9.3)	0.618	–	–
Alcohol drinker, <i>n</i> (%)	461 (36.2)	301 (42.9)	0.004	–	–
Systolic BP (mmHg)	124.2±0.44	131.1±0.60	<0.001	–	–
Diastolic BP (mmHg)	75.4±0.28	79.4±0.38	<0.001	–	–
Glucose (mg/dL) ^a	87.7±0.31	90.6±0.43	<0.001	–	–
Insulin (μIU/mL) ^a	7.44±0.11	9.34±0.15	<0.001	–	–
Triglyceride (mg/dL) ^a	115.3±1.84	135.2±2.65	<0.001	–	–
Free fatty acid (μEq/L) ^a	552.3±7.09	552.4±8.37	0.365	–	–
Total cholesterol (mg/dL) ^a	197.2±0.96	197.2±1.35	0.841	–	–
HDL cholesterol (mg/dL) ^a	55.2±0.43	51.0±0.48	<0.001	–	–
LDL cholesterol (mg/dL) ^a	119.4±0.91	119.8±1.23	0.852	–	–
LDL particle size (nm) ^a	23.7±0.02	23.4±0.03	<0.001	<0.001	0.004
Malondialdehyde (nmol/mL) ^a	8.65±0.10	9.14±0.14	0.005	0.828	0.558
Oxidized LDL (U/L) ^a	58.4±0.66	62.8±0.98	<0.001	0.012	0.505
Lp-PLA ₂ activity (nmol/mL/min) ^a	29.8±0.21	31.0±0.29	<0.001	0.006	0.005
8-Epi-PGF _{2α} (pg/mg creatinine) ^a	1602.3±19.5	1824.6±30.9	<0.001	<0.001	<0.001
Serum IL-1β (pg/mL) ^a	0.82±0.03	0.83±0.03	0.855	0.364	0.524
Serum TNFα (pg/mL) ^a	9.47±0.23	10.1±0.44	0.304	0.705	0.695
Serum IL-6 (pg/mL) ^a	3.51±0.07	4.02±0.11	<0.001	0.001	0.004
hs-CRP (mg/dL) ^a	1.14±0.07	1.67±0.12	<0.001	<0.001	0.316

The bold entries showed statistically significant *p*-value (*P*<0.05)

Means±SE; *P* values derived from independent *t* test; *P*₁ values derived from independent *t* test after adjusting for age, years since menopause, smoking, drinking, systolic and diastolic blood pressure, glucose, insulin, and serum lipid profiles; *P*₂ values derived from independent *t* test after adjusting for age, years since menopause, BMI, waist circumference, smoking, drinking, systolic and diastolic blood pressure, glucose, insulin, and serum lipid profiles

^a tested by logarithmic transformation

89 years old to elucidate the impact of age and BMI on plasma Lp-PLA₂ activity and ox-LDL levels, urinary 8-epi-PGF_{2α}, and serum IL-6 (Fig. 1). Overweight/obese women in the oldest age group, 70 to 89 years old, showed higher plasma Lp-PLA₂ activity than those of overweight/obese 50 to 54-year-old women, whereas there was no significant plasma Lp-PLA₂ activity

difference across these age groups in normal-weight women. Normal-weight women aged 60–89 years had higher plasma ox-LDL levels than those aged 50–54 years. Overweight/obese women aged ≥70 years showed higher ox-LDL than those aged 50–59 years. Normal-weight women aged ≥70 years had higher serum IL-6 levels than those aged 50–69 years, and

Fig. 1 Plasma Lp-PLA₂ activity and oxidized LDL level, serum IL-6 concentration, and urinary 8-epi-PGF_{2α} in normal-weight (white bars) and overweight/obese (gray bars) postmenopausal women according to age group. Mean±SE. †Tested by log-transformed. **P*<0.05 derived from independent *t* test within the each

age group. All alphabetical *P*<0.05 derived from ANOVA; no significant changes among age groups within the each group are marked with the same alphabet, and significant changes among age groups within each group are marked with a different alphabet

overweight/obese women aged 65–89 years showed higher circulating IL-6 levels compared to those aged 50–59 years. Additionally, normal-weight women aged ≥ 70 years had higher urinary 8-epi-PGF_{2 α} levels than those aged 50–59 years, and overweight/obese women aged 65–89 years showed higher urinary 8-epi-PGF_{2 α} compared to those aged 50–64 (Fig. 1).

Next, we compared these variables between normal-weight and overweight/obese women of the same age. Overweight/obese women aged 65 and over showed higher plasma Lp-PLA₂ activity and ox-LDL levels, and urinary 8-epi-PGF_{2 α} , than normal-weight controls of the same age. Similarly, overweight/obese women aged ≥ 60 years showed higher serum IL-6 levels than age-matched, normal-weight women (Fig. 1).

Relationship between age, BMI, HOMA-IR, LDL atherogenicity, hs-CRP, cytokines, and oxidative stress

Overall, age correlated positively with circulating hs-CRP ($r=0.219$, $P<0.001$), Lp-PLA₂ activity ($r=0.071$, $P=0.002$), ox-LDL ($r=0.174$, $P<0.001$), IL-1 β ($r=0.072$, $P=0.001$), TNF- α ($r=0.086$, $P<0.001$), IL-6 ($r=0.153$, $P<0.001$), and urinary 8-epi-PGF_{2 α} ($r=0.170$, $P<0.001$) and negatively with LDL particle size ($r=-0.202$, $P<0.001$). BMI correlated positively with HOMA-IR, hs-CRP, Lp-PLA₂ activity ($r=0.073$, $P=0.001$), ox-LDL ($r=0.103$, $P<0.001$), IL-6 ($r=0.090$, $P<0.001$), and urinary 8-epi-PGF_{2 α} ($r=0.109$, $P<0.001$) and negatively with LDL particle size. Other relationship among age, BMI, HOMA-IR, LDL atherogenicity, hs-CRP, cytokines, and oxidative stress are shown in Fig. 2.

Relationship between Lp-PLA₂ activity, ox-LDL, and IL-6 according to BMI

Figure 3 shows the relationships between plasma Lp-PLA₂ activity and ox-LDL levels and serum IL-6 levels in normal-weight and overweight/obese postmenopausal women. Lp-PLA₂ activity strongly and positively correlated with ox-LDL ($r=0.385$, $P<0.001$) and IL-6 ($r=0.293$, $P<0.001$) in overweight/obese postmenopausal women. Additionally, ox-LDL activity strongly and positively correlated with IL-6 in overweight/obese postmenopausal women ($r=0.303$, $P<0.001$).

Discussion

We found the highest plasma Lp-PLA₂ activity in the oldest age group (aged 70–89 years) of overweight/obese postmenopausal women but did not identify any age effect on Lp-PLA₂ activity in normal-weight postmenopausal women. Additionally, overweight/obese women aged ≥ 65 years showed higher plasma Lp-PLA₂ activity and ox-LDL levels, and urinary 8-epi-PGF_{2 α} , than age-matched, normal-weight controls. Furthermore, there were strong and positive correlations among Lp-PLA₂ activity, ox-LDL, and IL-6 in overweight/obese women but only weak correlations in normal-weight women. These results suggest that the oxidative and inflammatory roles of Lp-PLA₂ are enhanced in postmenopausal women who are overweight or obese versus women of normal weight.

Elevated Lp-PLA₂ activity in overweight/obese women aged 65–89 years is consistent with previous reports of a weak but significant correlation between age and Lp-PLA₂ activity (Hatoum et al. 2010; Koenig et al. 2004; Persson et al. 2007) and a favorable influence of normal weight (BMI ≤ 25 kg/m²) on Lp-PLA₂ activity (Hatoum et al. 2010; Persson et al. 2007). Indeed, Wassertheil-Smoller et al. (2008) identified an association between Lp-PLA₂ activity and ischemic stroke that was independent of CRP and traditional cardiovascular risk factors among postmenopausal, non-HRT women. The strongly positive correlations between Lp-PLA₂ activity, ox-LDL, and IL-6 in overweight/obese women are in agreement with findings in postmenopausal women with high ox-LDL (Paik et al. 2012a, b) and women with metabolic syndrome (Chae et al. 2011). However, Atik et al. (2010) found that neither serum nor plaque IL-6 correlated with serum Lp-PLA₂ activity, atherosclerotic plaque volume, or plaque Lp-PLA₂ grade among patients at risk for stroke. In addition, only a weak correlation between Lp-PLA₂ activity and IL-6 was previously observed in an elderly cohort (Furberg et al. 2008). Differences in subject characteristics and cohort numbers might have contributed to this discrepancy. We focused on non-HRT using postmenopausal women without any diagnosed disease.

Age-related increased circulating IL-6 levels have been reported in women aged 70–79 years, in whom IL-6 levels are ≈ 1.5 -fold higher than levels in middle-aged women (30–49 years) (Paik et al. 2012a, b). Similarly, in our study, postmenopausal normal-weight women only had significantly increased circulating IL-

Fig. 2 Correlation matrix among age, BMI, HOMA-IR, LDL atherogenicity, hs-CRP, cytokines, and oxidative stress in 1991 HRT-nonusing postmenopausal women. Supervised hierarchical clustering plot shows the 12 most important variables. Correlations were obtained by deriving a Spearman correlation coefficient. *Red* indicates a positive correlation and *purple* indicates a negative correlation

6 levels in the oldest group (aged 70–89 years). In postmenopausal overweight/obese women, however, significantly increased circulating IL-6 levels were already present at 65 years compared to subjects aged 50–54 years. Additionally, overweight/obese women aged ≥ 60 years showed higher plasma IL-6 than age-

matched, normal-weight controls. The main discrepancy between normal-weight and overweight/obese women in IL-6 levels seems to be due to differing amounts of age-associated increased central fat mass. Indeed, more than 25 % of circulating IL-6 is derived from fat tissue, and visceral fat tissue produces more IL-6 and TNF- α

Fig. 3 Correlations among Lp-PLA₂ activity, oxidized LDL, and IL-6 in normal-weight and overweight/obese postmenopausal women. Tested by Pearson correlation; r_0 =correlation coefficient

in normal-weight women; r_1 =correlation coefficient in overweight/obese women. [‡]Tested by log-transformation

than subcutaneous fat (Singh and Newman 2011; Brünnsgaard and Pedersen 2003; Michaud et al. 2013; Brinkley et al. 2012). In our study, no differences were found in circulating TNF- α and IL-1 β levels between normal-weight and overweight/obese women, and minimal age effects on these variables were found. These findings support the previous suggestion that locally produced TNF- α and IL-1 β do not escape into the circulation, although they induce a strong systemic IL-6 response (Paoanicolau and Vgontzas 2000).

In subgroup analysis according to BMI, the correlations between Lp-PLA₂ activity, ox-LDL, and IL-6 were more significant in overweight/obese women than in normal-weight women. This may be partly explained by the close relationship between oxidative stress, inflammation, and aging (de Gonzalo-Calvo et al. 2010). Aging and visceral fat accumulation play important roles in LDL atherogenicity including increased LDL oxidation and smaller LDL particle size, and oxidative stress activates inflammation and cytokine expression (Paik et al. 2012a, b; Chae et al. 2011; Hyun et al. 2008). Ox-LDL modifies IL-6 secretion (Shie et al. 2004; Shi et al. 2007) and stimulates Lp-PLA₂ expression in monocytes through the phosphatidylinositol 3-kinase and p38 mitogen-activating protein kinase pathways (Wang et al. 2010). Lp-PLA₂ is preferentially associated with small dense LDL (Tsimihodimos et al. 2002) and cleaves the oxidized phosphatidylcholine contained in the ox-LDL into two inflammatory mediators: lysoPC and oxidized free fatty acids (Macphée et al. 1999). LysoPC induces inflammatory cytokine expression, including interleukin IL-1 β , IL-6, and TNF- α (Carlquist et al. 2007). Therefore, it seems likely that aging and obesity, combined with oxidative stress, elicit the increased ox-LDL and smaller LDL particle size; thus, increased Lp-PLA₂ activity induced from LDL atherogenicity may contribute to inflammation process in postmenopausal overweight/obese women. Indeed, higher Lp-PLA₂ activity, 8-epi-PGF_{2 α} , IL-6, and smaller LDL particle size still remained significantly increased in postmenopausal overweight-obese women versus normal-weight women in this study after adjusting for numerous potential confounding factors including age, years since menopause, smoking and drinking habits, blood pressure, fasting glucose and insulin levels, serum lipid profiles, BMI, and waist circumference. Additionally, in agreement with a previous study (Kim et al. 2008), we identified a positive correlation between plasma Lp-PLA₂ activity and

urinary 8-epi-PGF_{2 α} , the most valid marker of oxidative stress (Patrono and FitzGerald 1997; Mehrabi et al. 2001). However, due to that the present study was designed for cross-sectional observation and not for prospective observation, it is not possible to determine potential cause and effect relationships among obesity, oxidative stress, inflammatory response, and Lp-PLA₂ activity. Therefore, clinical effects of inhibition of Lp-PLA₂ activity should be examined to determine whether Lp-PLA₂ was indeed contributed to inflammatory process in this subject group. In addition, other factors that potentially influence to Lp-PLA₂-associated inflammation, such as oxidant/antioxidant status, genetic background, and food intake, also should be examined in the future study.

In summary, the highest plasma Lp-PLA₂ activity was found in the oldest group (age 70–89 years) of overweight/obese women, but no age effect on Lp-PLA₂ activity was detected in normal-weight, postmenopausal women. Additionally, strong and positive correlations among Lp-PLA₂ activity, ox-LDL, and IL-6 were observed in overweight/obese women, but these correlations in normal-weight women were weak. These results suggest that aging and obesity-related oxidative and inflammatory mediators are associated with Lp-PLA₂ activity in overweight/obese postmenopausal women not using hormone therapy.

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