

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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Running Title: Fenofibrate and Postprandial Response

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ABSTRACT

Objectives

The aim of this study was to determine the effects of fenofibrate (160 mg/d) 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 mmol/L and < 6.9 mmol/L) and two or more of the Adult Treatment Panel III criteria of the metabolic syndrome were randomized to fenofibrate (160 mg/d) 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%, $p < 0.0001$) very low-density lipoprotein (VLDL) particles. Fasting total low-density lipoprotein (LDL) particle number was reduced in fenofibrate treated subjects (-19.0%, $p = 0.0033$) due primarily to reductions in small LDL particles (-40.3%, $p < 0.0001$); these treatment differences persisted postprandial. Fasting and postprandial oxidized fatty acids were reduced in fenofibrate-treated subjects compared to placebo (-15.3%, $p = 0.0013$ and 31.0%, $p < 0.0001$, respectively), and fenofibrate therapy lowered fasting and postprandial soluble VCAM-1 (-10.9%, $p = 0.0005$ and -12.0%, $p = 0.0001$, respectively) as well as fasting and postprandial soluble ICAM-1 (-14.8%, $p < 0.0001$ and -15.3%, $p < 0.0001$, respectively). Reduction in VCAM-1 and ICAM-1 was 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 anti-atherosclerotic effects were most highly correlated with reductions in large VLDL particles.

INTRODUCTION

The metabolic syndrome represents an agglomeration of interrelated risk factors that includes abnormally high fasting triglycerides (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 (RLP) are further enhanced postprandially among hypertriglyceridemia subjects (5). RLPs have been shown to increase intracellular oxidant concentrations and lipid peroxide levels in culture media, and activate NF- κ B (6). NF- κ B 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, NF- κ B activation and inflammatory cell infiltrates (T cells and macrophages). (7).

Although triglyceride levels are consistently measured on 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 inflammatory mediators' fibrinogen and CRP (10) however; the relationship between plasma lipoproteins, oxidatively modified fatty acids, and inflammatory mediators remains uncertain (11). In this randomized, placebo-controlled trial, we investigate the effects of fenofibrate therapy on fasting and postprandial lipoproteins, monohydroxy fatty acids (OH-FA), and cellular adhesion molecules in hypertriglyceridemic subjects with the metabolic syndrome and attempt to correlate the changes in lipids and lipoprotein particles with inflammatory mediators.

METHODS

Subject Selection

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 randomized to receive fenofibrate 160 mg QD 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 either due to withdrawal of consent ($n = 2$), relocation ($n = 1$), or gastrointestinal intolerance ($n = 1$). Males and postmenopausal females ≥ 18 years of age with fasting triglycerides ≥ 1.7 mmol/L 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 females and >102 cm in males); 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 types 1 or 2 diabetes, body mass index > 40 kg/m², use of lipid-lowering therapies, oral hypoglycemic therapies, insulin, aspirin > 81 mg daily, regular use of non-steroidal anti-inflammatory agents or cyclooxygenase-2 inhibitors, corticosteroids (oral and inhaled), anti-oxidants (including multivitamins), herbal or fiber supplements, recent 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 Institutional Review Board approved the protocol of this study. All subjects gave written informed consent prior to participating in this research trial.

Study Design

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. In order to monitor compliance, subjects were contacted twice during the six-week dietary lead-in period to obtain 24-hour diet recalls. Food recalls were analyzed for fat, calories, fiber, and alcohol intake using the Minnesota Nutrition Data System, version 2.93 (NCC, University of Minnesota, Minneapolis, MN, USA). At the end of the lead-in period, fasting blood lipids and blood glucose were measured to determine study eligibility. Eligible subjects returned within one week of the blood draw for randomization. A total of 59 eligible subjects were randomly assigned to fenofibrate 160mg QD or placebo using a block randomization design that stratified subjects by gender and decile of age. After a 12-hour 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/m²), and it was comprised 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 to 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 h and 8 h. Subjects were provided matching placebo or fenofibrate 160 mg daily, which they were instructed to take the evening of the randomization visit. After 3 months therapy, repeat laboratory studies were performed on the 55 subjects who completed the study protocol.

Laboratory Studies

Plasma lipids and chemistry panels were measured by standard procedures. For fasting triglycerides ≥ 4.52 mmol/L, LDL cholesterol (LDL-C) 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, Inc., Raleigh, NC, USA) (13). The following subclass categories were investigated: chylomicrons (> 200 nm), large very low-density lipoprotein (VLDL) (60-200 nm), intermediate VLDL (35-60 nm), small VLDL (27-35 nm), intermediate-density lipoprotein (IDL) (23-27 nm), large low-density lipoprotein (LDL) (21.2-23 nm), small LDL (18-21.2 nm), large high-density lipoprotein (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) $< 4\%$

were observed for total VLDL particle (VLDL-P), LDL particle (LDL-P), and HDL particle (HDL-P) 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 Ltd., Kuopio, Finland). CV for apoB was 2.0%. Plasma C18 hydroxy fatty acids were measured by gas chromatography/mass spectrometry (Jurilab Ltd., Kuopio, Finland) (14). CV for total OH-FA was <5%. Oxidized LDL (oxLDL) was measured by immunoassay (Mercodia, Inc., Winston Salem, NC, USA). 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 multi-analyte bead immunoassays (Linco Research, St. Charles, MO, USA, Luminex[®]). Inter-assay CVs were <12%.

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

Statistical Analyses

Subject demographic characteristics are reported as means \pm standard deviations while fasting and postprandial lipids and lipoproteins are reported as medians and interquartile ranges (IQR = $0.5 \times [75^{\text{th}} - 25^{\text{th}}$ percentile]). Postprandial responses were calculated as area under the curve (AUC) using the trapezoidal rule. The Wilcoxon

ranked 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 three-month therapy period for lipid and lipoprotein values as well as the AUC were calculated for each measurement. The medians of each parameter were compared between treatment groups using the Wilcoxon rank sum test. In order to account for baseline differences in fasting small VLDL particles and postprandial total VLDL particle numbers, a multivariate regression analyses was conducted on the ranks of the data. In the figures, 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, Inc., Cary, NC, USA).

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 (IBH, AWR) 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.

RESULTS

The clinical characteristics of the study populations are described in Table 1 (Online Appendix). This study included predominantly middle-aged men with central obesity, high fasting triglycerides, low levels of HDL-C, and hypertension. The high

proportion of men in the study may potentially introduce 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 regards 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 HOMA index ($p = 0.92$). There were group differences in baseline fasting small VLDL-P (median in fenofibrate was 59.1 nmol/L, median in placebo was 40.8 nmol/L, $p = 0.04$ and postprandial total VLDL-P (median AUC in fenofibrate was 1044 nmol/L, median AUC in placebo was 842 nmol/L, $p = 0.02$).

Aspirin (75-81 mg daily) was used by 33% of placebo-treated subjects and 24% of fenofibrate-treated subjects ($p = 0.56$). The subjects did not change their intake of dietary fats and calories. As determined from pill counts, the study subjects demonstrated high adherence to study medications with 97.6% of placebo-treated subjects and 97.1% of fenofibrate-treated subjects returning the correct number of tablets ($p = 0.65$). Weight change during treatment (kg) remained comparable ($p = 0.056$) stable in the placebo group (median, IQR, 101.8 kg [17.8 to, 103.2, 22.4] and the fenofibrate treatment group (median, IQR, 105.0 25.8 vs. 104.5, 26.2); $p = 0.056$). There were no differences in changes in waist circumference between placebo (median, IQR, 110.8, 12.2 to 111.8 13.8) and fenofibrate treatment groups (median, IQR, 109.2, 6.2, vs. 109.2 cm 10.2); $p = 0.10$). On trial, there were non-significant changes in fasting glucose levels (median, IQR, 5.45, 0.55 to 4.51, 0.88 in the placebo group vs 5.47, 0.36 to 4.57, 1.70 in the fenofibrate group) $p = 0.91$.

Fasting Lipoprotein and Hydroxy Fatty Acid Measurements

Fenofibrate therapy lowered fasting triglycerides (-46.1% versus -4.0% for placebo, $p < 0.0001$), non-HDL-C (-19.7% vs. $+0.3\%$, $p = 0.0048$) and total VLDL-P (-44.1% vs. $+4.9\%$, $p < 0.0001$). The reduction in total VLDL-P observed with fenofibrate is primarily due to reductions in large VLDL-P (-57.5% vs. -12.1% , $p = 0.0112$), medium VLDL-P (-54.2% vs. -11.2% , $p = 0.0008$) and small VLDL-P (-32.8% vs. $+19.6\%$, $p = 0.0026$). LDL-C was not lowered differentially between the treatment groups (-7.1% vs. -0.8% , $p = 0.48$). However, fenofibrate did lower LDL-P (-19.0% vs. $+1.5\%$, $p = 0.0033$) primarily due to reductions in the concentration of small LDL-P (-40.2% vs. $+10.1\%$, $p < 0.0001$). Consistent with the reduction in LDL-P, 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-C ($+21.8\%$ vs. $+11.1\%$, $p = 0.26$) or HDL-P ($+13.2\%$ vs. $+4.4\%$, $p = 0.12$) in fenofibrate treated subjects. Post-treatment levels of the lipid and lipoprotein results for each treatment group are presented in Figures 1-3 (online appendix: <http://care.diabetesjournals.org>). The p-values in these figures indicate the significance of the change from pre-treatment levels.

Fasting OH-FA levels were reduced in the fenofibrate group (-15.5%) compared with an increase in the placebo group ($+11.5\%$) ($p = 0.0013$), while oxLDL levels were lowered among fenofibrate-treated subjects compared to placebo (-14.3% vs. -3.1% , $p = 0.046$). Over the treatment phase of the study, mean percent changes in fasting OH-FA were most highly correlated with mean percent changes in small LDL-P ($r = 0.42$, $p < 0.01$) (Table 2),

and these associations were higher for oxLDL ($r = 0.54$, $p < 0.01$). Furthermore, oxLDL was inversely correlated with large LDL-P ($r = -0.39$, $p < 0.01$) and small HDL-P ($r = -0.27$, $p = 0.048$). The fasting OH-FA and oxLDL results for the fenofibrate-treated subjects are illustrated in Figure 4 (Online Appendix).

Post-treatment levels of the OH-FA and oxLDL results for each treatment group are presented in Figure 4. The p-values in this figure indicate the significance of the change from pre-treatment levels.

Postprandial Lipoprotein and Hydroxy Fatty Acids

Fenofibrate treatment lowered postprandial (AUC) triglyceride concentrations (-45.4%) compared with a rise in the placebo group (+1.4%) ($p < 0.0001$), and non-HDL-C (AUC) (-6.2%) compared with no change in the placebo group ($p = 0.0035$). Post-treatment levels for each time point are shown in Figure 1 (Online Appendix), together with an indication of the significance of the change in these values from pre-treatment levels. The reductions in postprandial (AUC) triglycerides resulted from a lowering in concentrations of large VLDL-P (-54.8% vs. -8.3%, $p = 0.0008$) and medium VLDL-P (-49.5% vs. -5.2%, $p < 0.0001$). After the test meal, LDL-C levels and LDL-P reflected changes in fasting specimens. The lowering in median absolute numbers of postprandial LDL-P (-2366 in fenofibrate versus +1725 in placebo) was due to a decrease in the small LDL-P (-4734 in fenofibrate vs +1633 in placebo) with an increase in large LDL-P (+2466 in fenofibrate vs +488 in placebo). The reduction in VLDL-P and LDL-P were reflected by the postprandial (AUC) lowering in apoB levels (-17.7% in fenofibrate vs -0.8 in placebo, $p = 0.01$). Post-treatment levels for VLDL and HDL for each time point are shown in Figures 2 and 3 (Online Appendix), together with an indication of the significance of the change in these values from pre-

treatment levels. Postprandially, there were significant increases in large HDL-P (+122.8% vs. +21.3%, $p = 0.003$) and total HDL-P (+19.4% vs. +2.1%, $p = 0.008$) with fenofibrate therapy as compared to placebo therapy.

Postprandial (AUC) OH-FA levels were reduced in the fenofibrate group (-31.0%) and increased in the placebo group (+7.5%, $p < 0.0001$). Post-treatment levels for OH-FA for each time point are shown in Figure 4. Changes (over the treatment phase) in postprandial OH-FA in the fenofibrate group were correlated with postprandial changes in triglycerides ($r = 0.73$, $p < 0.0001$), large ($r = 0.45$, $p < 0.01$), medium ($r = 0.48$, $p < 0.001$) and total VLDL particles ($r = 0.51$, $p < 0.01$); and small LDL-P ($r = 0.45$, $p < 0.01$) as shown in Table 2 (Online Appendix). In addition, there were inverse correlations between postprandial OH-FA changes and postprandial HDL-C changes ($r = -0.44$, $p < 0.01$), large ($r = -0.49$, $p < 0.01$), small ($r = -0.38$, $p < 0.01$) and total HDL-P ($r = -0.41$, $p < 0.003$).

Fasting and Postprandial Inflammatory Markers

Compared to placebo fenofibrate therapy reduced soluble VCAM-1 levels in fasting specimens (-10.9% vs. -1.5%, $p = 0.0005$), as well as postprandial (AUC) specimens (-12.0% vs. -0.8%, $p = 0.0001$). Soluble ICAM-1 levels were lowered by fenofibrate in both fasting specimens (-14.7% vs. +0.9%, $p < 0.0001$) and in postprandial specimens (-15.3% vs +1.0%, $p < 0.0001$). 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). In contrast, only changes in ICAM-1 were significantly associated with oxLDL ($r = 0.40$, $p < 0.01$), whereas changes in VCAM-1 were not. Post-treatment levels for VCAM-1 and

ICAM-1 for each time point are shown in Figure 5 (Online Appendix). In multivariate models that included, age, gender, weight change, small LDL and OH-FA, changes in VCAM-1 remained significantly correlated with fasting ($p = 0.02$) and postprandial large VLDL-P ($p = 0.006$). Similarly, ICAM-1 was associated with fasting ($p = 0.04$) and postprandial large VLDL-P ($p = 0.0003$).

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 group in aspartate aminotransferase ($p = 0.72$), alanine aminotransferase ($p = 0.10$), or creatine phosphokinase ($p = 0.16$).

DISCUSSION

This study reports several important findings: (1) postprandial hypertriglyceridemia is accompanied by persistent increased levels of small and total LDL-P 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-size VLDL-P and smaller-size LDL-P; (3) increased oxidative stress after a fatty meal as measured by oxidized fatty acids and oxidized LDL is abrogated by fenofibrate; and (4) the observed anti-oxidant 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 observed in fenofibrate-treated subjects resulted primarily from reductions in fasting and postprandial concentrations of large VLDL-P and medium VLDL-P.

In this study, fenofibrate significantly reduced fasting LDL-P compared to placebo (-19.0% versus +1.5%, $p = 0.0033$), which persisted postprandially (-21.0% versus +3.7%, $p = 0.0009$). Reductions in fasting and postprandial LDL-P 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-P compared to placebo. Although changes in LDL-C were not different between fenofibrate and placebo groups ($p = 0.38$ for fasting, $p = 0.24$ for postprandial) the LDL-P results suggest that fenofibrate therapy decreases the atherosclerotic potential of the LDL-C. The average cholesterol content of the LDL particles increased in the fenofibrate-treated subjects which explain the non-significant reduction in LDL-C. 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-P, and a graded decrease in the concentration of large LDL particles (17). On the other hand, overall, the cholesterol carried in LDL-P was not associated with the number of metabolic syndrome components, whereas there was a stepwise increase in total numbers of LDL-P. 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 VLDL-P, IDL-P, and LDL-P.

Elevated LDL-P identifies individuals at highest risk for atherosclerotic vascular disease (19) and cardiovascular events (20, 21). In the VA-HIT study, high levels of LDL-P and low levels of HDL-P were independent predictors of new coronary heart disease (CHD) events (16). Every 1 SD increment in total LDL-P was associated with a 1.19 incremental risk for CHD events in adjusted models (95% CI, 1.08-1.32, $p = 0.007$). Conversely, high HDL-P was associated with a reduced risk of CHD. For every 1 SD increment of HDL-P ($4.8 \mu\text{mol/L}$) at baseline, the relative risk for CHD events was 0.75 (95% CI, 0.67 to 0.84, $p < 0.0001$).

Small LDL-P has been suggested to be inherently more atherogenic due to more facile penetration in the arterial wall (22, 23) and a greater susceptibility to oxidative modification (24) than large LDL-P. In patients with metabolic syndrome, small LDL particle size was associated with increased intima-media area in carotid and femoral arteries (25). In our study, fenofibrate-treated subjects had large reductions in small LDL-P (AUC, -495 nmol/L), and smaller increases in large LDL-P relative to placebo (AUC, $+185 \text{ nmol/L}$) such that total LDL-P was 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 triglyceride-mediated lipid exchange between VLDL-P and LDL-P. In combined hyperlipidemia subjects, fenofibrate (200 mg/d 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 severe hypertriglyceridemia patients (mean triglycerides $5.08 \pm 2.84 \text{ mmol/L}$), fasting chylomicron and large VLDL triglycerides were reduced (-84.0% and -65.5% , respectively), as was small LDL-C (-42.0%).

The effect of gemfibrozil therapy on lipoprotein subclasses and cardiovascular events has been reported in the VA-HIT trial (16). Gemfibrozil therapy lowered LDL-P by 5% (mean \pm SD, 1352 ± 316 to $1290 \pm 331 \text{ nmol/L}$, $p < 0.0001$) due to a 20% reduction in small LDL-P that was offset by a 36% increase in large LDL-P. A 1 SD increase of LDL-P (350 nmol/L) during the trial were associated with a multivariable adjusted risk for CHD events of 1.28 (95% CI, 1.12 to 1.47, $p = 0.0003$), and small LDL-P were accompanied by a CHD event risk of 1.41 (95% CI, 1.14 to 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-P. Consistent with the antioxidant properties of HDL (29), we report an inverse correlation between OH-FA and small HDL-P in subjects receiving fenofibrate that were 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 superior lowering effect on plasma OH-FA compared to placebo. Furthermore, significant correlations were observed between changes in postprandial OH-FA and triglycerides ($p < 0.01$), and small LDL subclasses ($p < 0.01$). Previously it was reported that postprandial triglyceride levels distinguish CHD risk better than fasting triglyceride levels (34). The increased atherogenic risk associated with postprandial triglycerides 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 previously described (31). No changes in fasting lipid peroxides were seen in 20 patients with type 2 diabetes randomized 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 an increased oxidant stress, as measured by two-hour PMA-activated leukocyte superoxide anion production (4.09 ± 0.93 nmol/ 10^6 cells/min to 5.49 ± 1.19 nmol/10) (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 supports 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 (36). In the current study, oxidized LDL levels were reduced by 14.3% in fenofibrate-treated subjects; however, the magnitude of change in OH-FA was larger as the fatty acid content is higher on VLDL than 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 metabolic syndrome subjects (37).

Statin therapy reduces fasting and postprandial concentrations of triglyceride-rich lipoproteins (38, 39); however to our knowledge there are no studies that demonstrate that statins 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 (40), fenofibrate therapy resulted in a non-significant reduction in CHD death or nonfatal myocardial infarction; however these results were confounded by large group differences in non-study lipid lowering therapy (41). In contrast to our

study, the FIELD study included patients with lower baseline fasting triglycerides (1.73 [1.34-2.30] mmol/L vs. 2.94 [2.21-3.90] mmol/L) and higher baseline HDL-C levels (mean±SD, 1.10 ± 0.26 mmol/L vs. median [IQR], 0.84 [0.66-0.99] mmol/L). It has been previously demonstrated that baseline triglyceride levels influence the efficacy of fibrate therapy on reduction of cardiovascular events (42, 43). In our study, fasting and postprandial triglyceride levels that were predominately carried in large VLDL particles were most highly correlated with inflammatory markers.

CONCLUSION

Therapy with fenofibrate was accompanied by marked reductions in fasting and postprandial concentrations of large- and medium-sized VLDL and total VLDL-P. Although fenofibrate therapy did not lower fasting or postprandial LDL-C levels, substantial reductions were seen in LDL-P. The greater reduction in total LDL-P as compared to LDL-C resulted from reductions in fasting and postprandial small LDL-P 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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