Comparison of Postprandial Responses to a High-Fat Meal in Hypertriglyceridemic Men and Women before and after Treatment with Fenofibrate in the Genetics and Lipid Lowering Drugs and Diet Network (GOLDN) Study

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Context. The fenofibrate effect on the subclass size distribution of lipoproteins before and after a high-fat challenge is not well studied. Objective. To characterize the baseline and post-prandial response (PPL) to a high-fat challenge following fenofibrate therapy, on changes in LDL, HDL, and VLDL particle subclasses, number, and size in 271 hypertriglyceridemic participants. Methods. Participants from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study who conducted PPL studies both before and after three weeks of fenofibrate (160 mg/d) treatment were analyzed. Particle size distributions were determined using nuclear magnetic resonance imaging, and lipid determinations were measured at fasting (0 hr), 3.5 hours, and 6 hours after ingestion of a standardized high-fat meal. Analyses were stratified by gender. Changes in particle subclass distributions were assessed using repeated measures analysis of variance adjusted for pedigree. Results. Before PPL, fenofibrate in men (adjusted for age, field center, smoking status, diabetes, and weight circumference) lowered fasting and postprandial VLDL primarily due to reductions in postprandial levels of large and medium VLDL particles (9 SE +/− 0.7 to 4+/− 0.4 and 78 = /− 4 to 36 = /− 3 nmol/L, both P < .0001, resp.). Fenofibrate also reduced fasting and postprandial total LDL particles, primarily a result of reduced small LDL particles (1497 = /− 37 to 1088 = /− 36 nmol/L, P < .0001). Directional changes were similar in men and women but the magnitude of change was different for some parameters. Conclusion. Fenofibrate treatment resulted in a lower triglyceride excursion following a high-fat meal. This investigation provides new knowledge of the magnitude and time course of fenofibrate induced attenuation of Lipoprotein subclass size distribution following a postprandial lipid challenge.

1. Introduction

While measurement of lipoprotein cholesterol provides important information regarding cardiovascular health, lipoproteins such as VLDL, LDL, and HDL are each composed of a heterogeneous group of molecules with varying lipid and lipoprotein concentrations and size (e.g., subclasses) which carry differing cardiovascular disease risk [1, 2]. Some studies have demonstrated that measurement of the subclasses of LDL, HDL, and VLDL provides cardiovascular risk information beyond the measurement of the respective total LDL, HDL or VLDL concentration alone [3–5]. The most well described of the three lipoprotein subclasses is low-density lipoprotein (LDL). Studies indicate that the prevalence of the small dense LDL phenotype varies with a number of factors, including age, gender, hormonal status, and racial and ethnic background [3–5]. Also, data from in vitro and in vivo studies suggest that small dense LDL may
be particularly atherogenic, and that triglyceride levels are
strongly correlated with LDL particle size [6]. Studies have
also shown that larger HDL particle subclasses are inversely
associated with coronary heart disease (CHD) incidence,
while smaller HDL particles are positively correlated with
CHD [7, 8].

Although the measurement of fasting lipoproteins has
contributed greatly to our knowledge of their role in
cardiovascular risk, the relationship between nonfasting (i.e.,
post-prandial) lipoprotein measures [9], and the effect of
fenofibrate on these measures is not well described. There
are also only a small number of studies specifically designed
to evaluate the effect of postprandial lipemia (PPL) on the
relationship between differences in fasting and PPL and
cardiovascular risk. This latter point is important because
humans spend a considerable time during the day in
a postprandial state. Fenofibrate is an effective agent at
lowering fasting triglycerides and LDL cholesterol. However,
there have been few studies done to evaluate post-prandial
lipids in relation to fenofibrate treatment, and even fewer
small studies have examined the effect of fenofibrate on
postprandial lipoprotein subclasses in a large and diverse
population of subjects [10–13]. Therefore, the goal of this
paper is to characterize the effect of fenofibrate on post-
prandial lipids; namely LDL, HDL, and VLDL subclasses.
Specifically, we examine the response of particle number and
size to a standardized fat challenge before and after 3 weeks
of fenofibrate therapy in a population of subjects with a wide
range of baseline triglycerides. This represents one of the
largest studies addressing this question.

2. Study Population

The study population consisted of members from 189
families who were recruited from 3-generational pedigrees
ascertained from the NHBLI Family Heart Study Minneapo-
lis, MN and Salt Lake City, UT field center. Informed
consent was obtained from all participants. Of the initial
1328 subjects contacted, 271 hypertriglyceridemic men
and women completed the protocol (mean age was 53.3, range
18–87 years, Figure 1).

3. Inclusion and Exclusion Criteria

To be eligible to participate in the Genetics and Lipid
Lowering Drugs and Diet Network (GOLDN) postprandial
challenge and fenofibrate trial, individuals had to meet the
following eligibility criteria: white ethnicity, since the two
recruitment centers in Utah and Minnesota were nearly
all Caucasian male or female, ≥18 years of age, fasting
triglycerides (TGs) < 1500 mg/dL, willingness to participate
in the study and attend the scheduled clinic exams, ability
to obtain physician approval and willingness to discontinue
use of all prescription or over the counter medications which
may affect lipid levels, membership in a family with at
least two members in a sibship, AST and ALT tests within
normal range, and serum creatinine ≤ 2.0 mg/dL. Exclusion
criteria for the postprandial challenge and fenofibrate trial
included: TGs ≥ 1500 mg/dL, history of malabsorption,
liver, kidney, pancreas, or gall bladder disease, pregnancy
or women of childbearing potential not using an acceptable
form of contraception, use of warfarin or insulin, known
hypersensitivity to fenofibrate, or pancreatitis within 12
months prior to enrollment. Individuals using prescription
or OTC medications or nutraceuticals known to affect lipid
levels were asked to consult with their physician, obtain
consent for withdrawal, and discontinue these medications
four weeks prior to the study initiation.

4. Study Design

The GOLDN study recruited extended pedigrees from the
NHLBI Family Heart Study (FHS) and conducted two brief
interventions: a fat loading dietary challenge and a short-
term (3 week), open-label intervention trial of 160 mg once
daily fenofibrate (TriCor, Abbott Laboratories, Chicago, IL).
The high fat challenge (postprandial lipemia-PPL) followed
the protocol of Patsch et al. [14]. The caloric content
of the meal was 83% of calories from fat, 14% from
carbohydrates, and 3% from protein. Furthermore, the meal
was formulated to have a cholesterol content of 240 mg and
a polyunsaturated:saturated fat ratio of 0.06. Based on
this protocol, the average individual ingested 175 mL of
heavy whipping cream (39.5% fat) combined with 7.5 mL
powdered, instant, nonfat, dry milk, and blended with ice.
To increase palatability of the drink, 15 mL of chocolate-
or strawberry-flavored syrup was also added. On the day of
the PPL meal, participants presented after at least 8 hrs of
fasting. Immediately before ingestion, blood samples were drawn from all participants (0 hr, fasting), and then again at 3.5 and 6 hours after the high-fat meal.

The specific methodology of GOLDN is reported elsewhere [15]. For the subclass analysis addressed in this manuscript, we specifically assessed LDL, HDL, and VLDL particle size and number in response to these two interventions. Plasma lipids were measured twice (and averaged) after a minimum fast of 8 hours at two consecutive days for baseline visits and on the last two days of the 21-day treatment period. Specific dietary guidance regarding fat intake was not offered, but subjects were asked to continue their usual diets. The dietary fat challenge was conducted at baseline prior to fenofibrate therapy, and on the last day of the fenofibrate treatment period. The genetic variations which might predict fenofibrate response is currently being analyzed.

4.1. Biochemical Assays. Serum and EDTA-anticoagulant tubes were collected and processed using a standardized protocol [16]. The serum and plasma samples were aliquoted and stored at −70°C until time of use. Analysis of the stored samples was completed on all samples at the end of the study and all samples for an individual were processed in the same batch to reduce measurement error.

HDL-C was measured in plasma by precipitating the non-HDL-C fractions with magnetic 50,000 dextran sulfate and magnesium chloride. The remaining HDL-C fraction was determined on the Hitachi 911 using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). Triglycerides were calculated from plasma samples and LDL-cholesterol was directly measured.

4.2. Lipoprotein Subclass Analysis. Proton nuclear magnetic resonance (NMR) spectroscopy was used to determine LDL, HDL, and VLDL subclass distribution (Liposcience, Raleigh, NC). NMR measures lipoprotein subclasses by detecting and deconvoluting the signal emitted by lipoprotein methyl-group protons when subjected to a 400 MHz magnet. This method quantifies particle concentration by converting the characteristic signal amplitude generated by the methyl group to NMR signal of each lipoprotein subclass particle [17]. Using NMR, three LDL particle subclass concentrations were determined [17]. Concentrations of the subclasses are reported as nanomoles per liter. As an example, the sizes of the three LDL subclasses were determined as follows: intermediate density lipoprotein, LDL, (23–27 nm), large LDL (21.3–23 nm), and small LDL (18.3–21.2 nm). Total LDL particle concentration was determined to be the sum of the IDL, large and small LDL particle subclasses. This specific NMR technology groups IDL particles as a subclass of total LDL particles. Similar characterizations were performed for HDL and VLDL particle subclasses as follows: large HDL (≥8.8–13 nm), medium HDL (≥8.2–8.8 nm), and small HDL (7.3–8.2 nm); and large VLDL (≥60 nm), medium VLDL (35–65 nm), and small VLDL (27–35 nm).

5. Statistical Methods

Sample size calculations were based upon the parent study. To determine significant differences in percentages, we used the Pearson r² and Fisher’s tests. To compare crude means, we used ANOVA and the Students t-test. Results are expressed as least squares mean (standard error). Analyses of particle size concentrations were stratified by gender and baseline triglycerides (normal versus elevated (≥150 mg/dL)), as these covariates demonstrated significant interactions with fenofibrate treatment.

We examined changes in subclass particle size concentrations that occurred during the dietary fat challenge (baseline, 3.5 hours, and 6 hours), both before and after fenofibrate treatment. To estimate the overall response of subclass concentrations and components present in the plasma during the two post-prandial periods, we used repeated measures analysis of variance (ANOVA), adjusted for potential confounders (e.g., age, gender, BMI, smoking) as well as pedigree as a random effect. This assessed the overall differences in postprandial responses: treatment, time, and treatment by time effects. Student’s paired t-test was performed to assess differences between the lipid challenge before and after fenofibrate treatment. All statistical calculations were performed using SAS 9.1 (SAS Institute, Inc., Cary, USA).

As mentioned above, significant interaction was seen for gender and triglyceride status (normal and elevated) results are stratified for these variables. To be classified as having elevated triglyceride levels, the subject’s mean baseline values had to be >150 mg/dL. All lipid values are adjusted population means, with adjustment for BMI, diabetes, smoking status, waist circumference, age, and field center.

6. Results

Table 1 lists clinical characteristics and demographics of the 271 subjects who completed the whole fenofibrate protocol and were analyzed in this study stratified by gender and triglyceride status. Fifty % were obese, 15.3% had diabetes, 99% were white, and 44.3% were female. Figure 1 outlines the exclusionary cascade resulting in the 271 subjects analyzed in this study.

Fenofibrate therapy lowered total LDL cholesterol and triglycerides by 5 and 40%, respectively, and increased HDL by 10%. The % change was greater for women than for men (−13.3 versus +1.1% for LDL-C, P < .0001; −41.9 versus −37.6% for triglycerides (P = .0689), and +10.1 versus +9.3% for HDL-C, P = .5980), Figure 1. At 6 hours after the high fat meal, triglyceride levels rose from 239 (10.0) to 411 (17.3) in men and from 230 (7.9) to 405 (15.8) in women; following fenofibrate treatment, the triglyceride excursion at 6 hours was markedly less: triglycerides rose from 140 (7.1) to 228 (9.7) and from 138 (7.9) to 215 (9.5) mg/dL in men and women (Figure 2).

6.1. Lipoprotein Subclass Analysis. Table 2(a) and 2(b) describe the LDL, HDL, and VLDL particle subclasses for each of the three time points during the PPL study (0, 3.5
6.1.1. LDL-C Particle Number and Subclasses in Men and Women. In hypertriglyceridemic men, comparing before and after fenofibrate therapy, fenofibrate treatment resulted in a 12% decrease in total fasting LDL-C particle number from 1740 to 1528 nmol/L. At 6 hrs PPL, the total LDL-C particle number was essentially unchanged, and there was a similar sustained effect of fenofibrate. Among the subclasses, IDL was reduced by fenofibrate at baseline (0 time), and rose with PPL, although this rise was much less after fenofibrate treatment compared to before fenofibrate treatment (66% before versus 28% after fenofibrate, 0–3.5 hours). Large LDL rose from 181 to 393 nmol/L, and small LDL decreased from 1497 to 1088 nmol/L following 3 weeks of fenofibrate therapy. Evaluating the 6 hr PPL effect, large LDL increased by about 34% (Table 2a) pretreatment, but only 14% following fenofibrate treatment, while small LDL decreased by 8.6% and 5.1%, respectively. LDL particle size rose in the post-prandial state in the before fenofibrate-treatment period, but not during the after-fenofibrate treatment period. The directional changes for LDL-C particle number, subclasses, and size were similar in women but of different (usually larger) magnitude.

6.1.2. HDL-C Particle Number and Subclasses in Men and Women. A small increase in HDL-C particle number was observed after fenofibrate, and PPL resulted in small increases in HDL-C particle number before and after fenofibrate. In men, while there was little overall effect of fenofibrate on HDL subclasses, there were large effects of PPL on one of the HDL subclasses. Specifically, medium sized HDL increased over 254% compared to before-fenofibrate levels and increased by 148% following PPL at 6 hours after fenofibrate treatment. However, in absolute concentration terms these effects were small (e.g., 1.3 to 4.6 nmol/L 6 hours PPL before and 2.7 to 6.7 nmol/L change PPL after fenofibrate). Overall there was a very modest change in HDL particle size following fenofibrate treatment with no trends in particle size for the PPL response. In women, the directional changes were similar, but the % of change with PPL was smaller.

6.1.3. VLDL Particle Number and Subclasses in Men and Women. In men, VLDL particle number decreased from 128 to 74 nmol/L following fenofibrate. In the PPL state, large VLDL increased the most, and this increase was dramatically attenuated by fenofibrate. Small and medium VLDL were decreased after PPL, with a similar magnitude of attenuation of these changes by fenofibrate. VLDL particle size was reduced by fenofibrate exposure, and the particle size increase following PPL appeared attenuated over the 6-hour PPL study period. Again, the changes in direction were similar for women with small differences in the magnitude of the changes especially particle size changes for VLDL.

7. Discussion

We sought to describe the effect of fenofibrate exposure (a large sample of subjects who are apart of The Genetics...
(a) Mean Baseline Levels of NMR Lipoprotein Particle Size Concentrations for Hypertriglyceridemic Men. F: Fenofibrate main effect; T: Time main effect; F*T: Fenofibrate by time interaction. Presented +/- least squares means (SE).

| Characteristic                      | 0 Hr Before Fenofibrate | 0 Hr After Fenofibrate | 3.5 Hr Before Fenofibrate | 3.5 Hr After Fenofibrate | 6 Hr Before Fenofibrate | 6 Hr After Fenofibrate | Global P Fenofibrate | Global P Time | Global P F*T |
|-------------------------------------|-------------------------|------------------------|----------------------------|--------------------------|--------------------------|------------------------|-----------------------|---------------|-------------|
| LDL particle no., nmol/L            | 1740 (36)               | 1528 (31)              | 1691 (34)                  | 1540 (32)                | 1714 (35)                | 1530 (35)              | .0001                | .1243         | .003        |
| LDL particles, nmol/L               | 68 (5)                  | 53 (4)                 | 113 (7)                    | 68 (5)                   | 110 (7)                  | 55 (4)                 | .0001                | <.0001        | <.0001      |
| Large LDL particles, nmol/L         | 181 (15)                | 393 (21)               | 245 (16)                   | 414 (21)                 | 242 (17)                 | 449 (20)               | .0001                | <.0001        | .0004       |
| Small LDL particles, nmol/L         | 1497 (37)               | 1088 (36)              | 1337 (33)                  | 1064 (37)                | 1368 (37)                | 1033 (40)              | .0001                | <.0001        | <.0001      |
| LDL particle size, nm               | 19.9 (0.05)             | 20.6 (0.05)            | 20.1 (0.04)                | 20.6 (0.06)              | 20.1 (0.05)              | 20.7 (0.06)            | .0001                | <.0001        | <.0001      |
| HDL particle no., nmol/L            | 27.5 (0.4)              | 30.0 (0.5)             | 27.2 (0.4)                 | 30.3 (0.4)               | 28.8 (0.4)               | 31.6 (0.4)             | .0001                | <.0001        | 0.078       |
| Large HDL particles, nmol/L         | 2.9 (0.16)              | 3.4 (0.15)             | 2.3 (0.16)                 | 3.1 (0.17)               | 2.4 (0.18)               | 3.1 (0.19)             | .0001                | <.0001        | 0.0009      |
| Medium HDL particles, nmol/L        | 1.3 (0.2)               | 2.7 (0.3)              | 3.2 (0.3)                  | 4.8 (0.4)                | 4.6 (0.3)                | 6.7 (0.4)              | .0001                | <.0001        | 0.0111      |
| Small HDL particles, nmol/L         | 23.2 (0.4)              | 23.9 (0.5)             | 21.6 (0.4)                 | 22.4 (0.5)               | 21.8 (0.4)               | 21.7 (0.5)             | 0.2549               | <.0001        | 0.0033      |
| HDL particle size, nm               | 8.5 (0.03)              | 8.4 (0.02)             | 8.4 (0.03)                 | 8.4 (0.02)               | 8.5 (0.03)               | 8.5 (0.02)             | 0.9366               | <.0001        | <.0001      |
| VLDL particle no., nmol/L           | 128 (4)                 | 74 (3)                 | 110 (4)                    | 71 (3)                   | 103 (4)                  | 69 (3)                 | .0001                | <.0001        | <.0001      |
| Large VLDL particles, nmol/L        | 9 (0.7)                 | 4 (0.4)                | 13 (0.7)                   | 8 (0.5)                  | 14 (0.8)                 | 7 (0.5)                | .0001                | <.0001        | <.0001      |
| Medium VLDL particles, nmol/L       | 78 (4)                  | 36 (3)                 | 74 (3)                     | 44 (2)                   | 64 (3)                   | 35 (2)                 | .0001                | <.0001        | <.0001      |
| Small VLDL particles, nmol/L        | 41 (2)                  | 33 (2)                 | 21 (2)                     | 18 (1)                   | 24 (2)                   | 26 (1)                 | 0.0593               | <.0001        | <.0001      |
| Chylomicrons, nmol/L                | 0.03 (0.02)             | 0.03 (0.03)            | 0.8 (0.05)                 | 0.4 (0.03)               | 0.8 (0.06)               | 0.3 (0.04)             | .0001                | <.0001        | <.0001      |
| VLDL particle size, nm              | 53 (0.5)                | 52 (0.5)               | 59 (0.6)                   | 57 (0.5)                 | 61 (0.7)                 | 57 (0.5)               | .0001                | <.0001        | <.0001      |

(b) Mean Baseline Levels of NMR Lipoprotein Particle Size Concentrations for Hypertriglyceridemic Women. Adjusted for age, field center, smoking status, diabetes, waist circumference.

| Characteristic                      | 0 Hr Before Fenofibrate | 0 Hr After Fenofibrate | 3.5 Hr Before Fenofibrate | 3.5 Hr After Fenofibrate | 6 Hr Before Fenofibrate | 6 Hr After Fenofibrate | Global P Fenofibrate | Global P Time | Global P F*T |
|-------------------------------------|-------------------------|------------------------|----------------------------|--------------------------|--------------------------|------------------------|-----------------------|---------------|-------------|
| LDL particle no., nmol/L            | 1774 (46)               | 1423 (40)              | 1756 (43)                  | 1368 (39)                | 1774 (46)                | 1381 (40)              | .0001                | .0026         | 0.0335      |
| LDL particles, nmol/L               | 73 (6)                  | 51 (5)                 | 91 (7)                     | 52 (5)                   | 86 (7)                   | 47 (5)                 | .0001                | .002          | 0.0097      |
| Large LDL particles, nmol/L         | 388 (31)                | 481 (22)               | 465 (31)                   | 527 (23)                 | 506 (32)                 | 574 (25)               | 0.0026               | <.0001        | 0.1076      |
| Small LDL particles, nmol/L         | 1302 (57)               | 892 (45)               | 1191 (54)                  | 789 (43)                 | 1171 (58)                | 763 (45)               | .0001                | <.0001        | 0.9182      |
| LDL particle size, nm               | 20.4 (0.08)             | 20.9 (0.06)            | 20.6 (0.07)                | 21.1 (0.06)              | 20.7 (0.08)              | 21.1 (0.07)            | .0001                | <.0001        | 0.2751      |
and Lipid Lowering Drugs and Diet Network [GOLDN]) in hypertriglyceridemic men and women before and after ingestion of a high (83%) fat meal on LDL, HDL, and VLDL particle number and subclasses. For fenofibrate alone (i.e., results prior to the ingestion of the high fat meal), we found reductions in LDL and VLDL particle numbers, and reductions in IDL, small LDL, all VLDL subclasses, and increases in large LDL and medium HDL. PPL resulted in small changes in LDL and HDL particle numbers, but caused a large increase in VLDL particle number, all of which was attenuated by fenofibrate. Similarly, there were marked changes in LDL and VLDL particle classes with PPL, but their effects were also significantly attenuated by fenofibrate. Thus, the striking finding was that fenofibrate lowered fasting and postprandial VLDL primarily due to reductions in postprandial levels of large and medium VLDL particles. These changes were directionally similar to those found by Rosenson et al. in patients with the Metabolic Syndrome [10]. It is important to note that 80.8% of our 271 hypertriglyceridemic subjects had metabolic syndrome. Fenofibrate also reduced fasting and postprandial total LDL particles, primarily a result of reduced small LDL particles. Directional changes were similar in men and women but the magnitude of change was different for some parameters. Mora et al. conducted a study to prospectively evaluate whether NMR lipoprotein particles predict CVD (a study of initially healthy women). Compared to standard lipid measurements, the NMR-measured lipoprotein particle concentrations of total LDL and VLDL (but not HDL) were higher in women who developed CVD. Women with CVD had significantly smaller LDL and HDL particle sizes and larger VLDL particle size [18].

Fibrates have been used for the treatment of hypertriglyceridemia and mixed dyslipidemias for more than 30 years. Fenofibrate was introduced into the North American marketplace approximately one decade ago. It is now known that fibrates act by stimulating the activity of peroxisome proliferator-activated receptor (PPAR)-alpha [19]. Forcheron et al. studied the mechanism of fenofibrate in diabetic patients, and concluded that the plasma triglyceride lowering effect was explained (in part) by a decrease in hepatic lipogenesis, and by a reduction in the hepatic reesterification of plasma free fatty acids, along with a stimulation of reverse cholesterol transport and up-regulation of Apo A1 transcription (by its effect on key regulatory genes) [20]. The effect of fenofibrate on total cholesterol is less clear. We observed that fenofibrate had a positive effect, not only on fasting lipid measures, but on the lipid excursions that follow a high fat meal, suggesting that its effectiveness may be particularly useful among those hypertriglyceridemic individuals who chose to follow a higher-fat diet regimen.

Studies suggest that large (HDL₃) particles are more protective against atherosclerosis than small HDL particles. In individuals with relatively high serum triglycerides (>250 mg/dL), HDL₃/HDL₂ ratios are often low, and this pattern of HDL subclasses has been shown to be associated with increased risk of coronary artery disease (CAD) [21–23]. However, significantly higher small HDL particle

| Characteristic                          | 0 Hr Before Fenofibrate | 3.5 Hr After Fenofibrate | 6 Hr After Fenofibrate | Global P | Fenofibrate | Time | Fenofibrate*Time |
|----------------------------------------|-------------------------|--------------------------|------------------------|---------|-------------|------|-----------------|
| HDL particle no., nmol/L               | 33.1 (0.6)              | 35.8 (0.6)               | 32.5 (0.6)             |         | <.0001      |      |                 |
| Large HDL particles, nmol/L            | 6.1 (0.3)               | 5.7 (0.3)                | 5.5 (0.4)              |         | 0.7323      | 0.0019 |                 |
| Medium HDL particles, nmol/L           | 3.5 (0.5)               | 6.5 (0.6)                | 6.0 (0.5)              |         | 0.0001      | <.0001 | <.0001          |
| Small HDL particles, nmol/L            | 24 (0.7)                | 24 (0.8)                 | 21 (0.7)               |         | 0.0062      | <.0001 | <.0001          |
| HDL particle size, nm                   | 8.8 (0.04)              | 8.6 (0.03)               | 8.8 (0.04)             | <.0002  | <.0001      | <.0001 |                 |
| VLDL particle no., nmol/L              | 115 (5)                 | 53 (3)                   | 102 (4)                |         | <.0001      | <.0001 | <.0001          |
| Large VLDL particles, nmol/L           | 8 (0.6)                 | 4 (0.5)                  | 13 (0.7)               |         | <.0001      | <.0001 | <.0001          |
| Medium VLDL particles, nmol/L          | 63 (4)                  | 22 (2)                   | 60 (3)                 |         | <.0001      | <.0001 | <.0001          |
| Small VLDL particles, nmol/L           | 43 (3)                  | 28 (2)                   | 28 (2)                 | <.0001  | <.0001      | 0.0023 |                 |
| Chylomicrons, nmol/L                   | 0.02 (0.05)             | 0.02 (0.05)              | 0.5 (0.06)             |         | 0.0001      | <.0001 | 0.0188          |
| VLDL particle size, nm                  | 53 (0.6)                | 55 (0.8)                 | 60 (0.8)               |         | 0.7639      | <.0001 | 0.0006          |
concentrations have been reported in the VA-HIT [23] and BECAIT [24] studies, where participants were treated with gemfibrozil and bezafibrate, respectively. The increase in small HDL particle concentration was independent of HDL-C concentrations and was shown to be a strong and significant predictor of a reduction in CAD events [24].

Recently, Mora et al., compared nonfasting to fasting lipid measurements for predicting incident cardiovascular events [25]. They observed that some of the fasting measures were less useful predictors of CVD risk than nonfasting levels and suggested that lipid screening may need to consider these differences. These observations support the need for further defining the lipid responses to PPL and the corresponding treatment responses in order to more fully evaluate CVD risk.

There are several limitations to our study of the effects of fenofibrate on lipid particle number and size. Although our findings are consistent with others, we cannot be certain that the findings of our study apply to individuals who do not identify themselves as Caucasians. As our study only included Caucasians, our findings are indeed limited on that basis. Furthermore, the time window over which we studied the changes in lipid particles post PPL is limited to a 6 hour window. Finally, the duration of exposure to fenofibrate was limited to 3 weeks. Although confident that this time frame was sufficient long to observe the maximum effects on lipid fractions for most individuals, there is no way to be certain that this was true for all individuals. On the other hand, the size of our study population and its inclusion of a large percentage of women represent significant virtues relative to other such investigations.

In conclusion, we observed changes in lipid subspecies which are generally associated with antiatherogenic profiles as a function of fenofibrate therapy both on fasting and post-prandial states. As this was not an outcome study, our observations on lipid subspecies require long-term outcome trials to qualify their clinical relevance. However, as one of the largest studies to date characterizing the effect of fenofibrate on LDL, HDL, and VLDL subspecies in both the fasting and the postprandial states, these findings provide new and confirmatory data as to the observations which may be expected in a clinically relevant subgroup receiving this drug as monotherapy. These findings provide a foundation to pursue mechanistic insight into the relationship between lipid subspecies and the development or prevention of atherosclerosis through drug therapy.

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