

# Coronary Heart Disease

## 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

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**Background**—Changes in conventional lipid risk factors with gemfibrozil treatment only partially explain the reductions in coronary heart disease (CHD) events experienced by men in the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT). We examined whether measurement of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particle subclasses provides additional information relative to CHD risk reduction.

**Methods and Results**—This is a prospective nested case-control study of 364 men with a new CHD event (nonfatal myocardial infarction or cardiac death) during a 5.1-year (median) follow-up and 697 age-matched controls. Nuclear magnetic resonance (NMR) spectroscopy was used to quantify levels of LDL and HDL particle subclasses and mean particle sizes in plasma obtained at baseline and after 7 months of treatment with gemfibrozil or placebo. Odds ratios for a 1-SD increment of each lipoprotein variable were calculated with adjusted logistic regression models. Gemfibrozil treatment increased LDL size and lowered numbers of LDL particles (−5%) while raising numbers of HDL particles (10%) and small HDL subclass particles (21%). Concentrations of these LDL and HDL particles achieved with gemfibrozil were significant, independent predictors of new CHD events. For total LDL and HDL particles, odds ratios predicting CHD benefit were 1.28 (95% CI, 1.12 to 1.47) and 0.71 (95% CI, 0.61 to 0.81), respectively. Mean LDL and HDL particle sizes were not associated with CHD events.

**Conclusions**—The effects of gemfibrozil on NMR-measured LDL and HDL particle subclasses, which are not reflected by conventional lipoprotein cholesterol measures, help to explain the demonstrated benefit of this therapy in patients with low HDL cholesterol. (*Circulation*. 2006;113:1556-1563.)

**Key Words:** coronary disease ■ drugs ■ lipoproteins ■ risk factors ■ spectroscopy

The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) demonstrated that the fibric acid derivative gemfibrozil reduced the 5-year rate of new coronary heart disease (CHD) events in men with known CHD and low levels of both high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).<sup>1</sup> Overall, HDL-C was raised 6% and triglycerides lowered 31% by gemfibrozil, but only the increased HDL-C predicted the reduction in CHD events.<sup>2</sup> LDL-C concentrations neither predicted the development of new CHD events nor were lowered by gemfibrozil.

**Editorial p 1553**

**Clinical Perspective p 1563**

Individuals with low HDL-C and elevated triglycerides, such as those in VA-HIT, characteristically have LDL parti-

cles that are smaller and contain less cholesterol than average.<sup>3,4</sup> As a result, numbers of atherogenic LDL particles are frequently elevated even though LDL-C levels are not.<sup>5</sup> CHD risk in several prospective studies has been found to be better explained by concentrations of LDL particles, assessed by nuclear magnetic resonance (NMR) or plasma levels of apolipoprotein B (apoB), than by LDL-C.<sup>6-13</sup> Some evidence also suggests that LDL particle subclasses may differ in atherogenicity, with small, dense LDL usually,<sup>4</sup> but not always,<sup>14</sup> associated with greater CHD risk than large, buoyant LDL.

Angiographic trials with fibrates demonstrating reduced progression of coronary artery stenosis have shown that these drugs, although not appreciably changing the concentration of LDL-C, increase LDL particle size and decrease plasma apoB

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levels.<sup>15–17</sup> Furthermore, the increase in HDL-C brought about by fibrates results from increased levels of the smaller HDL<sub>3</sub> subclass.<sup>2,18,19</sup> As yet, there have been no trials with fibrates showing that a reduction in major clinical CHD events with these drugs may be related to changes in LDL or HDL subclass concentrations or particle size distributions.

In the present nested case-control substudy of VA-HIT, we assessed the effects of gemfibrozil treatment on LDL and HDL subclass particle numbers and mean particle sizes as measured by NMR spectroscopy. We also examined whether levels of these subclasses measured at baseline or during the trial were related to CHD events and whether these relations differed in subjects with diabetes or insulin resistance.

## Methods

### Subjects

The rationale, design, and methods for VA-HIT have been described previously.<sup>1</sup> Briefly, men younger than 74 years with an established diagnosis of CHD were recruited at 20 Veterans Affairs medical centers throughout the United States. Lipid eligibility criteria were HDL-C  $\leq$ 40 mg/dL, LDL-C  $\leq$ 140 mg/dL, and triglycerides  $\leq$ 300 mg/dL. A total of 2531 subjects were randomized to either gemfibrozil (1200 mg/d) or placebo and were treated for a median of 5.1 years. The primary end point was nonfatal myocardial infarction (MI) or CHD death.

For this analysis, the case subjects (n=364) were those study participants who experienced a nonfatal MI or CHD death during the trial from whom baseline and follow-up stored plasma samples were available. The control subjects (n=697) matched for age were selected from among the remaining study participants who remained free of CHD events during follow-up. An equal percentage (43%) of the 364 cases with a CHD event selected for this analysis and the 494 total CHD cases in the entirety of VA-HIT were treated with gemfibrozil. VA-HIT was approved by the Human Rights Committee of the Cooperative Studies Program Coordinating Center, by each of the 20 study site's institutional review boards, and by the Cooperative Studies Program Evaluation Committee. All subjects gave written, informed consent.

### Laboratory Analyses

Blood samples were collected from subjects, after a 12- to 14-hour fast, into tubes containing 0.1% EDTA. Plasma was isolated, frozen, and sent to the VA-HIT central laboratory (Tufts University, Boston, Mass) for lipid analyses and for long-term storage at  $-80^{\circ}\text{C}$  before subsequent analyses of apolipoproteins in the central laboratory and lipoprotein particle subclasses at LipoScience, Inc (Raleigh, NC). Unlike the lipid values reported for the entire VA-HIT population,<sup>2</sup> which were the averages of 2 baseline samples obtained 1 to 2 weeks apart and 4 follow-up samples obtained at 4, 7, 12, and 18 months during the trial, values reported here represent a single baseline and 7-month follow-up measurement, except for the follow-up apolipoprotein values, which were obtained at the 12-month visit.

Total cholesterol, HDL-C, triglycerides, and glucose were measured by standardized automated methods, and LDL-C was calculated by the Friedewald equation.<sup>20</sup> Insulin was determined as total immunoreactive insulin.<sup>21</sup> ApoA-1 and apoB levels were determined with immunoturbidimetric assays.<sup>22,23</sup> Diabetes was defined by history or by a fasting glucose  $\geq$ 126 mg/dL. Insulin resistance was defined by a homeostasis model assessment of insulin resistance value  $\geq$ 10.2, as previously described.<sup>21</sup> All insulin, glucose, and lipid assays had between-run coefficients of variation (CVs) of  $<$ 5%. Between-run CVs were 7% to 11% for apoB and  $<$ 4% for apoA-1.<sup>22,23</sup>

LDL and HDL subclass particle concentrations and mean LDL and HDL particle diameters were measured with an automated NMR spectroscopic assay as previously described<sup>24,25</sup> and recently modified.<sup>26</sup> In brief, the particle concentrations of lipoprotein subclasses

of different size are derived from the measured amplitudes of the distinct lipid methyl group NMR signals they emit. Concentrations (nmol/L for LDL particles and  $\mu\text{mol/L}$  for HDL particles) of the following subclasses were analyzed in this study: small LDL (18.0 to 21.2 nm), large LDL (21.2 to 23.0 nm), intermediate-density lipoprotein (IDL) (23.0 to 27.0 nm), large HDL (8.8 to 13.0 nm), medium HDL (8.2 to 8.8 nm), and small HDL (7.3 to 8.2 nm). Weighted-average lipoprotein particle sizes in nanometers were calculated from the subclass levels, and the diameters were assigned to each subclass. Very low-density lipoprotein (VLDL) subclass data are not included in this analysis because there was evidence that NMR detection of these triglyceride-rich particles was altered to a variable extent by the freeze-thaw cycles to which the plasma specimens were subjected. LDL and HDL subclass levels measured by NMR are unaffected by frozen storage<sup>25</sup> and multiple freeze-thaw cycles (J.D. Otvos, PhD, unpublished data, 2005).

Reproducibility of the NMR-measured lipoprotein particle parameters was determined by replicate analyses of plasma pools. Between-run CVs for low-normal concentrations were  $<$ 4% for total LDL and HDL particle concentrations,  $<$ 0.5% for LDL and HDL size,  $<$ 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. For all biochemical and NMR analyses, samples were handled in a fully blinded fashion such that investigators had no knowledge of case or control status. All results of laboratory analyses were sent to the VA Cooperative Studies Coordinating Center (West Haven, Conn) and entered into the VA-HIT centralized database.

### Statistical Analyses

Baseline characteristics were compared between cases and controls, and lipids, apolipoproteins, and NMR lipoprotein particle measures were compared by treatment with *t* tests. Odds ratios for 1-SD increments of baseline and on-trial concentrations of lipids, apolipoproteins, and NMR lipoprotein particle measures, as well as changes in these variables, were assessed with the use of logistic regression with adjustment for treatment group, age, hypertension, smoking, body mass index, and diabetes. Separate analyses were performed on the subset of subjects (n=395) with insulin resistance or diabetes (excluding those treated with insulin). Because of the multiple statistical tests that were performed, which could inflate the type 1 error rate, we used 0.01 as the criterion for statistical significance. To examine whether the relation of each lipid/lipoprotein variable to CHD differed by treatment group, we included interaction (product) terms in our regression models.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

## Results

Baseline characteristics of the cases and controls, shown in Table 1, were similar to those of the entire VA-HIT population. As previously documented, a relatively large proportion of subjects in this trial had diabetes or insulin resistance, with a greater prevalence in cases compared with controls. Lipid and apolipoprotein changes in the gemfibrozil treatment group (Table 2) were virtually identical to those reported previously for the whole study population.<sup>2</sup> Triglycerides decreased 30%, HDL-C increased 6%, and LDL-C was minimally increased. Both plasma apoB and non-HDL cholesterol (non-HDL-C), reflecting the combined levels of VLDL and LDL, underwent small reductions.

As Table 2 shows, the concentration of total LDL particles (LDL-P) measured by NMR was decreased 5% by gemfibrozil. This reduction was caused by a significant 20% decrease in the number of small LDL particles, from 967 to 777

**TABLE 1. Baseline Characteristics of Cases, Controls, and Entire VA-HIT Population**

Characteristic	Cases (n=364)	Controls (n=697)	Entire Population (n=2531)	P*
Age, y	64.3 (7.1)	64.5 (6.9)	64.2 (7.2)	0.64
Body mass index, kg/m <sup>2</sup>	29.2 (4.7)	29.1 (4.9)	29.0 (4.8)	0.43
Waist, cm	103 (12)	103 (12)	103 (12)	0.48
Hypertension, %	56.6	58.4	56.9	0.57
Diabetes, %	37.1	28.8	30.4	0.006
Insulin resistance, %	35.2	27.8	30.0	0.002
Current smoker, %	22.0	17.7	20.4	0.09
LDL-C, mg/dL	113.3 (21.4)	110.8 (23.1)	111.1 (22.2)	0.10
HDL-C, mg/dL	30.9 (5.5)	31.5 (5.2)	31.8 (5.3)	0.05
Triglycerides, mg/dL	166.9 (72.8)	161.4 (66.9)	160.6 (68.0)	0.41
Glucose, mg/dL	119 (40)	114 (34)	115 (37)	0.03

Values are mean (SD) or percentage. The prevalences of hypertension and smoking were obtained by history. Diabetes was defined by history or by a fasting glucose  $\geq 126$  mg/dL. Insulin resistance was defined by a homeostasis model assessment of insulin resistance value  $\geq 10.2$  and does not include subjects with diabetes being treated with insulin.<sup>21</sup>

\*P value is for the comparison of cases and controls. For body mass index and triglycerides, a nonparametric Wilcoxon test was used. For all other variables, *t* tests or  $\chi^2$  tests were used.

nmol/L, which was partially offset by a 36% increase in the number of large LDL particles. As a result of this alteration in LDL subclass composition, average LDL particle size increased significantly from 20.4 to 20.9 nm. Total HDL particles (HDL-P) in the gemfibrozil treatment group were increased as a result of increased numbers of small HDL

particles offsetting reductions in large- and medium-size HDL subclass particles.

Table 3 shows the odds ratios (ORs) for a new CHD event associated with a 1-SD increment of each lipid or lipoprotein particle variable measured at baseline and during the trial in the combined gemfibrozil and placebo treatment groups,

**TABLE 2. Treatment Effects on Lipids, Lipoprotein Particle Numbers, and Lipoprotein Particle Sizes**

Variable	Placebo (n=546)		Gemfibrozil (n=515)		P†
	Baseline	On-Trial*	Baseline	On-Trial	
<b>Lipids and apolipoproteins</b>					
LDL-C, mg/dL	111.6 (23.1)	112.1 (26.7)	111.7 (22.1)	114.7 (25.9)	0.14
HDL-C, mg/dL	31.2 (5.0)	31.3 (6.1)	31.5 (5.5)	33.4 (6.7)	<0.0001
Triglycerides, mg/dL	167.1 (69.5)	176.7 (84.2)	159.3 (68.3)	111.3 (59.3)	<0.0001
Non-HDL-C, mg/dL	144.6 (24.1)	147.0 (29.9)	143.6 (23.8)	137.0 (28.6)	<0.0001
ApoB, mg/dL	96.8 (20.6)	93.6 (17.9)	95.4 (21.7)	89.0 (19.4)	0.0002
ApoA-1, mg/dL	105.9 (15.9)	108.8 (16.2)	104.9 (16.6)	107.8 (16.4)	0.35
<b>NMR lipoprotein particle measures</b>					
LDL particle No., nmol/L	1364 (315)	1463 (342)	1352 (316)	1290 (331)	<0.0001
IDL particles, nmol/L	34 (25)	35 (27)	32 (24)	33 (28)	0.29
Large LDL particles, nmol/L	346 (214)	345 (239)	354 (217)	480 (231)	<0.0001
Small LDL particles, nmol/L	984 (391)	1083 (444)	967 (406)	777 (425)	<0.0001
LDL particle size, nm	20.4 (0.7)	20.3 (0.8)	20.4 (0.8)	20.9 (0.7)	<0.0001
HDL particle No., $\mu$ mol/L	25.2 (4.3)	26.6 (4.5)	25.1 (4.6)	27.6 (5.0)	0.0005
Large HDL particles, $\mu$ mol/L	2.7 (1.7)	2.3 (1.6)	2.7 (1.7)	2.3 (1.6)	0.71
Medium HDL particles, $\mu$ mol/L	1.9 (2.5)	2.0 (2.6)	1.9 (2.3)	0.7 (1.6)	<0.0001
Small HDL particles, $\mu$ mol/L	20.7 (5.2)	22.4 (5.3)	20.4 (5.2)	24.6 (5.4)	<0.0001
HDL particle size, nm	8.5 (0.3)	8.4 (0.3)	8.5 (0.3)	8.4 (0.3)	0.68

Values are mean (SD).

\*On-trial data are for samples obtained at the 7-month visit; apolipoproteins are the values at 12 months.

†P values are for comparison of on-trial values in the placebo vs gemfibrozil groups.

**TABLE 3. Lipids, Apolipoproteins, and Lipoprotein Particle Parameters as Individual Predictors of CHD Events**

Variable	Baseline		On-Trial	
	OR* (95% CI)	P	OR (95% CI)	P
<b>Lipids and apolipoproteins</b>				
LDL-C	1.10 (0.97–1.25)	0.15	1.08 (0.95–1.23)	0.25
HDL-C	0.91 (0.80–1.03)	0.14	0.95 (0.83–1.08)	0.42
Triglycerides	1.07 (0.94–1.22)	0.29	1.04 (0.90–1.19)	0.63
Non-HDL-C	1.14 (1.00–1.30)	0.05	1.09 (0.96–1.25)	0.17
Total cholesterol:HDL-C ratio	1.19 (1.05–1.35)	0.008	1.14 (1.01–1.30)	0.05
ApoB	1.12 (0.99–1.27)	0.08	1.07 (0.94–1.23)	0.31
ApoA-1	0.84 (0.74–0.96)	0.01	0.91 (0.80–1.04)	0.18
ApoB:apoA-1 ratio	1.24 (1.10–1.41)	0.0008	1.14 (0.99–1.30)	0.06
<b>NMR lipoprotein particle measures</b>				
LDL particle No.	1.20 (1.05–1.37)	0.006	1.28 (1.12–1.47)	0.0003
IDL particles	0.99 (0.87–1.12)	0.84	1.17 (1.03–1.33)	0.02
Large LDL particles	1.08 (0.95–1.23)	0.27	1.06 (0.93–1.22)	0.35
Small LDL particles	1.11 (0.98–1.27)	0.11	1.17 (1.02–1.34)	0.03
LDL particle size	0.97 (0.85–1.10)	0.64	0.96 (0.84–1.10)	0.57
HDL particles	0.78 (0.69–0.90)	0.0004	0.71 (0.61–0.81)	0.0001
Large HDL particles	0.98 (0.86–1.11)	0.74	0.94 (0.83–1.07)	0.37
Medium HDL particles	0.96 (0.84–1.09)	0.54	1.04 (0.92–1.19)	0.52
Small HDL particles	0.82 (0.72–0.94)	0.004	0.74 (0.64–0.85)	0.0001
HDL Particle Size	1.00 (0.88–1.14)	0.97	0.91 (0.79–1.04)	0.15

\*ORs (95% CIs) were calculated for a 1-SD increment in each lipid/lipoprotein variable in separate logistic regression models adjusted for treatment group, age, hypertension, smoking, body mass index, and diabetes. For placebo and gemfibrozil groups combined, n=1061 subjects.

assessed by separate logistic regression models that were adjusted for major nonlipid CHD risk factors and treatment group. Models that included the interaction between treatment and each lipid/lipoprotein variable indicated that the associations did not differ significantly between the placebo and gemfibrozil groups (data not shown). Neither baseline nor on-trial levels of HDL-C, triglycerides, or LDL-C were significant predictors of CHD risk. Among other lipid/apolipoprotein variables, baseline, but not on-trial, concentrations of apoA-1 and the ratios of apoB to apoA-1 and total cholesterol to HDL-C predicted a CHD end point.

Among NMR lipoprotein measures, both baseline and on-trial levels of LDL-P and HDL-P were strong, independent predictors of a new CHD event. A 1-SD increment of LDL-P (350 nmol/L) during the trial was associated with an OR of 1.28 (95% CI, 1.12 to 1.47;  $P=0.0003$ ), whereas the OR of a 1-SD increment of HDL-P (4.8  $\mu$ mol/L) was 0.71 (95% CI, 0.61 to 0.81;  $P<0.0001$ ). Further adjustment for LDL-C, HDL-C, and triglycerides did not appreciably change these relations (data not shown). The small HDL-P subclass, which constituted the majority of the total HDL particles in VA-HIT men, was also a significant predictor of CHD end points. On-trial numbers of small LDL particles were positively associated with events ( $P=0.03$ ) but did not achieve the  $P<0.01$  significance level. Neither the large nor the average LDL and HDL particle subclasses were related to

CHD events (Table 3). Additional analyses (results not shown) indicated that no change (by concentration or percentage) in any of the lipid, apolipoprotein, or lipoprotein particle variables was a significant predictor of CHD risk.

We also conducted separate analyses (not shown) identical to those in Tables 2 and 3 for the subset of subjects (n=395) with diabetes or insulin resistance. In agreement with results reported for the whole VA-HIT population,<sup>21</sup> gemfibrozil induced a somewhat smaller increase in HDL-C (3% versus 6%) and a smaller decrease in triglycerides (–26% versus –30%) in this subgroup. LDL-P also decreased less (–2% versus –5%), and the increase in LDL size was smaller (0.3 versus 0.5 nm). However, the gemfibrozil-induced changes in HDL-P and the HDL-P subclasses were very similar to those seen in the entire study population. Furthermore, relations of lipid, apolipoprotein, and lipoprotein particle parameters to CHD events in the diabetes/insulin resistance subgroup were virtually identical to those shown in Table 3. None of the on-trial lipid or apolipoprotein variables predicted CHD events in this subgroup, whereas both LDL-P (OR=1.30; 95% CI, 1.05 to 1.61) and HDL-P (OR=0.68; 95% CI, 0.54 to 0.86) did.

To assess the independence of relations of LDL and HDL particle subclasses with CHD events and correct for any confounding caused by the intercorrelations among these variables, all 6 LDL and HDL subclasses (including IDL)

**TABLE 4. NMR Lipoprotein Subclass Particle Parameters as Multivariable Predictors of CHD Events**

Variable	Baseline		On-Trial	
	OR* (95% CI)	P	OR (95% CI)	P
Large LDL particles	1.31 (1.09–1.57)	0.003	1.34 (1.11–1.62)	0.002
Small LDL particles	1.44 (1.20–1.73)	<0.0001	1.41 (1.14–1.73)	0.001
IDL particles	0.98 (0.86–1.12)	0.78	1.13 (0.97–1.30)	0.11
Large HDL particles	0.95 (0.82–1.11)	0.53	0.92 (0.79–1.07)	0.30
Medium HDL particles	0.82 (0.70–0.96)	0.02	0.82 (0.69–0.97)	0.02
Small HDL particles	0.71 (0.60–0.84)	<0.0001	0.67 (0.57–0.79)	<0.0001

\*ORs (95% CIs) were calculated for a 1-SD increment in each lipoprotein subclass parameter at baseline and on-trial with the use of logistic regression models that included all lipoprotein particle parameters in 1 model. All models were additionally adjusted for treatment group, age, hypertension, smoking, body mass index, and diabetes.

were included in the same regression models that were additionally adjusted for treatment and other risk factors (Table 4). Both large and small LDL subclass particle numbers were now strongly and independently predictive of CHD outcomes, both at baseline and during the trial. ORs for small and large LDL-P during the trial were 1.41 (95% CI, 1.14 to 1.73;  $P=0.001$ ) and 1.34 (95% CI, 1.11 to 1.62;  $P=0.002$ ), respectively. Stronger associations with CHD events were also seen for all 3 HDL subclasses when these variables were considered jointly with LDL subclasses in the same model (Table 4) rather than individually in separate models (Table 3).

The extent to which the risk of new CHD events is related to different measures of the ratio of atherogenic to antiatherogenic lipoprotein particles is shown in Table 5. The relative risk of those in the highest quartile of ratio of total cholesterol to HDL-C or ratio of apoB to apoA-1 was significantly elevated (relative risk=1.5; 95% CI, 1.1 to 2.0 for both lipid ratios). However, a stronger, graded risk relationship was seen for the ratio of LDL-P to HDL-P (relative risk=2.4 for

the highest versus lowest quartile; 95% CI, 1.8 to 3.3). Similar results were obtained when the gemfibrozil and placebo groups were examined separately (results not shown).

## Discussion

VA-HIT was undertaken to determine whether treatment aimed at raising HDL-C (and lowering triglycerides), rather than lowering LDL-C, would reduce CHD events in men with coronary disease. To simplify interpretation of the study results, 2 aspects of the study design were intended to “uncouple” HDL-C from LDL-C as contributors to any achieved treatment benefit.<sup>1</sup> First, subjects recruited into the trial had not only low HDL-C (mean, 32 mg/dL) but low-risk levels of LDL-C (mean, 111 mg/dL). Second, gemfibrozil was chosen as the treatment drug for its ability both to increase HDL-C and to not appreciably change levels of LDL-C. VA-HIT achieved these objectives. Not only did this treatment produce a significant 22% reduction in CHD events, but this result was accomplished without a decrease in LDL-C.<sup>1</sup> Subsequent regression analyses confirmed

**TABLE 5. Risk of CHD Events According to Quartile of Lipid/Lipoprotein Ratios**

Ratio	Quartile of Plasma Level				P for Trend
	1	2	3	4	
<b>TC:HDL-C</b>					
Median (range)	4.1 (2.1–4.6)	5.1 (4.6–5.5)	5.9 (5.5–6.5)	7.2 (6.5–12.5)	
Relative risk* (95% CI)	1	1.3 (0.9–1.7)	1.2 (0.9–1.6)	1.5 (1.1–2.0)	0.27
P		0.13	0.27	0.01	
<b>ApoB:ApoA-1</b>					
Median (range)	0.66 (0.26–0.73)	0.79 (0.73–0.85)	0.90 (0.85–0.97)	1.1 (0.97–1.9)	
Relative risk (95% CI)	1	1.3 (0.9–1.8)	1.6 (1.2–2.2)	1.5 (1.1–2.0)	0.37
P		0.14	0.004	0.02	
<b>LDL-P:HDL-P</b>					
Median (range)	34.4 (13.9–40.5)	46.4 (40.5–51.2)	55.3 (51.3–61.2)	71.0 (61.3–127.7)	
Relative risk (95% CI)	1	1.6 (1.2–2.3)	1.8 (1.3–2.6)	2.4 (1.8–3.3)	0.009
P		0.005	0.0005	<0.0001	

TC indicates triglycerides.

\*Logistic regression models used on-trial values of lipid/lipoprotein ratios and were adjusted for treatment group, age, hypertension, smoking, body mass index, and diabetes.

that LDL-C levels at baseline and during the trial were not related to new CHD events.<sup>2</sup> In contrast, levels of HDL-C achieved with therapy predicted CHD outcomes and explained some of the gemfibrozil treatment benefit.

Measuring the cholesterol content of LDL and HDL particles (LDL-C and HDL-C) is the traditional way that levels of these atherogenic and antiatherogenic lipoproteins are assessed. In this study we used an alternative technique, NMR spectroscopy, which “counts” the numbers of LDL and HDL particles (LDL-P and HDL-P) and their subclasses.<sup>24</sup> With this different measure of LDL and HDL concentration, we gained new insights into the lipoprotein particle altering effects of fibrate therapy in VA-HIT and how these alterations might affect clinical outcomes. Gemfibrozil significantly reduced total LDL particle numbers by changing the subclass distribution, lowering numbers of small LDL particles while raising to a lesser extent numbers of the larger, more cholesterol-rich particles (Table 2). Despite the decrease in LDL-P, LDL-C remained unchanged because the cholesterol content of the LDL had increased as a result of the gemfibrozil-induced shift toward larger particles. Similarly, although gemfibrozil raised HDL cholesterol levels only modestly (6%), there was a more substantial 10% increase in total HDL particle number and a 21% increase in numbers of the small, relatively cholesterol-poor HDL subclass.

Notably, we found that both LDL and HDL particle numbers measured during the trial had significant, independent associations with new CHD events, whereas LDL and HDL cholesterol levels did not. A possible reason is that LDL and HDL cholesterol levels have more sources of variability, with levels differing either because of differences in cholesterol composition (amount of cholesterol per particle due to particle size or core lipid compositional differences),<sup>5</sup> differences in particle number, or some combination of the two. Because only men with low levels of LDL-C and HDL-C were enrolled in VA-HIT, the range of lipid levels was restricted compared with the general population. As a result, the impact of lipoprotein cholesterol compositional heterogeneity on relations of lipid levels with CHD risk was likely magnified in this study population. Whatever the reason, our results clearly indicate that in this secondary prevention study of men with low HDL and LDL, CHD risk is better reflected by numbers of lipoprotein particles than by the amount of cholesterol these particles contain. The same conclusion applies to the subset of men in VA-HIT who had diabetes or insulin resistance. It was shown previously that these subjects, despite experiencing smaller changes in HDL-C, derived substantially more benefit from gemfibrozil treatment than those without insulin resistance.<sup>21</sup> We found that on-trial levels of HDL-P and LDL-P were equally predictive of CHD events in this subgroup and the study population as a whole.

Other studies have also reported that CHD risk appears to be more strongly related to the number of circulating LDL particles than to the measured concentration of cholesterol in LDL.<sup>6,11–13</sup> Many of these have used plasma apoB concentration to approximate LDL particle number because, despite

apoB being present on VLDL as well as LDL particles, at least 90% of apoB is on LDL even in hypertriglyceridemic patients.<sup>27</sup> In VA-HIT, gemfibrozil comparably lowered levels of both apoB (6%) and NMR-measured LDL-P (5%), yet in regression analyses previously conducted on the entire study population<sup>2</sup> and in this nested case-control substudy, levels of apoB with gemfibrozil were not significantly related to the development of a CHD event. Two other studies in which both apoB and LDL-P were measured have also reported stronger disease associations for LDL-P.<sup>7,28</sup> We are unsure why apoB is less strongly related to CHD outcomes than LDL-P in VA-HIT. One contributing factor may be the greater analytic reproducibility of the LDL-P measurement compared with the apoB immunoassay. Another is that, as previously mentioned, plasma apoB provides only an estimate of LDL particle number because some apoB resides on non-LDL particles. Of note, LDL-P and apoB were correlated less strongly in this study ( $r=0.56$ ) than in the Framingham Offspring Study ( $r=0.86$ )<sup>22</sup> and Women’s Health Study ( $r=0.70$ ),<sup>7</sup> in part because the range of LDL concentrations in VA-HIT was more restricted.

Although gemfibrozil appreciably increased average LDL particle size in VA-HIT by 0.5 nm, this parameter did not predict a significant reduction in CHD events. This observation is consistent with the results of several observational and intervention studies showing that quantitative measures of LDL particle number, assessed by either NMR or apoB measurement, are more strongly associated with CHD than is small LDL size.<sup>6–8,10,16,17</sup> Although large LDL-P in our study was not related to CHD events when considered individually (Table 3), it became strongly predictive when confounding due to its correlations with small LDL-P and the HDL subclasses was overcome by inclusion of these interrelated variables in the same model (Table 4). Similar observations have been made by others.<sup>14</sup>

Our findings with regard to prediction of CHD events by LDL and HDL particle subclasses are highly concordant with those of the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT), an angiographic trial that studied a similar population of male CHD patients with low HDL-C.<sup>17</sup> The only on-trial predictors of angiographic outcomes were levels of the small HDL subclass (HDL<sub>3</sub>-C) and apoB. Significantly, and in agreement with VA-HIT, despite the large impact of fibrate treatment on triglyceride levels and LDL particle size, neither of these parameters was related to the angiographic outcomes.

In agreement with the results of other studies with fibrates, we found that increased levels of the small HDL subclass accounted for the increase in HDL-C<sup>18,19</sup> brought about by drug treatment and that on-trial numbers of the small HDL particles, but not large HDL particles, predicted CHD events.<sup>17,29</sup> Although it is unclear by which mechanism(s) the increase in numbers of HDL particles with this therapy leads to clinical benefit, it may be suggested that higher numbers of HDL particles might promote greater cholesterol efflux and protection of LDL from oxidative changes.<sup>30</sup>

In this nested case-control substudy, HDL-C levels at baseline (31.5 mg/dL) and on-trial levels (33.4 mg/dL)

matched exactly the mean values reported for the entire study population of VA-HIT.<sup>2</sup> However, although the findings in the whole trial showed that achieved levels of HDL-C were predictive of CHD events (RR=0.89; 95% CI, 0.81 to 0.97), we found a weaker, nonsignificant association (RR=0.95) in this substudy. We have no certain explanation for this difference but speculate that it may be because we used only a single on-trial measure of HDL-C, whereas the original study used the mean of 4 on-trial determinations.<sup>2</sup> Random measurement variability, which is partially offset by averaging multiple determinations, would be expected to attenuate associations with clinical outcomes.

In summary, this case-control substudy nested within VA-HIT shows that fibrate therapy produces favorable changes in the numbers of plasma LDL and HDL subclass particles that can occur independently of any change in the cholesterol content of these lipoproteins. These lipoprotein particle changes may help to explain the reduction in major CHD events and CHD death achieved in this trial. We believe this demonstration of a significant beneficial effect of fibrate therapy in a population with low LDL-C should provide impetus for considering the potential benefit of this kind of therapy even in the absence of substantial changes in conventional lipid measures.

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### Disclosures

Drs Otvos and Shalaurova are employees of LipoScience, Inc. Dr Schaefer has been a consultant to LipoScience, Inc. The other authors report no conflicts.

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### CLINICAL PERSPECTIVE

A highly prevalent lipid abnormality in patients with coronary heart disease (CHD) is low high-density lipoprotein cholesterol (HDL-C) coupled with a relatively low low-density lipoprotein cholesterol (LDL-C). The Veterans Affairs HDL Intervention Trial (VA-HIT) has been one of the few clinical trials to address specifically the risk associated with low HDL-C in conjunction with low LDL-C. Men with established CHD and low levels of both HDL-C and LDL-C were treated with the fibric acid derivative gemfibrozil, which raised HDL-C and lowered triglycerides without affecting levels of LDL-C. This treatment resulted in a significant reduction in major CHD events, a benefit only partially explained by the HDL-C increase and triglyceride decrease. In this case-control substudy of VA-HIT, we used NMR spectroscopy to investigate the effects of gemfibrozil on numbers of LDL and HDL subclass particles. Despite having no effect on LDL-C, gemfibrozil markedly increased the size of LDL particles and reduced their overall number. LDL-C levels were unrelated to CHD events, but LDL particle numbers at baseline and during the trial were strong, independent predictors of a new CHD event. LDL particle size, in contrast, was not related to events. Total HDL particle numbers and numbers of the small HDL particle subclass were increased by gemfibrozil, and on-trial concentrations of both of these HDL particle parameters predicted CHD events. In summary, gemfibrozil produced favorable changes in both LDL and HDL particle numbers that were not reflected by changes in the cholesterol content of these lipoproteins but that predicted a significant reduction in CHD events.