Fenofibrate reduces lipoprotein associated phospholipase A_2 mass and oxidative lipids in hypertriglyceridemic subjects with the metabolic syndrome

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Background Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a macrophage-synthesized lipase that is primarily bound to small electronegative low-density lipoproteins (LDLs). Lipoprotein-associated phospholipase A_2 oxidatively modifies LDL and generates the proinflammatory byproducts oxidized fatty acids (ox-FAs) and lysophosphatidylcholine. Fenofibrate reduces Lp-PLA_2 mass; however, it remains unknown whether the anti-inflammatory effects of fenofibrate are related to changes in LDL subclasses.

Methods This was a randomized, double-blind, controlled clinical trial designed to investigate the effects of 3-month treatment with fenofibrate (160 mg/d) on Lp-PLA_2 mass, LDL subclasses, and ox-FAs among 55 hypertriglyceridemic (≥1.7 and <6.78 mmol/L) subjects with the metabolic syndrome.

Results Fenofibrate treatment lowered fasting Lp-PLA_2 mass by 13.2% (–19.0 to –7.7) versus placebo (2.3% [–5.0 to 4.1], $P = .0002$) and total ox-FA by 15.5% (–34.2 to +1.4) versus an 11.5% increase with placebo ($P = .0013$). In age-, sex-, and treatment-adjusted models, changes in Lp-PLA_2 mass were associated with reductions in chemical LDL cholesterol ($r = 0.59$, $P < .01$) and measured total LDL particles (LDL-Ps) ($r = 0.64$, $P < .01$) and small LDL-Ps ($r = 0.57$, $P < .01$). In models that included small LDL, effects of fenofibrate on Lp-PLA_2 mass were attenuated ($P = .125$), but not in models that included LDL cholesterol ($P < .0001$) and LDL-Ps ($P = .005$). Changes in Lp-PLA_2 mass were not significantly associated with changes in ox-FA or inflammatory markers.

Conclusions Among hypertriglyceridemic subjects with the metabolic syndrome, fenofibrate therapy reduced Lp-PLA_2 mass, and these changes were associated with fewer small LDL-Ps. (Am Heart J 2008;155:499.e9-499.e16.)

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a predominantly macrophage-synthesized lipase that hydrolyzes oxidatively modified phosphatidylcholine on low-density lipoprotein (LDL) and yields a more highly oxidized LDL particle (LDL-P) and the two biologically active inflammatory mediators lysophosphatidylcholine (lyso-PC) and oxidized nonesterified fatty acids (ox-NEFA).[1] These mediators are soluble within atherosclerotic lesions and elicit proatherogenic effects in endothelial cells, monocytes/macrophages, T cells, neutrophils, and smooth muscle cells. For example, they increase the expression of adhesion molecules and chemokines, induce activation of inflammatory cytokines, and activate proteolytic enzymes that impair the structural integrity of the fibrous plaque.[2,3] Studies have shown that Lp-PLA_2 concentrations are higher among patients with the metabolic syndrome,[4,5] and increased levels of Lp-PLA_2 are associated with incremental components of the metabolic syndrome in insulin-treated patients with type 2 diabetes.[5] In the cross-sectional analysis, hypertriglyceridemia, which is highly and inversely correlated with LDL size, was the only multivariate-adjusted metabolic syndrome component associated with Lp-PLA_2 mass.

Fenofibrate, a synthetic ligand that activates peroxisome proliferator activator receptor α, has anti-inflammatory properties that involve inhibition of nuclear factor κB activation.[6] Treatment with fenofibrate has been shown to reduce Lp-PLA_2 mass in controlled[7] and uncontrolled trials[8,9] and reduce Lp-PLA_2 activity in

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obese patients with the metabolic syndrome.[10] This study was designed to investigate whether fenofibrate reduces Lp-PLA_2 mass and the proinflammatory byproduct oxidized fatty acid (ox-FA) in hypertriglyceridemic patients with ≥2 additional features of the metabolic syndrome. Second, we examined the relationships between Lp-PLA_2 mass, ox-FA, soluble cellular adhesion molecules, and chemokine production. Third, we examined whether the changes in Lp-PLA_2 mass correlated with alterations in the distribution of LDL subclasses.

Methods

Selection of subjects

The study population consisted of 55 subjects (25 intervention subjects and 30 control subjects) with the metabolic syndrome and hypertriglyceridemia as previously described.[11] Men and postmenopausal women >18 years of age with fasting triglycerides ≥1.7 and <6.78 mmol/L were included in the study. Subjects were required to have ≥2 of the following additional Adult Treatment Panel III[12] criteria of the metabolic syndrome: abdominal obesity (waist circumference >89 cm in women and >102 cm in men), low high-density lipoprotein cholesterol (HDL-C) (<1.3 mmol/L in women and <1.0 mmol/L in men), hypertension (systolic blood pressure ≥130 or diastolic blood pressure ≥85 mm Hg) or current drug therapy for hypertension, and impaired fasting glucose (≥6.1 mmol/L and <7.0 mmol/L). Exclusion criteria included diabetes, body mass index >40 kg/m^2, use of lipid-lowering therapies, aspirin >81 mg daily, regular use of nonsteroidal anti-inflammatory agents or cyclooxygenase-2 inhibitors, corticosteroids (oral and inhaled), antioxidants (including multivitamins), herbal or fiber supplements, changes in type or formulation of hormone replacement therapy in the last 6 months, alcohol intake >3 drinks per day, untreated hypothyroidism or recent change (within 2 months) of thyroid replacement therapy, and cigarette smoking (current or within the last 6 months).

The local ethics committees approved the protocol of this study. All subjects gave written informed consent before participating in this research trial.

Study design

A registered dietitian counseled subjects on the American Heart Association Step 2 diet and instructed to maintain the diet throughout the study. At the end of a 6-week run-in period, fasting lipids and glucose were measured to determine study eligibility, and within 1 week, subjects returned for randomization. Subjects were randomized to fenofibrate 160 mg daily or placebo for the next 3 months. Fasting blood specimens were obtained after a 12-hour fast at both randomization and study completion.

Laboratory studies

Lipids and lipoproteins. Plasma lipids and serum chemistry analyses were measured by standard procedures. Lipoprotein subclass profiles were measured with an automated nuclear magnetic resonance (NMR) spectroscopic assay using a modification of the method described previously (LipoScience, Inc, Raleigh, NC).[13] The lipoprotein subclasses investigated in this study included large (21.2-23 nm) and small LDL (18-21.2 nm).

Reproducibility of the NMR-measured lipoprotein particle parameters was determined by replicate analyses of plasma pools. The coefficients of variance (CVs) for total LDL-P concentration, LDL-P sizes, and large and small LDL subclasses were <4%, <0.5%, and <8%, respectively.

Oxidized lipids. Plasma C18-hydroxy-fatty acids (ox-FA) were measured by gas chromatography/mass spectrometry (Jurilab Ltd, Kuopio, Finland).[14] Plasma concentrations were calculated for total C18-ox-FA concentration and for all the single ox-FAs (8-, 9-, 10-, 11-, 12-, 13-, 15-, and 16-ox-FAs). The CV was 5% for total ox-FA and ≤11% for individual single ox-FAs (8- to 16-ox-FAs). Oxidized LDL (ox-LDL) was measured by immunoassay (Mercodia, Inc, Winston-Salem, NC), and the CV was 8.3%.

Lipoprotein-associated phospholipase A_2 mass. Lipoprotein-associated phospholipase A_2 mass measurements were performed with the PLAC Test (diaDexus, Inc, San Francisco, CA). The test resembles a sandwich enzyme immunoassay with two specific monoclonal antibodies combined with a horseradish peroxidase–tetramethylbenzidine detection system, as described by Caslake et al.[15] All samples were analyzed in duplicate with an average CV of <5.0%.

Inflammatory markers. Levels of vascular cell adhesion molecule–1 (VCAM-1), intercellular adhesion molecule–1 (ICAM-1), monocyte chemotactic protein–1 (MCP-1), and macrophage inflammatory protein–1α (MIP-1α) were assayed by monoclonal antibody–based multianalyte bead immunoassays (Luminex; Linco Research, St Charles, MO). Interassay CVs were <10%.

Statistical analyses

Subject demographic characteristics as well as fasting lipids and lipoproteins are reported as medians and interquartile ranges (IQRs). The IQR gives the 25th and 75th percentile. Wilcoxon rank sum and Fisher exact tests were used to compare continuous and dichotomous variables, respectively, between treatment groups. Person-specific percent changes over the 3-month therapy period were calculated for lipid and lipoprotein values, and median levels of these quantities were compared between treatment groups using the Wilcoxon rank sum test. Percent change over the treatment phase among Lp-PLA_2 mass, ox-FA, LDL cholesterol (LDL-C), and LDL subclasses was correlated using Spearman correlations. Treatment groups were combined for these correlations, which were performed both unadjusted and adjusted for age, sex, and treatment group. Multivariate Spearman correlations were also calculated as partial correlations on residual ranks to examine the effect of fenofibrate on Lp-PLA_2 mass and activity in the presence of small LDL, LDL-C, and total LDL-P. All statistical analyses were performed with SAS package 2003 (SAS Institute, Inc, Cary, NC).

This study was an investigator-initiated clinical trial and was conceived and managed independent of the sponsor. Statisticians (AWR, IBH) employed by Northwestern University performed the statistical analysis based on specific requests of the investigator.

Results

The baseline characteristics of the study populations are described in Table I. Aspirin (75-81 mg/d) was used in 50% of placebo-treated subjects and 32% of fenofibrate-