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Relation of gemfibrozil treatment and high-density lipoprotein subpopulation profile with cardiovascular events in the Veterans Affairs High-Density Lipoprotein Intervention Trial

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Abstract

The significant cardiovascular disease (CVD) event reduction in the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) could not be fully explained by the 6% increase in high-density lipoprotein (HDL) cholesterol with the fibrate gemfibrozil. We examined whether measurement of HDL subpopulations provided additional information relative to CVD risk reduction. The HDL subpopulations were characterized by 2-dimensional gel electrophoresis in subjects who were treated with gemfibrozil (n = 754) or placebo (n = 741). In this study, samples obtained at the 3-month visit were used; and data were analyzed prospectively using CVD events (coronary heart disease death, myocardial infarction, or stroke) during the 5.1 years of follow-up. Analyses in the gemfibrozil arm showed that subjects with recurrent CVD events had significantly higher preβ-1 and had significantly lower α-1 and α-2 HDL levels than those without such events. Preβ-1 level was a significant positive predictor; α-1 and α-2 levels were significant negative risk factors for future CVD events. α-2 level was superior to HDL cholesterol level in CVD-risk assessment after adjustment for established risk factors. Gemfibrozil treatment was associated with 3% to 6% decreases in the small, lipid-poor preβ-1 HDL and in the large, lipid-rich α-1 and α-2 HDL and with increases in the small α-3 (3%) and preα-3 (16%) HDLs. Although the use of gemfibrozil has been associated with reduction in CVD events in VA-HIT, HDL subpopulation analysis indicates that gemfibrozil-mediated improvement in CVD risk might not be the result of its effects on HDL. It is quite possible that much of the cardiovascular benefits of gemfibrozil are due to a much wider spectrum of effects on metabolic processes that is not reflected by changes in blood lipids and HDL subpopulations.

1. Introduction

The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) demonstrated that gemfibrozil therapy significantly reduced the 5-year incidence of major coronary heart disease (CHD) events and produced a significant reduction in stroke in men with known CHD and low high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels [1]. High-density lipoprotein cholesterol was raised by 6% with gemfibrozil, and this increase partially predicted the reduction in CHD events [2]. Participants in VA-HIT had a high prevalence of diabetes and the features of the metabolic syndrome. The VA-HIT provided an opportunity for investigating the relationship between HDL-related parameters and cardiovascular risk in a well-characterized cohort of CHD patients selected with low HDL-C and low LDL-C levels.

High-density lipoprotein is a heterogeneous class of lipoprotein particles with subspecies that differ in apolipoprotein and lipid composition, size, density, and charge. The different subspecies of HDL appear to have different physiologic functions [3-6]. In the last decade, several methods have been developed to identify specific HDL subspecies and

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explore their role in the etiology of cardiovascular disease (CVD) including quantitative nondenaturing 2-dimensional (2D) gel electrophoresis and image analysis [7,8]. Apolipoprotein (apo) A-I–containing HDL subpopulations were assessed in a subset (n = 1495) of VA-HIT by 2D gel electrophoresis, which characterizes plasma HDL subpopulations by electrophoretic mobility (preβ, α, and preα mobility) and size (4-17 nm). The apo A-I content in the different particles was quantitatively determined by image analysis. It was demonstrated that those subjects who developed new CVD events had higher concentrations of apo A-I in the poorly lipidated, small α-3 and preβ-1 HDL particles and less apo A-I in the more lipidated and larger α-1 and α-2 HDL particles as compared with subjects who did not develop such events [9]. Indeed, for every 1–standard deviation (SD) increase in α-1 and HDL-C, the hazard for new CVD events decreased by 18% and 15%, respectively.

The present analysis was performed to assess the effects of gemfibrozil treatment on the apo A-I–containing HDL subpopulations in a subset of VA-HIT to determine whether the reduction in CVD events in this trial could be related to a change in the HDL subpopulation profile that was a consequence of gemfibrozil therapy.

2. Methods

2.1. Study population

The VA-HIT study design and population have been described in detail [10]. Briefly, men were recruited at 20 VA medical centers throughout the United States. Eligibility for the trial required a documented history of CHD (including previous myocardial infarction [MI], coronary revascularization, or angiographic evidence of stenosis >50% of the luminal diameter in ≥1 major epicardial coronary artery), an absence of serious coexisting conditions, an HDL-C level ≤40 mg/dL (1 mmol/L), an LDL-C level ≤140 mg/dL (3.6 mmol/L), a triglyceride (TG) level ≤300 mg/dL (3.4 mmol/L), and an age <74 years. Participants in VA-HIT were randomly assigned to 2 groups receiving either gemfibrozil or matching placebo treatment. In these analyses, all subjects with recurrent CVD events (CVD+) (CHD death, MI, or stroke) were included in both arms; and subjects without recurrent events (CVD−) were randomly selected in numbers based on power calculations. The HDL subpopulations were determined in 741 (n = 230 CVD+) subjects in the placebo and in 754 (n = 168 CVD+) subjects in the gemfibrozil arm. All measurements were performed in samples obtained at the 3-month visit, and subjects were followed for an average of 5.1 years. We had a prospective study design; therefore, the selection of an early time point (3 months) provided a long follow-up and better compliance.

2.2. Laboratory measurements

Total cholesterol, TG, and HDL-C concentrations were determined by standard enzymatic methods. High-density lipoprotein cholesterol was isolated from the supernatant after dextran sulfate–magnesium precipitation. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula. Total plasma apo A-I concentrations were measured with a turbidimetric immunoassay (Wako Diagnostics, Richmond, VA) on a Hitachi 911 analyzer (Hitachi, Tokyo, Japan). Apolipoprotein A-I–containing HDL subpopulations were determined by 2D nondenaturing gel electrophoresis, immunoblotting, and image analysis as described [7,11]. Apolipoprotein A-I levels in the individual HDL subpopulations were calculated by multiplying plasma apo A-I levels by the subpopulation percentiles. Because each HDL particle has a fixed number of apo A-I molecules, the change in apo A-I levels in each HDL subpopulation is proportional to changes of particle numbers. The inter- and intraassay coefficients of variation were <4% for the lipid measurements and <10% for the apo A-I and HDL subpopulation determinations. All plasma samples were stored at −80°C and were never thawed until analysis. The effects of long-term storage on HDL subspecies showed no significant changes in the values obtained after measurements of the same samples fresh and after short- and long-term storage [7].

2.3. Statistical analysis

Descriptive statistics including means ± SD for continuous variables or proportions for categorical variables were computed for all study variables: (1) in subjects in the placebo arm vs subjects in the gemfibrozil arm and (2) in subjects with and without new CVD events (CHD death, MI, or stroke) in the gemfibrozil arm. The distribution of the variables was compared using 2-sample *t* tests for continuous variables and χ^2 tests for categorical variables. Cox proportional hazard models were used to determine the hazard ratios (HRs) for new CVD events in follow-up (5.1 years) based on a 1-SD increase in lipid and HDL subpopulation variables in the gemfibrozil arm. These variables were divided into quartiles for further analysis, with the upper quartile compared with the lower quartile in Cox models adjusted for CHD risk factors (age, smoking, hypertension, body mass index [BMI], and diabetes). Cochran-Armitage trend tests were performed using the percentage of CVD events in each quartile. A 4-model approach was used to compare the risk of 1-SD increase in α-2 with HDL-C: in model 1, data were unadjusted; in model 2, data were adjusted for nonlipid CHD risk factors (age, smoking, hypertension, BMI, and presence of diabetes); in model 3, data were adjusted for lipid (HDL-C, LDL-C, and TG) and nonlipid risk factors; and in model 4, data were further adjusted for either α-2 for HDL-C or HDL-C for α-2. Finally, a receiver operating curve (ROC) analysis was performed using HDL-C, TG, and HDL subpopulations (preβ-1, α-1, α-2, and α-3) as variables in the gemfibrozil and in the placebo arm of the study, with a threshold of >0.75 set for significance.

The SAS statistical package version 9.1 (SAS, Cary, NC) was used in all analyses. Results with P values < .05 were considered statistically significant.

All measurements were conducted in a blinded fashion, and data were analyzed at the VA Cooperative Studies Coordinating Center (West Haven, CT). The study was approved and continually monitored by the subcommittee on human studies at Tufts University/New England Medical Center. All subjects gave written informed consent.

3. Results

None of the parameters were different at baseline between the 2 arms because the gemfibrozil and placebo arms were evenly matched for clinical characteristics and laboratory values at baseline in VA-HIT [2]. Therefore, we have compared the gemfibrozil arm to the placebo arm at the 3-month visit to assess the influence of gemfibrozil on the measured variables in this substudy (Table 1). The average age of the subjects was 64 years; about 35% of them had diabetes, and about 60% had hypertension. Subjects who received gemfibrozil treatment had higher mean HDL-C (6%, P < .001) and lower mean TG (−33%, P < .001) levels than subjects who received placebo. Subjects receiving gemfibrozil had lower mean preβ-1 (−6%, P < .05), α-1 (4.5%, not significant), and α-2 (−3%, P < .05) and higher mean α-3 (3%, P < .05) and preα-3 (16%, P < .001) HDL subpopulation levels than those receiving placebo.

Table 1
Characteristics of subjects after 3 months of treatment with placebo or gemfibrozil in VA-HIT

Variable	Placebo	Gemfibrozil
	n = 741	n = 754
Age (y)	64.2 ± 7.0	64.0 ± 7.4
BMI (kg/m^2)	29.4 ± 4.7	29.1 ± 5.0
Hypertension (%)	59.9	56.8
Diabetes (%)	35.0	34.5
Smoking (%)	17.8	19.1
Total cholesterol	175.5 ± 27.0	166.8 ± 27.7 *
TG [a]	163.7±76.6	109.5 ± 58.7 *
LDL-C	111.6 ± 25.0	111.6 ± 25.2
HDL variables		
HDL-C	31.5 ± 5.7	33.3 ± 6.4 *
Apo A-I	109.5 ± 17.6	109.6 ± 17.8
Preβ-1	12.7 ± 6.6	12.0 ± 6.4 **
Preβ-2	2.1 ± 1.3	2.2 ± 1.4
α-1	9.0 ± 5.0	8.6 ± 4.8
α-2	31.8 ± 8.3	30.8 ± 8.5 **
α-3	40.3 ± 9.9	41.4 ± 9.8 **
Preα-1	2.8 ± 2.3	2.9 ± 2.5
Preα-2	5.2 ± 2.3	5.4 ± 2.4
Preα-3	5.5 ± 2.2	6.4 ± 2.5 *

Values are mean ± SD (in milligrams per deciliter) or as indicated. Conversion factor from milligrams per deciliter to millimoles: for cholesterol, divide numbers by 38.88; for TG, divide numbers by 86.88.
[a] Statistical test performed using log-transformed values.
* P < .001
** P < .05.

Table 2
Characteristics of subjects in the gemfibrozil arm with and without new CVD events (nonfatal MI, CHD death, and stroke) in the 5.1-year follow-up

Variable	CVD (+)	CVD (−)
	n = 168	n = 586
Age (y)	64.8 ± 7.0	63.7 ± 7.5
BMI (kg/m^2)	29.0 ± 5.0	29.1 ± 5.0
Hypertension (%)	63.0	54.9
Diabetes (%)	38.7	33.3
Smoking (%)	23.8	17.7
Total cholesterol	169.2 ± 27.5	166.2 ± 27.8
LDL-C	113.0 ± 24.5	111.2 ± 25.4
TG [a]	119.2 ± 69.9	106.8 ± 54.8
HDL variables		
HDL-C	32.5 ± 6.0	33.6 ± 6.4
Apo A-I	107.5 ± 16.9	110.2 ± 18.0
Preβ-1	13.3 ± 7.0	11.6 ± 6.1 *
Preβ-2	2.0 ± 1.3	2.3 ± 1.4 **
α-1	7.9 ± 4.3	8.8 ± 4.9 **
α-2	28.9 ± 8.2	31.4 ± 8.5 *
α-3	41.7 ± 9.1	41.3 ± 10.0
Preα-1	2.7 ± 2.3	2.9 ± 2.5
Preα-2	5.0 ± 2.4	5.5 ± 2.4 **
Preα-3	6.1 ± 2.4	6.5 ± 2.5

Values are mean ± SD (in milligrams per deciliter) or as indicated.
[a] Statistical test performed using log-transformed values.
* P < .01.
** P < .05.

Table 2 compares subjects with new CVD events to those without such events in the gemfibrozil arm of the study. Among the 754 subjects studied in the gemfibrozil arm, 168 subjects experienced an MI, stroke, or CHD death in the 5.1-year follow-up. There were no significant differences in the measured lipid parameters between the 2 groups; however, there were significant differences in the mean concentrations of several HDL subpopulations: subjects with new CVD events had higher level of preβ-1 (14%, P < .01) and lower levels of preβ-2 (−13%, P < .05), α-1 (−10%, P < .05), α-2 (−8%, P < .01), and preα-2 (−9%, P < .05) HDL subpopulations than those without new CVD events.

Cox proportional hazard models were used to determine the HRs for new CVD events in follow-up for 1-SD unit increase in the measured parameters in the gemfibrozil arm of the study (Table 3). These analyses indicated that HDL-C (HR = 0.83, P < .01), TG (HR = 1.21, P < .008), preβ-1 (HR = 1.18, P < .01), preβ-2 (HR = 0.80, P < .01), and α-2 (HR = 0.81, P < .01) were independent predictors of new CVD events after adjusting data for established nonlipid CHD risk factors (age, smoking, hypertension, BMI, and diabetes). High-density lipoprotein cholesterol lost power (P = .23) to predict new CVD events when data were further adjusted for LDL-C and logTG (Table 4, model 3). α-2 HDL lost power (P < .06) to predict new CVD events after data were adjusted for the above parameters as well as HDL-C (Table 4, model 4).

Table 3
Hazard ratios for lipids, apo A-I, and HDL subpopulations in predicting cardiovascular end points (n = 168) in the gemfibrozil arm (n = 754)

	Unadjusted analysis		Adjusted analysis[a]	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Total cholesterol	1.09 (0.94-1.26)	.24	1.13 (0.95-1.33)	.13
LDL-C	1.05 (0.91-1.22)	.53	1.08 (0.93-1.26)	.33
TG	1.20 (1.05-1.38)	.008	1.21 (1.05-1.38)	.008
HDL variables				
HDL-C	0.81 (0.70-0.93)	.003	0.83 (0.72-0.96)	.01
Apo A-I	0.88 (0.75-1.03)	.12	0.88 (0.75-1.03)	.12
Preβ-1	1.19 (1.05-1.36)	.008	1.18 (1.04-1.35)	.01
Preβ-2	0.81 (0.68-0.96)	.02	0.80 (0.67-0.95)	.01
α-1	0.85 (0.72-1.01)	.06	0.86 (0.72-1.01)	.07
α-2	0.79 (0.68-0.93)	.004	0.81 (0.69-0.96)	.01
α-3	1.02 (0.88-1.19)	.79	0.99 (0.86-1.16)	.95
Preα-1	0.93 (0.79-1.09)	.38	0.93 (0.79-1.10)	.42
Preα-2	0.85 (0.72-0.99)	.05	0.88 (0.75-1.04)	.13
Preα-3	0.88 (0.75-1.03)	.11	0.88 (0.75-1.04)	.12

Hazard ratios were estimated using Cox proportional hazard models. *Cardiovascular end points* were defined as stroke, nonfatal MI, or CHD death. The 95% confidence interval is given 1 SD unit for each variable. CI indicates confidence interval.

[a] Data were adjusted for age, smoking, hypertension, BMI, and diabetes.

The relationship between HDL subpopulation quartiles and CVD event rate is shown in Table 5. Subjects with the lowest preβ-1 level had 47% lower relative risk (RR) for recurrent CVD events than subjects with the highest preβ-1 level (RR = 1.95, P = .005). In contrast, subjects with the highest levels of preβ-2 (57%, RR = 0.55, P = .02), α-2 (83%, RR = 0.57, P = .002), and preα-2 (70%, RR = 0.58, P = .02) had lower RR than subjects with the lowest levels of these parameters. The Cochran-Armitage trend test indicated a positive association between recurrent CVD events and preβ-1 (P = .003) and an inverse trend for preβ-2 (P = .04), α-1 (P = .05), α-2 (P = .0002), and preα-2 (P = .03).

The ROC curve analysis using HDL-C, TG, and the major HDL subpopulations (preβ-1, α-1, α-2, and α-3) as variables did not distinguish between subjects with and without new CVD events either in the placebo or in the gemfibrozil arm. None of the *c* values (area under the curve) were higher than 0.580 (0.75 is the minimum for a positive threshold effect [data not shown]).

4. Discussion

The VA-HIT was the first lipid intervention trial to test whether increasing HDL-C concentrations in men selected with established CHD, low LDL-C levels, and low HDL-C levels decrease CVD risk [2]. The VA-HIT investigators concluded that the gemfibrozil-mediated reduction (22%) in new coronary events was partly dependent on an HDL-C increase because the benefit was independent of changes in the concentration of TGs or LDL-C as well as other major risk factors [1]. In contrast to VA-HIT, in the Fenofibrate Intervention in Event Lowering in Diabetes trial, only a nonsignificant 11% reduction in coronary events was observed [12]. As pointed out in a recent review, there have been substantial differences in results of fibrate trials; and all fibrates may not have equivalent clinical benefit [13].

We examined whether measurement of HDL subpopulations by 2D gel electrophoresis provided additional information relative to CVD-risk reduction by gemfibrozil in the VA-HIT arm alone. Gemfibrozil treatment was associated with 3% to 6% decreases in the small, lipid-poor preβ-1 HDL and in the large, lipid-rich α-1 and α-2 HDL and with increases in the small α-3 (3%) and preα-3 (16%) HDLs.

Data generated in this study are in agreement with previous assessments of HDL subfractions: increases in HDL_3-C but not in HDL_2-C estimated after separation by differential polyanion precipitation were significantly related to the development of new CVD events in VA-HIT [2]. In a subset of VA-HIT, HDL particle number was assessed by nuclear magnetic resonance (NMR), which indicated a 10% increase in total HDL particle number and a 21% increase in the number of the small HDL subclasses in the gemfibrozil arm compared with the placebo arm [14]. High-density lipoprotein subpopulation analysis by 2D gel electrophoresis revealed differences in the effects of gemfibrozil on the small, lipid-poor HDL particles, which cannot be differentiated by polyanion precipitation or by NMR. Among the 3 varieties of small HDL particles (preβ-1, α-3, and preα-3), only preβ-1 concentration was significantly lower, whereas the concentrations of α-3 and preα-3 were significantly higher in the gemfibrozil arm compared with the placebo arm. Data generated by polyanion precipitation and NMR cannot be directly compared with data generated by 2D gel electrophoresis because the former methods measure lipid content and the latter one measures apo A-I in HDL. However, we have compared ultracentrifugally separated HDL subclasses (HDL2 and HDL3) with 2D gel electrophoresis [7]. We have shown that HDL3 was a composite of α-2– and the small α-3– and preβ-1–sized particles; HDL2 was composed mainly of the large α-1 and preα-1 particles.

Table 4
Hazard ratios as calculated for α-2 HDL and HDL-C in predicting cardiovascular end points (n = 168) in the gemfibrozil arm (n = 754)

Model[a]	HR for each 1-SD increase in α-2 (8.51)		HR for each 1-SD increase in HDL-C (6.37)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Model 1	0.79 (0.68-0.93)	.004	0.81 (0.70-0.93)	.003
Model 2	0.81 (0.69-0.96)	.01	0.83 (0.72-0.96)	.01
Model 3	0.83 (0.71-0.98)	.03	0.90 (0.76-1.07)	.23
Model 4	0.82 (0.66-1.01)	.06	0.93 (0.76-1.14)	.50

Hazard ratios were estimated using Cox proportional hazard models. *Cardiovascular end points* were defined as stroke, nonfatal MI, or CHD death. Confidence interval is given 1 SD unit in α-2 (8.51) and HDL-C (6.37).

[a] Model 1: data were unadjusted; model 2: data were adjusted for nonlipid CHD risk factors (age, smoking, hypertension, BMI, and diabetes); model 3: data were further adjusted for LDL-C and logTG; model 4: for α-2, data were further adjusted for HDL-C; and for HDL-C, data were further adjusted for α-2.

Table 5
Quartile analysis for evaluating the RR for recurrent CVD events (percentage) in the gemfibrozil arm of VA-HIT

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	RR (95% CI)	P[a]	Trend test[b] P =
	% New CVD events						
Preβ-1[c]	14.9	22.2	24.1	27.9	1.95 (1.22-3.11)	.005	.003
Preβ-2	23.4	26.6	24.2	14.9	0.55 (0.33-0.89)	.02	.04
α-1	24.9	25.5	21.3	17.5	0.70 (0.44-1.11)	.13	.05
α-2	29.1	27.7	16.5	15.9	0.57 (0.36-0.90)	.02	.0002
α-3	19.1	23.4	24.5	22.2	1.03 (0.66-1.63)	.89	.43
Preα-1	24.9	20.7	26.5	17.0	0.69 (0.37-1.09)	.11	.19
Preα-2	28.0	20.0	24.6	16.5	0.58 (0.37-0.92)	.02	.03
Preα-3	23.8	27.7	18.0	19.7	0.80 (0.52-1.25)	.33	.10

n = 754 including 168 subjects with recurrent CVD events (stroke, nonfatal MI, or CHD death).

[a] P values for RR were calculated by comparing data in quartile 4 with data in quartile 1. Data were adjusted for age, smoking, hypertension, BMI, and diabetes.

[b] Cochran-Armitage trend test.

[c] The trend between particle concentrations and recurrent CVD events is positive.

Preβ-1 promotes cellular phospholipids and cholesterol efflux via the adenosine triphosphate–binding cassette transporter A1 metabolic pathway, and these additional lipids transform preβ-1 (mean diameter, 5.4 nm) into more lipidated α-3 HDL particles (mean diameter, 6.7 nm) [15]. The fractional catabolic rate of apo A-I is inversely correlated with HDL particle size [16]; thus, transformation of preβ-1 into α-3 increases HDL residence time. In case-control studies, high preβ-1 level is associated with prevalent CHD [17]. High preβ-1 level was a significant predictor for recurrent CVD events in subjects in the placebo and gemfibrozil arms combined [9] as well as in the gemfibrozil arm alone. We hypothesize that the gemfibrozil-mediated transformation of preβ-1 into α-3 is beneficial if the newly formed α-3 can further maturate into more lipidated α-2 and α-1 HDL particles, which promote selective cholesterol uptake in the liver via the scavenger receptor class B type 1 pathway [15,18]. However, in this study, concentrations of the large, cholesterol-rich particles (α-1 and α-2) were lower in the gemfibrozil arm than in the placebo arm, indicating a defect in full maturation of HDL particles. Previously, we have reported that a low α-1 level was a significant predictor for first CHD events in male participant of the Framingham Offspring Study and that a low level of α-2 was a significant predictor for recurrent CVD events in subjects in the combined arms of VA-HIT [8,9].

Using NMR, Otvos et al [14] have reported that gemfibrozil-mediated increase in small HDL particle number was significantly associated with decreased CVD events in a subgroup of VA-HIT. In our assessment, only α-3 and preα-3 concentrations increased among small HDL particles, which have been shown to be positively associated with CVD risk [7-9]. These seemingly conflicting results make it difficult to explain the beneficial effects of gemfibrozil on CVD risk by its effects on HDL. Moreover, an ROC curve analysis indicated that neither HDL-C, TG, nor the major HDL subpopulation levels distinguished between subjects with new events and subjects without new events because none of the c values reached the minimum of 75% for positive threshold effect.

Our findings suggest that gemfibrozil has a significantly different effect on the HDL subpopulation profile than do other lipid-modifying drugs we have investigated (statins and nicotinic acid) [19-21]. Statins and nicotinic acid significantly decrease preβ-1 levels and significantly increase α-1 and α-2 HDL levels in subjects with CHD, resulting in a shift in the HDL subpopulation profile toward normal. In the HDL-Atherosclerosis Treatment Study, we [19] have documented that the increase in α-1 level was significantly correlated with the decrease in coronary artery stenosis. We [20,21] and others [22] have similarly found that statins increase HDL size or, more specifically, α-1 concentrations and suggested that this might be a consequence of a reduction in TG concentrations that could, in turn, decrease cholesteryl ester transfer protein (CETP) activity [22]. Data on subjects treated with a specific CETP inhibitor clearly support the above statement [23]. In contrast, gemfibrozil treatment was associated with decreases in the large (α-1 and α-2) and increases in the small, lipid-poor HDL particles (α-3 and preα-3) despite a 33% reduction in TG, suggesting that a decrease in TG levels is not necessarily accompanied by a decrease in CETP activity and an increase in the concentration of large HDL particles. This assumption is supported by reports on fibrate mechanism indicating that gemfibrozil did not significantly decrease CETP [24].

Based on this and previous studies, we believe that several HDL subpopulations are involved in the development of CVD but probably via different mechanisms. High-density lipoprotein particles may possess several potentially atheroprotective properties, not necessarily distributed evenly among the different HDL particles. The most important and experimentally verified atheroprotective functions of HDL are (1) mediating reverse cholesterol transport, (2) inhibiting LDL oxidation, (3) improving endothelial function, and (4) decreasing inflammation in the vessel wall. More studies are needed to elucidate the effects of gemfibrozil on other

possibly beneficial changes in HDL function, as fibrates have been shown to act as anti-inflammatory agents by inhibiting the nuclear factor–κB (NF-κB) inflammatory cascade [25], which is the master regulator of production of several proinflammatory proteins. Peroxisome proliferator–activated receptor α, which is up-regulated by gemfibrozil, binds to the P65 unit of NF-κB and inhibits the translocation of NF-κB into the nucleus; therefore, NF-κB cannot activate genes of proinflammatory proteins [26]. Moreover, fibrates improve coagulation and fibrinolysis [27,28], increase LDL size in diabetic patients [29], and increase insulin sensitivity by decreasing free fatty acid production by decreasing NF-κB–mediated lipoprotein lipase production.

These data confirm that apo A-I–containing HDL subpopulations are related to CVD risk, but do not support earlier analyses of the same subjects that HDL played a significant role in the gemfibrozil-mediated CVD risk reduction. It is quite possible that much of the cardiovascular benefits of gemfibrozil are due to the considerable decrease in small dense LDL [14] concentrations or a much wider spectrum of effects on metabolic processes that is not reflected by changes in blood lipids and HDL subpopulations, such as decreases in inflammation.

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