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Original Articles

Fenofibrate Effectively Reduces Remnants, and Small Dense LDL, and Increases HDL Particle Number in Hypertriglyceridemic Men — A Nuclear Magnetic Resonance Study

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Hypertriglyceridemia is often associated with small dense low density lipoprotein (LDL), elevated remnants, and decreased high density lipoprotein (HDL)-cholesterol (C), which comprise the dyslipidemic triad. The objective of this study was to investigate the effect of fenofibrate on the lipoprotein subfraction profile and inflammation markers in hypertriglyceridemic men. Twenty hypertriglyceridemic men were administered fenofibrate, 200 mg daily, for 8 weeks. Lipoprotein subclasses were measured by nuclear magnetic resonance (NMR) spectroscopy. Inflammation markers including C-reactive protein (CRP), interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) were also determined. Fenofibrate lowered triglyceride (TG) by 58% and increased HDL-C by 18%. NMR analysis revealed that very low density lipoprotein (VLDL), particularly large VLDL, intermediate density lipoprotein (IDL), and small LDL, were significantly decreased, and LDL distribution shifted towards the larger particles. HDL distribution was altered; there was an increase in small HDL and a decrease in large HDL, resulting in a significant decrease in HDL particle size, from 9.1 to 8.9 nm, as well as a 27% increase in HDL particle number. Among inflammation markers, CRP was significantly decreased by 42%. In conclusion, fenofibrate effectively improves atherogenic dyslipidemia by reducing remnants and small LDL, as well as by increasing HDL particles. These effects, together with the favorable effect on inflammation, might provide a clinical benefit in hypertriglyceridemic subjects. *J Atheroscler Thromb, 2004; 11: 278–285.*

Key words: Fenofibrate, Lipoprotein, Nuclear magnetic resonance, Inflammatory marker

Introduction

Besides elevated low density lipoprotein (LDL)-cholesterol (C), there is a growing body of evidence that small dense LDL, increased triglyceride (TG)-rich lipoproteins (remnants), and decreased high density lipoprotein (HDL)-C comprise a new cluster of atherogenic dyslipidemia. This type of dyslipidemia, called the dyslipidemic triad, is often associated with abdominal obesity, insulin resistance, and more importantly an increase in the risk for coronary artery disease (CAD) (1).

Fenofibrate, one of the fibrate derivatives, has been considered to be highly effective in treating hypertriglyceridemia. In addition to previous interventional studies in which fibrate derivatives reduced the risk in CAD patients (2, 3), fenofibrate reduced the progression of CAD in diabetic patients with high TG and low HDL-C levels (4). Effects of fenofibrate on lipoproteins are characterized as decreased remnants, increased HDL-C, and altered

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LDL particle size in favor of reducing small dense LDL. While previous studies have all employed laborious ultracentrifugation and gradient gel electrophoresis methodologies to quantify lipoprotein subclasses, a new technology utilizing proton nuclear magnetic resonance (NMR) has drawn more attention recently (5–7).

The current concept of atherosclerosis stresses an important role of inflammation (8). Fenofibrate has been shown to suppress inflammation, as evidenced by the reduction of CRP (9, 10). Staels *et al.* (9) demonstrated that fenofibrate suppressed IL-6, an effect attributable to the activation of peroxisome proliferator-activated receptor (PPAR)-α with a consequent reduction of NF-$\kappa\beta$ activation.

Therefore, in the present study, we investigated the effects of fenofibrate on lipoprotein subclass distribution as analyzed by NMR, and on inflammatory markers in hypertriglyceridemic men.

Methods

Twenty hypertriglyceridemic men were recruited for this study from an outpatient clinic of Jikei University School of Medicine Hospital. Eligibility criteria included age between 30 and 80 years, body mass index (BMI) less than 30 kg/m^2, and fasting TG values $\geq$ 150 mg/dl both at screening and at the end of the run-in period. None of the subjects had poorly controlled diabetes or evidence of thyroid, liver, or renal dysfunction (creatinine > 2mg/dl), and none were taking lipid-modifying medications. With regard to apoE phenotype, 14 patients were E3/3, 4 were E4/3, and 1 each was E3/2 and E4/4, respectively. All subjects were instructed to maintain their diet, exercise, and alcohol intake throughout the study period, including the run-in period. The study was approved by the Ethics Committee of Jikei University School of Medicine. All subjects gave written informed consent to the study protocol.

After a 4–8-week run-in period, the patients received fenofibrate at 200 mg daily for 8 weeks. Fasting blood samples (12-hour overnight) were drawn from each subject at the end of the run-in period and after 8-week fenofibrate treatment, into tubes containing N_2EDTA at a final concentration of 0.1%. Plasma was immediately separated by centrifugation (3,000 g, 20 mins at 4°C), then plasma concentrations of total cholesterol (TC), TG, and HDL-C (11) were freshly measured by the automated enzymatic technique using a Toshiba TBA-80FR autoanalyzer. LDL-C was directly measured using a homogeneous enzymatic assay (12) from Daiichi Pure Chemicals (Tokyo, Japan). Plasma apoliporotein concentrations were quantified using immunoturbidometric assays (13). Remnant-like particle (RLP)-C was measured by an immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan) as reported previously (14). Aliquots of plasma were stored at – 80°C for the following assays.

Malondialdehyde-modified LDL (MDA-LDL) was measured by ELISA using monoclonal antibody (ML25) as previously described (15). Interleukin (IL)-6 and monocyte chemotactic protein (MCP)-1 levels were measured with an immunoassay using specific monoclonal antibodies (R&D Systems, Mineapolis, MN, USA). Measurement of CRP levels was obtained by high sensitivity latex-enhanced immunonephelometry on a Behring BN II analyzer (Dade Behring, Marburg, Germany) (16). Frozen plasma was shipped to LipoScience, Inc. (Raleigh, NC, USA) for proton NMR analysis as previously reported (5, 7). This new method is based on the signals emitted from methyl group protons of the lipids, which were then calculated by linear least-square method to quantify lipoprotein subclass lipid concentrations (TG for VLDL, C for IDL, LDL, and HDL subclasses).

A total of 16 subclasses were simultaneously measured: six VLDL subclasses (VLDL6 150 $\pm$ 70 nm, VLDL5 70 $\pm$ 10 nm, VLDL4 50 $\pm$ 10 nm, VLDL3 38 $\pm$ 3 nm, VLDL2 33 $\pm$ 2 nm, and VLDL1 29 $\pm$ 2 nm), 4 LDL subclasses (IDL 25 $\pm$ 2 nm, LDL3 22 $\pm$ 0.7 nm, LDL2 20.5 $\pm$ 0.7 nm, and LDL1 19 $\pm$ 0.7 nm), and 5 HDL subclasses (HDL5 11.5 $\pm$ 1.5 nm, HDL4 9.4 $\pm$ 0.6 nm, HDL3 8.5 $\pm$ 0.3 nm, HDL2 8.0 $\pm$ 0.2 nm, and HDL1 7.5 $\pm$ 0.2 nm). LDL and HDL particle concentrations (nanomoles per liter for LDL and μmoles per liter for HDL) were calculated as the sum of the subclass concentrations, including IDL for the former and the sum of HDL subclasses for the latter.

Average lipoprotein particle sizes were determined by weighting the mass percentage of each subclass by its diameter. The LDL sizes by NMR method were closely correlated with those by the gradient gel electrophoresis (GGE) method, but were smaller by 5 nm. This resulted in 20.5 nm being a good cutoff point for distinguishing individuals with pattern A (mainly large LDL) and those with pattern B (mainly small LDL), giving good agreement to the phenotyping by the original GGE method.

ApoE phenotype was determined by immunoblot using a specific goat anti-apoE polyclonal antibody as reported previously (17).

Among lipid parameters, TG showed a skewed distribution. Therefore, logarithmically transformed TG values were used for comparison. Changes in biochemical parameters with fenofibrate treatment were analyzed by the paired t-test. Distribution of LDL size (patterns A and B) was tested by a χ-square test. Pearson's correlation coefficients were calculated to examine the relationship between lipid parameters. A p value of less than 0.05 was considered to be significant. All statistical procedures were performed using SPSS software (version 9.1, SPSS Inc., Chicago, IL, USA).

Table 1. Characteristics of study subjects and changes in lipids with fenofibrate.

	Baseline	Fenofibrate	Change (%)
N	20		
Age (yrs)	52 ± 14		
BMI (kg/m^2)	25.5 ± 4.4		
Current smoker (%)	40		
Hypertension (%)	35		
Diabetes (%)	30		
TC (mg/dl)	232 ± 62	209 ± 37	– 10.0*
TG (mg/dl)	480 ± 251	200 ± 109	– 58.4††
HDL-C (mg/dl)	40.9 ± 7.3	48.3 ± 13.1	18.2*
LDL-C (mg/dl)	99.3 ± 33.8	100.1 ± 26.5	0.8
ApoA-I (mg/dl)	134 ± 20	143 ± 26	6.3†
ApoA-II (mg/dl)	29.0 ± 5.1	34.5 ± 6.9	18.9††
ApoB (mg/dl)	118 ± 34	107 ± 23	– 9.3*
ApoC-II (mg/dl)	9.8 ± 5.0	7.3 ± 2.4	– 26.1*
ApoC-III (mg/dl)	25.9 ± 7.9	19.0 ± 4.9	– 26.1††
ApoE (mg/dl)	7.5 ± 3.0	4.6 ± 1.0	– 38.9††
RLP-C (mg/dl)	36.9 ± 33.5	9.1 ± 5.0	– 75.4†

* $p < 0.05$, † $p < 0.01$ †† $p < 0.001$

Results

Baseline characteristics of the study subjects are summarized in Table 1. The study subjects were on average 52 years old and had BMI of 25.5 kg/m^2, thus representing mildly obese patients. The percentages of hypertension, diabetes, and current smokers were 35%, 30%, and 40%, respectively. All patients, except for two, were free from CAD as evidenced by clinical symptoms, electrocardiogram, or laboratory data abnormalities. The plasma lipid and lipoprotein concentrations at baseline and fenofibrate treatment are also listed in Table 1. The mean TC, TG, HDL-C, and LDL-C were 232, 480, 40.9, and 98 mg/dl, respectively. Fenofibrate significantly reduced TC and TG by 10.0% ($p < 0.05$) and 58.4% ($p < 0.001$), respectively, and significantly increased HDL-C by 18.2% ($p < 0.05$). LDL-C, however, remained unchanged (0.8%). Both apoA-I and apoA-II, major protein constituents of HDL, were significantly increased by 6.3 and 18.9%, respectively. The resulting apoA-I/apoA-II ratios were significantly decreased from 4.62 to 4.18, indicating that fenofibrate altered the HDL composition in favor of LpA-I: A-II predominance. ApoB was modestly but significantly decreased by 9.3%. ApoC-II, apoC-III, and apoE were all significantly decreased by 26 to 39%, a finding consistent with decreased TG-rich lipoprotein.

The effects of fenofibrate on lipoprotein subclasses evaluated by NMR are summarized in Table 2. Fenofibrate significantly reduced VLDL particle TG concentrations, particularly those of large VLDL (VLDL 5, 6), resulting in a significant reduction of mean VLDL particle size by 19%. Fenofibrate also significantly lowered IDL by 62.8%. Large (LDL3) and intermediate (LDL2) LDL were in-

Table 2. Effects of fenofibrate on lipoprotein subclass parameters by NMR.

	Baseline	Fenofibrate	Change (%)
VLDL (mg/dl triglyceride)			
VLDL6 (large)	48.8 ± 61.5	7.8 ± 11.9	– 84.0†
VLDL5	155.8 ± 117.6	53.7 ± 75.6	– 65.5†
VLDL4	97.8 ± 39.2	58.7 ± 30.5	– 39.9†
VLDL3	21.6 ± 40.9	10.9 ± 12.9	– 49.3
VLDL2	5.4 ± 7.4	7.7 ± 6.3	41.2
VLDL1 (small)	3.7 ± 5.2	7.2 ± 7.4	95.4
VLDL particle size (nm)	60.5 ± 11.9	49.0 ± 9.4	– 18.9††
LDL (mg/dl cholesterol)			
IDL	12.3 ± 9.8	4.6 ± 4.1	– 62.8†
LDL3 (large)	18.1 ± 37.8	35.6 ± 30.6	97.0*
LDL2	25.4 ± 40.4	39.5 ± 29.7	55.2
LDL1 (small)	64.8 ± 45.9	37.6 ± 31.3	– 42.0*
LDL particle size (nm)	19.7 ± 0.8	20.4 ± 0.7	3.8††
LDL particle number (nmol/l)	1567 ± 606	1396 ± 393	– 10.9
LDL phenotype§			
Pattern A (*n*)	4	9	$p = 0.088$
Pattern B (*n*)	16	11	
HDL (mg/dl cholesterol)			
HDL5 (large)	8.3 ± 4.5	6.3 ± 4.5	– 24.0*
HDL4	7.9 ± 4.2	11.3 ± 7.3	43.7*
HDL3	8.9 ± 5.1	10.2 ± 6.8	15.3
HDL2	14.9 ± 4.9	18.5 ± 4.3	24.0†
HDL1 (small)	0.9 ± 2.1	2.0 ± 2.8	109.5
HDL particle size (nm)	9.1 ± 0.3	8.9 ± 0.3	– 2.2†
HDL particle number (μmol/l)	27.4 ± 5.0	34.9 ± 6.8	27.2††

* $p < 0.05$, † $p < 0.01$ †† $p < 0.001$

§ The average LDL particle size of 20.5 nm as shown by NMR was used as the cutoff point for distinguishing individuals with pattern A (mainly large LDL) and those with pattern B (mainly small LDL).

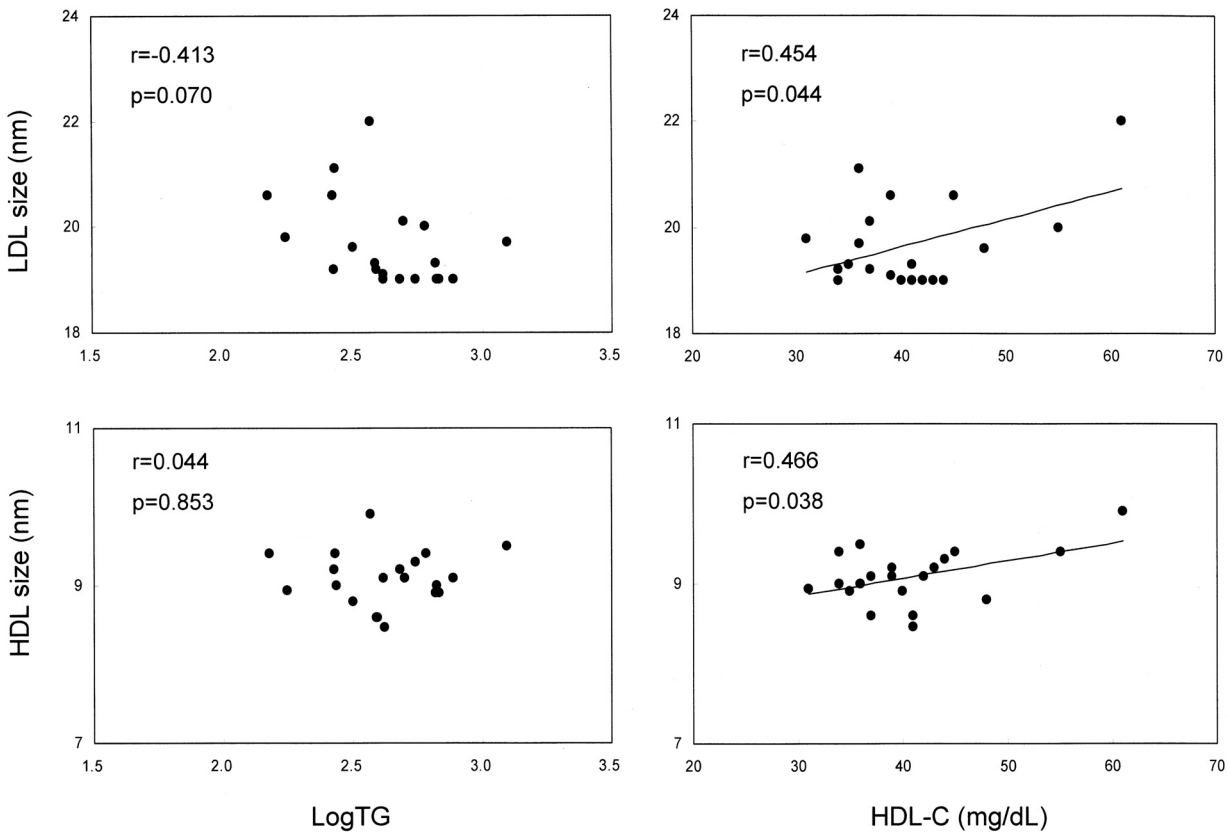

Fig. 1. Correlation between lipid parameters (LogTG and HDL-C) and particle sizes (LDL and HDL) in study subjects.

creased, but small LDL (LDL1) was significantly decreased by 42.0%, by fenofibrate treatment. This altered LDL subclass distribution resulted in a significant increase in particle size (19.7 to 20.4 nm) and a modest but non-significant reduction of LDL particle number. The former change is comparable to a trend toward a decrease in LDL phenotype pattern B (80% to 55%, $p = 0.088$). Among HDL, only HDL5 was significantly decreased, with mild to marked increases in other HDL subclasses, by fenofibrate. The average HDL particle was significantly decreased from 9.1 nm to 8.9 nm. This observation, together with a 19.9% increase in HDL-C, resulted in a 27.2% increase in HDL particle number ($p < 0.001$) by fenofibrate treatment.

Next, we correlated TG and HDL-C levels with LDL and HDL particle size to gain insight into possible roles of TG and HDL in lipoprotein particle size. As shown in Fig. 1, plasma log-transformed TG showed a trend toward negative correlation with LDL size ($r = -0.413$, $p = 0.07$), but not with HDL size. On the other hand, HDL-C level showed significant and positive correlations with both LDL and HDL particle size. We then associated changes in particle size with changes in lipid parameters by fenofibrate treatment (Table 3). Although marginally fail-

Table 3. Correlation coefficient between changes in LDL and HDL sizes, and those in lipid parameters by fenofibrate.

	Δ LDL size	Δ HDL size
	r	
Δ TC	0.169	0.223
Δ TG (LogTG)	– 0.222	– 0.011
Δ HDL-C	0.388	– 0.037
Δ LDL-C	0.182	0.193
Δ RLP-C	– 0.551*	– 0.061
Δ Large VLDL (VLDL5, 6)	– 0.369	0.098
Δ Intermediate VLDL (VLDL3, 4)	– 0.253	– 0.165
Δ Small VLDL (VLDL1, 2)	0.378	0.175
Δ IDL	– 0.426	– 0.113
Δ Large LDL (LDL3)	0.685†	– 0.210
Δ Intermediate LDL (LDL2)	0.281	0.465*
Δ Small LDL (LDL1)	– 0.761††	– 0.341
Δ Large HDL (HDL4, 5)	0.364	0.279
Δ Intermediate HDL (HDL3)	0.221	– 0.037
Δ Small HDL (HDL1, 2)	0.138	– 0.332

Δ; delta, * $p < 0.05$, † $p < 0.01$ †† $p < 0.001$

ing to reach a statistically significant level, the percentage change in LDL particle size was negatively correlated with that in TG, but positively with that in HDL-C. Interestingly, LDL particle size change was negatively correlated with change in RLP-C ($r = -0.551$, $p = 0.012$). The change in HDL particle size was not correlated with change in any lipid parameters, except LDL2 ($r = 0.465$, $p = 0.039$). Further, the response of large VLDL (VLDL5, 6) was positively correlated with responses of IDL ($r = 0.630$, $p = 0.003$) and RLP-C ($r = 0.685$, $p = 0.001$), both representing proatherogenic remnants.

Finally, the effects of fenofibrate on inflammation markers are summarized in Table 4. Although small LDL (LDL1) was significantly decreased by NMR analysis, neither MDA-LDL nor MDA-LDL/LDL-C was changed by fenofibrate treatment. Among inflammation markers, CRP showed a significant reduction (– 41.9%, $p = 0.04$), whereas IL-6 and MCP-1 remained unchanged by fenofibrate treatment.

Discussion

In the present study, we investigated the effects of 8 weeks of fenofibrate treatment on lipoprotein subclass distribution, evaluated by proton NMR spectroscopy, and on inflammation markers, in 20 hypertriglyceridemic male patients. Our results demonstrated that fenofibrate effectively improved atherogenic dyslipidemia by reducing remnants and small LDL, as well as by increasing the HDL particle number. Therefore, fenofibrate may have a clinical benefit in patients with elevated TG and decreased HDL-C levels, who are considered to be at increased risk for CAD. Furthermore, fenofibrate demonstrated an anti-inflammatory function, as evidenced by the significant reduction of CRP, potentially providing an additional benefit.

Fenofibrate is a potent PPAR-α activator, activating lipoprotein lipase activity and HDL apolipoprotein synthesis, and suppressing apoC-III synthesis (18). Several studies have focused on the effects of fenofibrate on lipoprotein distribution, demonstrating that it enlarged LDL (19–22) but reduced HDL particle size (23). These studies, however, all employed laborious ultracentrifugation or GGE methodologies to determine lipoprotein sizes and subclass concentrations. In this regard, it is worth commenting that we utilized a new technology, proton NMR, to analyze these effects in greater detail. This new method uses signals emitted from methyl groups of lipids (5, 24). The overall NMR signals from these lipids were computationally decomposed to yield 16 lipoprotein subclass concentrations, together with particle numbers and sizes. The NMR-determined lipoprotein subclass concentrations corresponded well to those by the established methods (25). We have previously demonstrated, using bezafibrate, findings similar to those in the present study (26).

Small dense LDL has drawn attention as a new member of independent risk factors for CAD (27). In this regard, LDL particle size can provide a better risk assessment of patients, as compared with LDL-C level alone. In this study, LDL phenotype pattern B was dominant (80%) prior to the fenofibrate treatment, as typically observed in hypertriglyceridemic patients, but was decreased to 55% at the end of the 8-week treatment, mirroring a significant decrease in small LDL (LDL1) as well as increases in intermediate (LDL2) and large LDL (LDL 3). This is consistent with the previous findings in which LDL particle size was evaluated by the GGE method (22, 28). However, the reduced small LDL was not accompanied by a reduction of MDA-LDL in this study. Enlargement of LDL is supposedly translated to be less atherogenic. Indeed, LDL size was directly associated with progression of CAD in the Diabetes Atherosclerosis Intervention Study (28). Although not measured in this study, Yoshida *et al.* demonstrated that gemfibrozil, another fibrate derivative, rendered *in vitro* susceptibility to oxidation (29). Therefore, the overall effect of fenofibrate on LDL should be considered by this factor, together with LDL-C change.

In the present study, we observed a favorable and potent effect of fenofibrate on HDL metabolism, as evidenced by the 18.2% increase in HDL-C levels, a finding consistent with numerous previous studies. NMR analysis further detailed this effect by demonstrating that fenofibrate markedly increased intermediate and small HDL subclasses, with the average HDL size being significantly decreased from 9.1 to 8.9 nm, thus confirming the previous observation by GGE (23). HDL remodeling in favor of smaller particle size seems comparable with the metabolic consequence of increased hepatic lipase activity (23). These small HDL are considered better acceptors for cholesterol as compared with larger counterparts, as evidenced by a finding that the free cholesterol effluxing capacity was markedly increased by fenofibrate treatment (30). Collective data, including kinetic studies,

Table 4. Effects of fenofibrate on MDA-LDL concentration and inflammation markers.

	Baseline	Fenofibrate	Change (%)
MDA-LDL (U/l)	100.7 ± 35.7	99.8 ± 46.0	– 0.9
MDA-LDL/LDL-C (U/mg/10^{-1})	1.113 ± 0.561	1.027 ± 0.616	– 7.7
CRP (mg/l)	1.25 ± 1.46	0.73 ± 0.57	– 41.9*
IL-6 (pg/ml)	2.34 ± 2.72	1.91 ± 1.45	– 18.6
MCP-1 (pg/ml)	176 ± 100	165 ± 91	– 5.9

* $p < 0.05$

indicate that increased HDL-C by fenofibrate is likely due to an increased production of apoA-I (31, 32), together with decreased cholesteryl ester transfer protein activity (19) and increased ABCA1 (33). Overall, its observed alteration of HDL makes fenofibrate an ideal agent for strengthening the anti-atherogenic properties of HDL, when used in hypertriglyceridemic patients.

Another major finding was the marked reduction of large VLDL and IDL, shown by NMR analysis. The latter observation was further supported by the significant reduction of RLP-C, a clinical parameter of remnants. The underlying metabolic mechanisms for these reductions are likely due to the increased catabolic rate, as consistently demonstrated by two recent kinetics studies (31, 32). Although TG-rich lipoproteins are heterogeneous in size and composition, whereby in association with atherosclerosis, large VLDL, which possesses the highest capacity for inducing macrophage lipid loading, is preferentially reduced by fenofibrate (34).

The correlation study revealed findings which are worth noting. Although TG level has been established as being negatively correlated with LDL size, HDL-C level was a stronger determinant of LDL and HDL particle size as compared with TG level at baseline. The exact reason for this discrepancy is not clear at present. The association of changes in particle sizes with those in lipid parameters, however, was more evident in LDL size; they were negatively and positively correlated with TG and HDL-C change, respectively, thus confirming results of previous studies (22, 35). The changes in HDL size did not show any meaningful association with those in lipid parameters. Although this may simply be due to a greater percentage of change in LDL size (3.8%) than in HDL size (2.2%), other factors, including baseline and percentage changes in lipids and study duration should be taken into consideration. Finally, the positive correlation of the changes in large VLDL (VLDL5, 6) with the changes in IDL and RLP-C was interpreted as indicating that the precursor of remnants was large VLDL, not small VLDL, in our hypertriglyceridemic subjects.

In this study, several inflammation markers were monitored, revealing a significant reduction of CRP by fenofibrate treatment. This effect on CRP compares favorably with results in previous studies (9, 10). Staels *et al.* (9) demonstrated that fenofibrate suppressed IL-6, an effect attributable to the activation of PPAR-α, with a consequent reduction of NF-$\kappa\beta$ activation. This mechanism was not fully exercised in this study, since the IL-6 levels remained unchanged. This could be due to the relatively small sample size of this study, thus necessitating a future study.

There were some limitations in the present study. First, this study lacked a control group to which the fenofibrate group could be compared. We therefore installed a run-in period to maintain a steady state throughout the study period. Nonetheless, a placebo-controlled study should be performed to draw a solid conclusion in the future. Second, the study subjects were men, thus it should be cautious to extend our results to hypertriglyceridemic women. Third, the duration of the treatment was relatively short (8 weeks). In this regard, our results are considered to be short-term effects which may be different from long-term effects.

In summary, the results of the present study demonstrated that, in addition to its potent TG-lowering effect, fenofibrate effectively improved proatherogenic lipoprotein profiles by reducing remnants and small LDL, as well as increasing small HDL particles. Therefore, fenofibrate may provide a benefit in hypertrigylceridemic patients at risk for CAD, typically patients with metabolic syndrome.

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