Fenofibrate Therapy Ameliorates Fasting and Postprandial Lipoproteinemia, Oxidative Stress, and the Inflammatory Response in Subjects With Hypertriglyceridemia and the Metabolic Syndrome

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OBJECTIVE — The aim of this study was to determine the effects of fenofibrate (160 mg/day) on fasting and postprandial lipoproteins, oxidized fatty acids, and inflammatory mediators in subjects with hypertriglyceridemia and the metabolic syndrome.

RESEARCH DESIGN AND METHODS — Fifty-nine subjects with fasting hypertriglyceridemia (≥1.7 and <6.9 mmol/l) and two or more of the Adult Treatment Panel III criteria for the metabolic syndrome were randomly assigned to fenofibrate (160 mg/day) or placebo in a double-blind, controlled clinical trial.

RESULTS — Fenofibrate treatment lowered fasting triglycerides (−46.1%, $P < 0.0001$) and postprandial (area under the curve) triglycerides (−45.4%, $P < 0.0001$) due to significant reductions in postprandial levels of large (−40.8%, $P < 0.0001$) and medium (−49.5%, $P < 0.0001$) VLDL particles. The number of fasting total LDL particles was reduced in fenofibrate-treated subjects (−19.0%, $P = 0.0033$) primarily due to reductions in small LDL particles (−40.3%, $P < 0.0001$); these treatment differences persisted postprandially. Fasting and postprandial oxidized fatty acids were reduced in fenofibrate-treated subjects compared with placebo-administered subjects (−15.3%, $P = 0.0013$, and 31.0%, $P < 0.0001$, respectively), and fenofibrate therapy lowered fasting and postprandial soluble vascular cell adhesion molecule-1 (VCAM-1) (−10.9%, $P = 0.0005$, and −12.0%, $P = 0.0001$, respectively) as well as fasting and postprandial soluble intercellular adhesion molecule-1 (ICAM-1) (−14.8%, $P < 0.0001$, and −15.3%, $P < 0.0001$, respectively). Reductions in VCAM-1 and ICAM-1 were correlated with reductions in fasting and postprandial large VLDL particles ($P < 0.0001$) as well as postprandial oxidized fatty acids ($P < 0.0005$).

CONCLUSIONS — Triglyceride-lowering therapy with fenofibrate reduced fasting and postprandial free fatty acid oxidation and inflammatory responses, and these antiatherosclerotic effects were most highly correlated with reductions in large VLDL particles.

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The metabolic syndrome represents an agglomeration of interrelated risk factors that include abnormally high fasting triglyceride levels (1,2). In a recent analysis, hypertriglyceridemia (≥1.7 mmol/l) represented the component of the metabolic syndrome most strongly associated with a history of myocardial infarction and stroke (3). Furthermore, elevated fasting triglyceride levels (≥1.4 mmol/l) and an enlarged waist circumference (>89 cm) were the two characteristics of the metabolic syndrome that were associated with the greatest increased risk for all-cause mortality and cardiovascular deaths among postmenopausal women (4).

Hypertriglyceridemia signifies the presence of increased plasma triglyceride–remnant lipoproteins, and the concentrations of remnant-like lipoproteins are further enhanced postprandially among hypertriglyceridemic subjects (5). Remnant-like proteins have been shown to increase intracellular oxidant concentrations and lipid peroxide levels in culture media and to activate nuclear factor-κB (NF-κB) (6). NF-κB is a redox-sensitive transcription factor that increases expression of multiple inflammatory genes. In human carotid endarterectomy specimens, there are established concentration-dependent associations between oxidized LDL (oxLDL), NF-κB activation, and inflam-

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The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written. Statisticians (I.B.H. and A.W.R.) employed by Northwestern University performed the statistical analysis based on specific requests of the investigators. Final data were made available to the authors who, independent of the sponsor, assessed the data analysis as well as the interpretation and writing of the results.

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Abbreviations: apoB, apolipoprotein B; AUC, area under the curve; CHD, coronary heart disease; ICAM-1, intercellular adhesion molecule-1; IDL, intermediate-density lipoprotein; NF-κB, nuclear factor-κB; NMR, nuclear magnetic resonance; OH-FA, monohydroxy fatty acid; oxLDL, oxidized LDL; VA-HIT, Veterans Administration HDL Intervention Trial; VCAM-1, vascular cell adhesion molecule-1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

matory cell infiltrates (T-cells and macrophages) (7). Although triglyceride levels are consistently measured in fasting blood specimens, accumulating evidence indicates that postprandial triglycerides further increase oxidative stress (8) and are an under-recognized contributing factor to endothelial dysfunction, atherosclerosis, and cardiovascular events (9).

Fenofibrate therapy results in significant reductions in fasting triglyceride concentrations and has been shown to reduce fibrinogen and C-reactive protein in inflammatory mediators (10); however, the relationship between plasma lipoproteins, oxidatively modified fatty acids, and inflammatory mediators remains uncertain (11). In this randomized placebo-controlled trial, we investigated the effects of fenofibrate therapy on fasting and postprandial lipoproteins, monohydroxy fatty acids (OH-FAs), and cellular adhesion molecules in hypertriglyceridemic subjects with the metabolic syndrome and attempted to correlate the changes in lipids and lipoprotein particles with inflammatory mediators.

RESEARCH DESIGN AND METHODS

The study population consisted of 59 subjects with hypertriglyceridemia and the metabolic syndrome. Study subjects were recruited from consecutive patients referred to a preventive cardiology outpatient clinic or from radio or print advertisements. A total of 59 subjects meeting inclusion and exclusion criteria were randomly assigned to receive fenofibrate 160 mg every day or placebo. Of these, 55 (25 fenofibrate and 30 control subjects) completed the study and were included in the primary analysis. Four subjects did not complete the protocol because of withdrawal of consent ($n = 2$), relocation ($n = 1$), or gastrointestinal intolerance ($n = 1$). Men and postmenopausal women ≥18 years of age with fasting triglyceride levels ≥1.7 and <6.9 mmol/l and two or more of the following Adult Treatment Panel III (1) criteria for the metabolic syndrome were included in the study: abdominal obesity (waist circumference >89 cm in women and >102 cm in men); low HDL cholesterol levels (<1.3 mmol/l in women and <1.0 mmol/l in men); hypertension (systolic blood pressure ≥130 or diastolic blood pressure ≥85 mmHg) or current drug therapy for hypertension; and impaired fasting glucose (≥6.1 and <7.0 mmol/l). Exclusion criteria included type 1 or 2 diabetes; BMI >40 kg/m^2; use of lipid-lowering therapies, oral hypoglycemic therapies, insulin, or aspirin >81 mg daily; regular use of nonsteroidal anti-inflammatory agents or cyclooxygenase-2 inhibitors, corticosteroids (oral and inhaled), antioxidants (including multivitamins), or herbal or fiber supplements; recent changes in type or formulation of hormone replacement therapy (in the last 6 months); alcohol intake >3 drinks/day; untreated hypothyroidism or a recent change (within 2 months) in thyroid replacement therapy; and cigarette smoking (current or within the last 6 months).

The institutional review board approved the protocol of this study. All subjects gave written informed consent before participating in this research trial.

Subjects were counseled by a registered dietitian on the American Heart Association Step 2 Diet and were instructed to maintain the diet throughout the study. To monitor compliance, subjects were contacted twice during the 6-week dietary lead-in period to obtain 24-h diet recalls. Food recalls were analyzed for fat, calories, fiber, and alcohol intake using the Minnesota Nutrition Data System, version 2.93 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). At the end of the lead-in period, fasting blood lipids and blood glucose were measured to determine study eligibility. Eligible subjects returned within 1 week of the blood drawing for randomization. A total of 59 eligible subjects were randomly assigned to fenofibrate 160 mg every day or placebo using a block randomization design that stratified subjects by sex and decile of age. After a 12-h fast, baseline blood specimens were collected. An oxidative challenge was administered as a test meal consisting of a milkshake, which included a standardized fat content (68% of energy) that was adjusted to body surface area (50 g/m^2); it was composed of ice cream, cream of coconut, and pasteurized egg (12). The energy load of the test meal varied from 6,378 to 10,226 kJ (1,524–2,443 kcal) and the total fat content varied from 116 to 186 g based on the body surface area of each subject. After completion of the baseline measurements, subjects had repeat phlebotomies performed at 3.5 and 8 h. Subjects were provided matching placebo or 160 mg fenofibrate daily, which they were instructed to take the evening of the randomization visit. After 3 months of therapy, repeat laboratory studies were performed for the 55 subjects who completed the study protocol.

Laboratory studies

Plasma lipid and chemistry panel measurements were obtained by standard procedures. For fasting triglyceride levels ≥4.52 mmol/l, LDL cholesterol values were measured using nuclear magnetic resonance (NMR). Lipoprotein subclass profiles were measured with an automated NMR spectroscopic assay using a modification of the method described previously (LipoScience, Raleigh, NC) (13). The following subclass categories were investigated: chylomicrons (>200 nm), large VLDL (60–200 nm), intermediate VLDL (35–60 nm), small VLDL (27–35 nm), intermediate-density lipoprotein (IDL) (23–27 nm), large LDL (21.2–23 nm), small LDL (18–21.2 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm).

Reproducibility of the NMR-measured lipoprotein particle parameters was determined by replicate analyses of plasma pools. Coefficients of variation (CV) observed were <4% for total VLDL, LDL, and HDL particle concentrations, <0.5% for VLDL, LDL, and HDL particle sizes, <10% for chylomicrons and VLDL subclasses, <8% for large and small LDL subclasses, and <5% for large and small HDL subclasses. Higher CVs for IDL (<20%) and medium HDL (<35%) subclasses reflect their typically low concentrations.

Apolipoprotein B (apoB) levels were measured by immunoassay (Jurilab, Kuopio, Finland). The CV for apoB was 2.0%. Plasma C18 hydroxy fatty acids were measured by gas chromatography/mass spectrometry (Jurilab) (14). The CV for total OH-FAs was <5%. oxLDL was measured by immunoassay (Mercodia, Winston Salem, NC). The CV for oxLDL was <9%.

Levels of soluble cellular adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) were assayed by monoclonal antibody–based multianalyte bead immunoassays (Luminex; Linco Research, St. Charles, MO). Interassay CVs were <12%.

Serum insulin levels were measured using a human-specific insulin radioimmunoassay (Linco Research). The CV for insulin was <6%. Insulin resistance was estimated from the homeostasis model (15).

Statistical analyses

Subject demographic characteristics are reported as means ± SD, while fasting and postprandial lipids and lipoproteins are reported as medians and interquartile ranges (0.5 × [75th – 25th percentile]). Postprandial responses were calculated as area under the curve (AUC) using the trapezoidal rule. The Wilcoxon rank-sum test and Fisher's exact test were used to compare continuous and dichotomous demographic variables, respectively, between treatment groups. Subject-specific percent changes from baseline over the 3-month therapy period for lipid and lipoprotein values as well as AUC were calculated for each measurement. The medians of each parameter were compared between treatment groups using the Wilcoxon rank-sum test. To account for baseline differences in fasting small VLDL particles and postprandial total VLDL particle numbers, a multivariate regression analysis was conducted on the ranks of the data. In the figures (available in an online appendix at http://dx.doi.org/10.2337/dc07-0015), *P* values are for between-group percent changes at each time point using the Wilcoxon signed rank-sum test. Percent change over the treatment phase between lipid/lipoprotein levels, OH-FA, oxLDL, and soluble cellular adhesion molecules were correlated using Spearman correlations, and both treatment groups were combined for these correlations. All statistical analyses were performed with SAS package 2003 (SAS Institute, Cary, NC).

RESULTS — The clinical characteristics of the study populations are described in Table 1 of the online appendix. This study included predominantly middle-aged men with central obesity, high fasting triglyceride levels, low levels of HDL cholesterol, and hypertension. The high proportion of men in the study may potentially introduce an unexpected bias; however, the distribution between groups was well balanced. Only two subjects had fasting glucose levels ≥6.1 mmol/l. Treatment groups were well balanced with regard to baseline demographics and laboratory values; in particular, there were no differences between treatment groups in baseline blood glucose ($P = 0.88$), insulin concentration ($P = 0.77$), or homeostasis model assessment index ($P = -0.92$). There were group differences in baseline fasting small VLDL particles (median in fenofibrate 59.1 nmol/l and median in placebo 40.8 nmol/l, $P = 0.04$) and postprandial total VLDL particles (median AUC in fenofibrate 1,044 nmol/l and median AUC in placebo 842 nmol/l, $P = 0.02$).

Aspirin (75–81 mg daily) was used by 33% of placebo-administered subjects and 24% of fenofibrate-treated subjects ($P = 0.56$). The subjects did not change their intake of dietary fat and calories. As determined from pill counts, the study subjects demonstrated high adherence to study medications, with 97.6% of placebo-administered subjects and 97.1% of fenofibrate-treated subjects returning the correct number of tablets ($P = 0.65$). Weight change during treatment (kilograms) remained comparably ($P = 0.056$) stable in the placebo group (median [interquartile range] 101.8 [17.8] to 103.2 [22.4] kg) and the fenofibrate treatment group (105 [0 25.8] to 104.5 [26.2] kg; $P = 0.056$). There were no differences in changes in waist circumference between placebo (110.8 [12.2] to 111.8 [13.8] cm) and fenofibrate treatment groups (109.2 [6.2] to 109.2 [10.2] cm); $P = 0.10$). On trial, there were nonsignificant changes in fasting glucose levels (5.45 [0.55] to 4.51 [0.88] mmol/l in the placebo group vs. 5.47 [0.36] to 4.57 [1.70] mmol/l in the fenofibrate group; $P = 0.91$).

Fasting lipoprotein and OH-FA measurements

Fenofibrate therapy lowered fasting triglycerides (−46.1 vs. −4.0% for placebo; $P < 0.0001$), non-HDL cholesterol (−19.7 vs. +0.3%; $P = 0.0048$), and total VLDL particles (−44.1 vs. +4.9%; $P < 0.0001$). The reduction in total VLDL particles observed with fenofibrate is primarily due to reductions in large VLDL particles (−57.5 vs. −12.1%; $P = 0.0112$), medium VLDL particles (−54.2 vs. −11.2%; $P = 0.0008$), and small VLDL particles (−32.8 vs. +19.6%; $P = 0.0026$). LDL cholesterol was not lowered differentially between the treatment groups (−7.1 vs. −0.8%; $P = 0.48$). However, fenofibrate did lower LDL particles (−19.0 vs. +1.5%; $P = 0.0033$) primarily due to reductions small LDL particles (−40.2 vs. +10.1%; $P < 0.0001$). Consistent with the reduction in LDL particles, apoB levels were also reduced in the fenofibrate-treated group (−13.5 vs. −1.7%; $P = 0.02$). Fenofibrate therapy did not significantly lower IDL levels (−30.3 vs. +10.8%; $P = 0.12$), and there was no significant increase in either HDL cholesterol (+21.8 vs. +11.1%; $P = 0.26$) or HDL particles (+13.2 vs. +4.4%; $P = 0.12$) in fenofibrate-treated subjects. Posttreatment levels of the lipid and lipoprotein results for each treatment group are presented in Figs. 1–3 of the online appendix. *P* values in these figures indicate the significance of the change from pretreatment levels.

Fasting OH-FA levels were reduced in the fenofibrate group (−15.5%) compared with an increase in the placebo group (+11.5%) ($P = 0.0013$), whereas oxLDL levels were lowered among fenofibrate-treated subjects compared with placebo-administered subjects (−14.3 vs. −3.1%; $P = 0.046$). Over the treatment phase of the study, mean percent changes in fasting OH-FAs were most highly correlated with mean percent changes in small LDL particles ($r = 0.42$, $P < 0.01$) (Table 2 of the online appendix), and these associations were higher for oxLDL ($r = 0.54$, $P < 0.01$). Furthermore, oxLDL was inversely correlated with large LDL particles ($r = -0.39$, $P < 0.01$) and small HDL particles ($r = -0.27$, $P = 0.048$). The fasting OH-FA and oxLDL results for the fenofibrate-treated subjects are illustrated in Fig. 4 of the online appendix.

Posttreatment levels of the OH-FA and oxLDL results for each treatment group are presented in Fig. 4 of the online appendix. *P* values in this figure indicate the significance of the change from pretreatment levels.

Postprandial lipoprotein and OH-FAs

Fenofibrate treatment lowered postprandial (AUC) triglyceride concentrations (−45.4%) compared with a rise in the placebo group (+1.4%) ($P < 0.0001$) and lowered non-HDL cholesterol (AUC) (−6.2%) compared with no change in the placebo group ($P = 0.0035$). Posttreatment levels for each time point are shown in Fig. 1 of the online appendix, together with an indication of the significance of the change in these values from pretreatment levels. The reductions in postprandial (AUC) triglyceride levels resulted from a lowering in concentrations of large VLDL particles (−54.8 vs. −8.3%; $P = 0.0008$) and medium VLDL particles (−49.5 vs. −5.2%; $P < 0.0001$). After the test meal, LDL cholesterol levels and LDL particles reflected changes in fasting specimens. The lowering in median absolute numbers of postprandial LDL particles (−2,366 in the fenofibrate group vs. +1,725 in the placebo group) was due to a decrease in the small LDL particles (−4,734 in the fenofibrate group vs.

+1,633 in the placebo group) with an increase in large LDL particles (+2,466 in the fenofibrate group vs. +488 in the placebo group). The reductions in VLDL and LDL particles were reflected by the postprandial (AUC) lowering in apoB levels (−17.7% in the fenofibrate group vs.−0.8 in the placebo group; $P = 0.01$). Posttreatment levels for VLDL and HDL for each time point are show in Figs. 2 and 3 of the online appendix, together with an indication of the significance of the change in these values from pretreatment levels. Postprandially, there were significant increases in large HDL particles (+122.8 vs. +21.3%; $P = 0.003$) and total HDL particles (+19.4 vs. +2.1%; $P = 0.008$) with fenofibrate therapy compared with placebo.

Postprandial (AUC) OH-FA levels were reduced in the fenofibrate group (−31.0%) and increased in the placebo group (+7.5%; $P < 0.0001$). Posttreatment levels for OH-FAs for each time point are shown in Fig. 4 of the online appendix. Changes (over the treatment phase) in postprandial OH-FA levels in the fenofibrate group were correlated with postprandial changes in triglyceride levels ($r = 0.73$, $P < 0.0001$) and in large ($r = 0.45$, $P < 0.01$), medium ($r = 0.48$, $P < 001$), and total VLDL particles ($r = 0.51$, $P < 0.01$) and in small LDL particles ($r = 0.45$, $P < 0.01$) as shown in Table 2 of the online appendix. In addition, there were inverse correlations between postprandial OH-FA changes and postprandial HDL cholesterol changes ($r = −0.44$, $P < 0.01$) and in large ($r = −0.49$, $P < 0.01$), small ($r = −0.38$, $P < 0.01$), and total HDL particles ($r = −0.41$, $P < 0.003$).

Fasting and postprandial inflammatory markers

Compared with placebo, fenofibrate therapy reduced soluble VCAM-1 levels in fasting specimens (−10.9 vs. −1.5%; $P = 0.0005$), as well as in postprandial (AUC) specimens (−12.0 vs. −0.8%; $P = 0.0001$). Soluble ICAM-1 levels were lowered by fenofibrate in both fasting (−14.7 vs. +0.9%; $P < 0.0001$) and postprandial (−15.3 vs +1.0%; $P < 0.0001$) specimens. The changes in fasting and postprandial levels of VCAM-1 and ICAM-1 in fenofibrate-treated subjects were correlated with reductions in both large VLDL particles and OH-FA levels (Table 2 of the online appendix). In contrast, changes in ICAM-1 were significantly associated with oxLDL ($r = 0.40$, $P < 0.01$), whereas changes in VCAM-1 were not. Posttreatment levels for VCAM-1 and ICAM-1 for each time point are shown in Fig. 5 of the online appendix. In multivariate models that included, age, sex, weight change, small LDL, and OH-FA, changes in VCAM-1 remained significantly correlated with fasting ($P = 0.02$) and postprandial large VLDL particles ($P = 0.006$). Similarly, ICAM-1 was associated with fasting ($P = 0.04$) and postprandial large VLDL particles ($P = 0.0003$).

Safety studies

During the treatment period, there were no treatment-related adverse events that occurred more frequently in either treatment group. There were no significant differences between the placebo and fenofibrate groups in aspartate aminotransferase ($P = 0.72$), alanine aminotransferase ($P = 0.10$), or creatine phosphokinase ($P = 0.16$).

CONCLUSIONS — This study reports several important findings. *1*) Postprandial hypertriglyceridemia is accompanied by persistent increased levels of small and total LDL particles and enhanced oxidative stress as evidenced by a marked increase in oxidized fatty acids and a sustained increase in the inflammatory response as indicated by elevated levels of soluble adhesion molecules. *2*) Treatment with fenofibrate markedly reduced postprandial increases in larger-sized VLDL particles and smaller-sized LDL particles. *3*) Increased oxidative stress after a fatty meal as measured by oxidized fatty acids and oxLDL is abrogated by fenofibrate. *4*) The observed antioxidant and anti-inflammatory effects of fenofibrate appear to involve reduced oxidative modification of phospholipid-rich large VLDL particles.

In this cohort of hypertriglyceridemic subjects with the metabolic syndrome, fenofibrate therapy resulted in a reduction in fasting triglyceride levels (−46%) and a comparable reduction in postprandial triglyceride levels (−45%). The triglyceride-lowering effect observed in fenofibrate-treated subjects resulted primarily from reductions in fasting and postprandial concentrations of large VLDL particles and medium VLDL particles.

In this study, fenofibrate significantly reduced fasting LDL particles compared with placebo (−19.0 vs. +1.5%; $P = 0.0033$), which persisted postprandially (−21.0% vs. +3.7%; $P = 0.0009$). Reductions in fasting and postprandial LDL particles with fenofibrate resulted primarily from a large decrease in small cholesterol-depleted LDL particles and a smaller increase in the number of larger, more cholesterol-rich LDL particles compared with placebo. Although changes in LDL cholesterol were not different between fenofibrate and placebo groups ($P = 0.38$ for fasting vs. $P = 0.24$ for postprandial), the LDL particle results suggest that fenofibrate therapy decreases the atherosclerotic potential of LDL cholesterol. The average cholesterol content of the LDL particles increased in the fenofibrate-treated subjects, which explains the nonsignificant reduction in LDL cholesterol. Similar observations were reported in the Veterans Administration HDL Intervention Trial (VA-HIT) with gemfibrozil therapy (16).

Lipoprotein subclass abnormalities contribute to the increased vascular risk for patients with the metabolic syndrome (2). In the Framingham Offspring Study, an increasing number of components of the metabolic syndrome were associated with a graded increase in the concentration of small LDL particles and a graded decrease in the concentration of large LDL particles (17). On the other hand, overall, the cholesterol carried in LDL particles was not associated with the number of metabolic syndrome components, whereas there was a stepwise increase in total numbers of LDL particles. This association correlates with the severity of insulin resistance, as more severe states of insulin resistance were associated with a progressively larger VLDL particle size, smaller LDL particle size, and smaller HDL particle size (18). Furthermore, subjects with insulin resistance had higher concentrations of VLDL, IDL, and LDL particles.

Elevated LDL particle levels identifies individuals at highest risk for atherosclerotic vascular disease (19) and cardiovascular events (20,21). In the VA-HIT study, high levels of LDL particles and low levels of HDL particles were independent predictors of new coronary heart disease (CHD) events (16). Every 1 SD increment in total LDL particles was associated with a 1.19 incremental risk for CHD events in adjusted models (95% CI 1.08–1.32, $P = 0.007$). Conversely, a high HDL particle level was associated with a reduced risk of CHD. For every 1 SD increment of HDL particles (4.8 μmol/l) at baseline, the relative risk for

CHD events was 0.75 (0.67–0.84, $P < 0.0001$).

Small LDL particles have been suggested to be inherently more atherogenic because of their more facile penetration in the arterial wall (22,23) and a greater susceptibility to oxidative modification (24) than large LDL particles. In patients with metabolic syndrome, small LDL particle size was associated with increased intima-media area in the carotid and femoral arteries (25). In our study, fenofibrate-treated subjects had large reductions in small LDL particles (AUC −495 nmol/l) and smaller increases in large LDL particles relative to placebo-administered subjects (AUC +185 nmol/l) such that total LDL particles were lowered by 19% ($P = 0.0033$). The observed differences in fasting LDL subclasses between groups persisted after the meal. Although the lipid content of the lipoprotein particles was not measured, these data are consistent with inhibition of the triglyceride-mediated lipid exchange between VLDL and LDL particles. In combined hyperlipidemic subjects, fenofibrate (200 mg/day for 8 weeks) reduced cholesteryl ester transfer protein–mediated cholesteryl ester transfer from HDL to VLDL, inducing a shift from dense LDL to intermediate-dense LDL subspecies (26).

Our findings on NMR-measured lipoprotein subclasses are consistent with an earlier open-label report of fenofibrate (200 mg daily) therapy (27). Among 20 patients with severe hypertriglyceridemia (mean triglyceride levels 5.08 ± 2.84 mmol/l), fasting chylomicron and large VLDL triglyceride levels were reduced (−84.0 and −65.5%, respectively), as was small LDL cholesterol (−42.0%).

The effect of gemfibrozil therapy on lipoprotein subclasses and cardiovascular events was reported in the VA-HIT trial (16). Gemfibrozil therapy lowered LDL particles by 5% (mean ± SD 1,352 ± 316 to 1,290 ± 331 nmol/l; $P < 0.0001$) due to a 20% reduction in small LDL particles that was offset by a 36% increase in large LDL particles. A 1 SD increase of LDL particles (350 nmol/l) during the trial was associated with a multivariable adjusted risk for CHD events of 1.28 (95% CI 1.12–1.47, $P = 0.0003$), and small LDL particles were accompanied by a CHD event risk of 1.41 (1.14–1.73, $P = 0.001$) (16).

Oxidative modification of lipoprotein particles is considered an essential process for lipoprotein retention in the vessel wall, activation of redox-sensitive inflammatory gene transcription, and recognition by macrophage scavenger receptors (28). In this study, on-trial changes in fasting and postprandial OH-FA were most highly correlated with small LDL particles. Consistent with the antioxidant properties of HDL (29), we report an inverse correlation between OH-FA and small HDL particles in subjects receiving fenofibrate that was stronger in the postprandial state.

Oxidant stress impairs endothelial function, and this abnormality is more pronounced after a fatty meal (30–33). Postprandially, fenofibrate therapy had a lowering effect on plasma OH-FA superior to that with placebo. Furthermore, significant correlations were observed between changes in postprandial OH-FA and triglyceride levels ($P < 0.01$) and small LDL subclasses ($P < 0.01$). Previously it was reported that postprandial triglyceride levels distinguished CHD risk better than fasting triglyceride levels (34). The increased atherogenic risk associated with postprandial triglyceride levels may be mediated by a prolonged endothelial exposure to oxidative stress. In this situation, fenofibrate therapy may be particularly beneficial in ameliorating endothelial dysfunction and the atherogenicity of postprandial hypertriglyceridemia. The reductions in postprandial levels of VCAM-1 and ICAM-1 in fenofibrate-treated subjects are consistent with this hypothesis.

Associations between postprandial triglycerides and lipid peroxides have been described previously (31). No changes in fasting lipid peroxides were seen in 20 patients with type 2 diabetes randomly assigned to ciprofibrate or placebo. Fibrate therapy resulted in a 31% reduction in postprandial lipid peroxides. Fasting lipid peroxides did not correlate with VLDL, LDL, or HDL subfractions separated by density-gradient ultracentrifugation; however, postprandial lipid peroxides were strongly correlated with VLDL triglycerides ($r = 0.53$, $P = 0.03$). In another study, hypertriglyceridemic and type 2 diabetic subjects administered a high-fat meal (53.4 g fat) recorded increased oxidant stress, as measured by 2-h phorbol myristic acid–activated leukocyte superoxide anion production (4.09 ± 0.93 to 5.49 ± 1.19 nmol · 10^6 cells^{-1} · min^{-1}) (30). The increased production of superoxide anion correlated with postprandial triglyceride levels ($r = 0.798$, $P < 0.001$).

Although improved insulin sensitivity would be expected to reduce fatty acid oxidation and potentially diminish activation of inflammatory pathways (35), there were no baseline or on-trial group differences in fasting glucose levels or body weight in our study. The data from our study support our hypothesis concerning involvement of fatty acid oxidation in inflammatory gene activation.

Fenofibrate (267 mg daily) was previously shown to reduce oxLDL by 30.4% ($P < 0.001$) in subjects with impaired fasting glucose (11). In the current study, oxLDL levels were reduced by 14.3% in fenofibrate-treated subjects; however, the magnitude of change in OH-FAs was larger as the fatty acid content is higher on VLDL than on LDL particles. We used an oxLDL method that recognizes antigenic determinants on apoB, whereas OH-FA measures oxidized phospholipids present on multiple lipoprotein particles. These data suggest that the higher fatty acid–containing large VLDL particles contribute to increased oxidative stress in subjects with metabolic syndrome (6).

Statin therapy reduces fasting and postprandial concentrations of triglyceride-rich lipoproteins (36,37); however, to our knowledge there are no studies demonstrating that stains reduce postprandial inflammatory markers. Our study provides some of the first data demonstrating this potential benefit with lipid-altering drugs.

The fenofibrate-mediated changes in lipoprotein and anti-inflammatory markers would be expected to reduce atherosclerosis and cardiovascular events. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study (38), fenofibrate therapy resulted in a nonsignificant reduction in CHD death or nonfatal myocardial infarction; however, these results were confounded by large group differences in nonstudy lipid-lowering therapy (39). In contrast with our study, the FIELD study included patients with lower baseline fasting triglycerides (median 1.73 [IRQ 1.34–2.30] vs. 2.94 [2.21–3.90] mmol/l) and higher baseline HDL cholesterol levels (mean ± SD 1.10 ± 0.26 mmol/l vs. 0.84 [0.66–0.99] mmol/l). It has been demonstrated previously that baseline triglyceride levels influence the efficacy of fibrate therapy on reduction of cardiovascular events (40,41). In our study, fasting and postprandial triglyceride levels that were predominately carried in large VLDL

particles were most highly correlated with inflammatory markers.

In summary, therapy with fenofibrate was accompanied by marked reductions in fasting and postprandial concentrations of large- and medium-sized VLDL and total VLDL particles. Although fenofibrate therapy did not lower fasting or postprandial LDL cholesterol levels, substantial reductions were seen in LDL particles. The greater reduction in total LDL particles compared with that in LDL cholesterol resulted from reductions in fasting and postprandial small LDL particle levels. The effect of fenofibrate therapy on VLDL and LDL subclasses, in addition to reduced oxidative stress and inflammatory response, represents an important aspect of this agent in reducing atherosclerotic risk in hypertriglyceridemic patients with the metabolic syndrome.

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References

1. Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–2497
2. Rosenson RS: New approaches in the intensive management of cardiovascular risk in the metabolic syndrome. *Curr Probl Cardiol* 30:241–279, 2005
3. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS: Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation* 109:42–46, 2004
4. Tanko LB, Bagger YZ, Qin G, Alexandersen P, Larsen PJ, Christiansen C: Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. *Circulation* 111:1883–1890, 2005
5. Ooi TC, Cousins M, Ooi DS, Steiner G, Uffelman KD, Nakajima K, Simo IE. *J Clin Endocrinol Metab* 86:3134–3142, 2001
6. Doi H, Kugiyama K, Ohgushi M, Sugiyama S, Matsumura T, Ohta Y, Oka H, Ogata N, Hirata A, Yamamato Y, Yasue H: Membrane active lipids in remnant lipoproteins cause impairment of endothelium-dependent vasorelaxation. *Arterioscler Thromb Vasc Biol* 19:1918–1924, 1999
7. Cipollone F, Iezzi A, Fazia M, Zucchelli M, Pini B, Cuccurullo C, DeCesare D, DeBlasis G, Muraro R, Bei R, Chiarelli F, Schmidt AM, Cuccurullo F, Mezzetti A: The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control. *Circulation* 108:1070–1077, 2003
8. Bae JH, Bassenge E, Kim KB, et al: Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 155:517–523, 2001
9. Ceriello A: The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabete Metab Res Rev* 16: 125–132, 2000
10. Koh KK, Han SH, Quon MJ, Yeal Ahn J, Shin EK: Beneficial effects of fenofibrate to improve endothelial dysfunction and raise adiponectin levels in patients with primary hypertriglyceridemia. *Diabetes Care* 28:1419–1424, 2005
11. Okopien B, Krysiak R, Herman ZS: Effects of Short-Term Fenofibrate Treatment on Circulating Markers of Inflammation and Hemostasis in Patients with Impaired Glucose Tolerance. *J Clin Endocrinol Metab* 91:1770–1778, 2006
12. Tangney CC, Hafner JM, McQuiston BD, Domas AJ, Rosenson RS: Postprandial changes in plasma and serum viscosity and plasma lipids and lipoproteins after an acute test meal. *Am J Clin Nutr* 65:36–40, 1997
13. Freedman DS, Otvos JD, Jeyarajah EJ, Shalaurova I, Cupples LA, Parise H, D'Agostino RB, Wilson PW, Schaefer EJ: Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem* 50:1189–1200, 2004
14. Kaikkonen J, Tuomainen TP, Nyyssonen K, Morrow JD, Salonen JT: C18 hydroxy fatty acids as markers of lipid peroxidation ex vivo and in vivo. *Scand J Clin Lab Invest* 64:457–468, 2004
15. Wallace TM, Matthews DR: The assessment of insulin resistance in man. *Diabet Med* 19:527–534, 2002
16. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ: Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation* 113:1556–1563, 2006
17. Kathiresan S, Otvos JD, Sullivan LM, Keyes LM, Schaefer EJ, Wilson PW, D'Agostino RB, Vasan RS, Robins SJ: Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation* 113:20–29, 2006
18. Garvey WT, Kwon S, Zheng D, Shaughnessey S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y: Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 52:453–462, 2003
19. Rosenson RS, Otvos JD, Freedman DS: Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. *Am J Cardiol* 90:89–94, 2002
20. Kuller L, Arnold A, Tracy R, Otvos J, Burke G, Psaty B, Siscovick D, Freedman DS, Kronmal R: Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol* 22:1175–1180, 2002
21. Blake GJ, Otvos JD, Rifai N, Ridker PM: Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation* 106:1930–1937, 2002
22. Bjornheden T, Babyi A, Bondjers G, Wiklund O: Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis* 123:43–56, 1996
23. Nordestgaard BG, Zilversmit DB: Comparison of arterial intimal clearances of LDL from diabetic and nondiabetic cholesterol-fed rabbits. Differences in intimal clearance explained by size differences. *Arteriosclerosis* 9:176–183, 1989
24. Tribble DL, Holl LG, Wood PD, Krauss RM: Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93:189–199, 1992
25. Hulthe J, Bokemark L, Wikstrand J, Fagerberg B: The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. *Arterioscler Thromb Vasc Biol* 20: 2140–2147, 2000
26. Guerin M, Bruckert E, Dolphin PJ, Turpin G, Chapman MJ: Fenofibrate reduces plasma cholesteryl ester transfer from HDL to VLDL and normalizes the atherogenic, dense LDL profile in combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 16:763–772, 1996
27. Ikewaki K, Tohyama J, Nakata Y, Wakikawa T, Kido T, Mochizuki S: Fenofibrate effectively reduces remnants, and small dense LDL, and increases HDL particle number in hypertriglyceridemic men: a nuclear magnetic resonance study. *J Atheroscler Thromb* 11:278–285, 2004
28. Rosenson RS: Statins in atherosclerosis:

lipid-lowering agents with antioxidant capabilities. *Atherosclerosis* 173:1–12, 2004
29. Kontush A, de Faria EC, Chantepie S, Chapman MJ: Antioxidative activity of HDL particle subspecies is impaired in hyperalphalipoproteinemia: relevance of enzymatic and physicochemical properties. *Arterioscler Thromb Vasc Biol* 24:526–533, 2004
30. Bae JH, Bassenge E, Lee HJ, Park KR, Park CG, Park KY, Schwemmer M: Impact of postprandial hypertriglyceridemia on vascular responses in patients with coronary artery disease: effects of ACE inhibitors and fibrates. *Atherosclerosis* 158:165–171, 2001
31. Evans M, Anderson RA, Graham J, Ellis GR, Morris K, Davies S, Jackson SK, Lewis MJ, Frenneaux MP, Rees A: Ciprofibrate therapy improves endothelial function and reduces postprandial lipidemia and oxidative stress in type 2 diabetes mellitus. *Circulation* 101:1773–1779, 2000
32. Marchesi S, Lupattelli G, Lombardini R, Roscini AR, Siepi D, Vaudo G, Pirro M, Sinzinger H, Schillaci G, Mannarino E: Effects of fenofibrate on endothelial function and cell adhesion molecules during post-prandial lipidemia in hypertriglyceridemia. *J Clin Pharm Ther* 28:419–424, 2003
33. Vogel RA, Corretti MC, Plotnick GD: Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 79:350–354, 1997
34. Uiterwaal CS, Grobbee DE, Witteman JC, et al: Postprandial triglyceride response in young adult men and familial risk for coronary atherosclerosis. *Ann Intern Med* 121:576–583, 1994
35. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome: The Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102:42–47, 2000
36. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M, The FIELD study investigators: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 366: 1849–1861, 2005
37. Boquist S, Karpe F, Danell-Toverud K, Hamsten A: Effects of atorvastatin on postprandial plasma lipoproteins in postinfarction patients with combined hyperlipidaemia. *Atherosclerosis* 162: 163–170, 2002
38. Battula SB, Fitzsimons O, Moreno S, Owens D, Collins P, Johnson A, Tomkin GH: Postprandial apolipoprotein B48- and B100-containing lipoproteins in type 2 diabetes: Do statins have a specific effect on triglyceride metabolism? *Metabolism: Clinical and Experimental* 49:1049–1054, 2000
39. Rosenson RS: Field of confusion: future prospects for fibrate therapy in cardiovascular disease. *Curr Atheroscler Rep* 8:219–222, 2006
40. The Bezafibrate Infarction Prevention (BIP) study Group. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. *Circulation* 102:21–27, 2000
41. Manninen V, Tenkanen L, Koskinen P, et al: Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* 85:37–45, 1992