

Fenofibrate induces HDL-associated PAF-AH but attenuates enzyme activity associated with apoB-containing lipoproteins

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Abstract Human plasma platelet-activating factor acetylhydrolase (PAF-AH) is an enzyme associated mainly with the apolipoprotein B (apoB)-containing lipoproteins and primarily with LDL. A small proportion of enzymatic activity is also associated with HDL. Plasma paraoxonase 1 (PON1) is an esterase exclusively associated with HDL. The effect of fenofibrate on PAF-AH and PON1 activities in patients with dyslipidemias of Types IIA, IIB, and IV were studied. Fenofibrate reduced plasma PAF-AH activity in all patient groups. In Type IIA patients, this reduction was mainly due to a fall in enzyme activity associated with the dense LDL subspecies, whereas in Type IIB and Type IV patients, it was due to the decrease in PAF-AH activity associated with both the VLDL+IDL and dense LDL subspecies. Drug therapy in Type IIB and Type IV patients significantly increased the HDL-associated PAF-AH activity due to the increase in enzyme activity associated with the HDL-3c subfraction. Fenofibrate did not affect serum PON1 activities toward paraoxon and phenylacetate in either patient group. The fenofibrate-induced elevation of HDL-associated PAF-AH activity in dyslipidemic patients of Type IIB and Type IV, as well as the reduction in enzyme activity associated with atherogenic apoB-containing lipoproteins in all patient groups, may represent a new and important antiatherogenic effect of this potent lipid-modulating agent.—Tsimihodimos, V., A. Kakafika, A. P. Tambaki, E. Bairaktari, M. J. Chapman, M. Elisaf, and A. D. Tselepis. Fenofibrate induces HDL-associated PAF-AH but attenuates enzyme activity associated with apoB-containing lipoproteins. *J. Lipid Res.* 2003. 44: 927–934.

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Platelet-activating factor (PAF) is a potent proinflammatory lipid mediator that is implicated in atherogenesis (1). In plasma, PAF is hydrolyzed and inactivated by PAF-

acetylhydrolase (PAF-AH) (EC 3.1.1.47), an enzyme associated mainly with the apolipoprotein B (apoB)-containing lipoproteins and primarily with LDL (2). A small proportion of circulating enzyme activity is also associated with HDL (2, 3). PAF-AH exhibits a α/β hydrolase conformation (4) and has broad substrate specificity toward lipid esters containing short acyl chains (5). Thus, PAF-AH can hydrolyze short-chain diacylglycerols, triacylglycerols, and acetylated alkanols, but also displays phospholipase A₁ and A₂ activities, as well as transacetylase activity (5, 6). Among them, the Ca²⁺-independent phospholipase A₂ activity of PAF-AH has been principally studied, and thus this enzyme has been denoted as lipoprotein-associated phospholipase A₂ (7). Indeed, PAF-AH has marked preference for phospholipids with short-chain moieties at the *sn*-2 position and, with the exception of PAF, can hydrolyze proinflammatory and proatherogenic oxidized phospholipids produced by peroxidation of phosphatidylcholines containing an *sn*-2 polyunsaturated fatty acyl residue (8).

The role of PAF-AH in atherosclerotic disease is controversial. Data from the West of Scotland Coronary Prevention Study (Scotland, 1989–1995) trial suggest that plasma levels of PAF-AH mass, which mainly reflects the LDL-associated enzyme, represent an independent risk factor for coronary artery disease (9). In contrast, recent findings in the Women's Health Study (US, 1992 to present) suggest that plasma PAF-AH is not a strong predictor of cardiovascular risk in apparently healthy middle-aged women over a mean follow-up of 3 years (10). Nonetheless, loss of plasma PAF-AH activity due to a G⁹⁹⁴→T mutation in the PAF-AH gene may constitute a genetic determinant of atherosclerotic disease in the Japanese population (11). Despite conflicting observations concerning the potential relevance of total plasma- and LDL-associated PAF-AH to

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atherosclerotic disease, several lines of evidence suggest that HDL-associated PAF-AH activity, although present at low levels, may contribute to the antiatherogenic effects of this lipoprotein (12). Thus, adenoviral transfer of human plasma PAF-AH gene in apoE^{-/-} mice significantly reduced macrophage adhesion and homing (13), and inhibited injury-induced neointima formation and spontaneous atherosclerosis (14). A contributory role in the HDL-associated PAF-AH activity may be played by paraoxonase 1 (PON1), an enzyme that is present in plasma exclusively associated with this lipoprotein (15). Indeed, PON1 exhibits PAF-AH-like catalytic activity in addition to its paraoxon and phenyl acetate hydrolytic activities (16).

We recently demonstrated that patients with primary hypercholesterolemia exhibit an alteration in the relative distribution of PAF-AH between LDL and HDL particles, resulting in a decrease in the ratio of HDL-PAF-AH to plasma-PAF-AH [or to LDL-cholesterol (LDL-C)] levels, which is proportional to the severity of the hypercholesterolemia (17). Furthermore, atorvastatin therapy partially restored such an altered PAF-AH distribution by reducing both plasma LDL-C levels and LDL-associated PAF-AH activity, although this statin did not affect plasma levels of HDL-C or HDL-associated PAF-AH activity (18).

Considered together, the above findings support the convention that HDL-associated PAF-AH may play an antiatherogenic role. It remains indeterminate, however, as to whether drugs that modify plasma levels of HDL could influence PAF-AH activity associated with this lipoprotein. Fibrates are a family of hypolipidemic drugs that may reduce plasma LDL-C levels but equally induce elevation in HDL-C levels (19). We therefore undertook the present study to investigate the effect of a potent fibrate, fenofibrate, on HDL-associated PAF-AH relative to its effects on enzyme activity associated with apoB-containing lipoproteins in atherogenic dyslipidemias of Types IIA, IIB, and IV. This question is of special interest as this fibrate induces a shift in LDL particle profile from small, dense LDL to large, buoyant particles; indeed, PAF-AH is primarily associated with dense LDL particles in plasma (20).

METHODS

Patients

Unrelated hyperlipidemic patients (n = 71) attending the Outpatient Lipid Clinic of the University Hospital of Ioannina participated in the study. Secondary causes of dyslipidemia (hypothyroidism, diabetes mellitus, liver or renal diseases, alcoholism, etc.) were excluded by personal history, physical examination, and appropriate laboratory tests. None of the study participants was obese (BMI > 30 kg/m²), hypertensive (blood pressure > 140/90 mmHg on repeated measurements), or was taking medications known to interfere with lipid metabolism. No patient had any clinical or ECG evidence of cardiovascular disease. After the initial screening, patients gave informed consent and were advised to follow the National Cholesterol Education Program Step 1 diet for 3 months. At the end of this period, a complete laboratory baseline analysis was performed. According to their lipid levels, patients were divided into the following groups: 1) primary

hypercholesterolemia (Type IIA dyslipidemia), consisting of 18 patients (mean age 54.2 ± 10.8 years, BMI 25.8 ± 3.9 kg/m², seven active smokers, eight males), exhibiting plasma LDL-C levels >160 mg/dl. 2) Combined hyperlipidemia (Type IIB dyslipidemia), consisting of 23 patients (mean age 51.5 ± 11.4 years, BMI 26.6 ± 4.5 kg/m², 10 active smokers, 14 males), exhibiting plasma LDL-C levels >160 mg/dl and triglyceride levels >200 mg/dl. 3) Primary hypertriglyceridemia (Type IV dyslipidemia), consisting of 30 patients (mean age 51.1 ± 11.7 years, BMI 28.1 ± 4.4 kg/m², 12 active smokers, 13 males), exhibiting plasma triglyceride levels >200 mg/dl, and LDL-C levels <160 mg/dl. Patients with familial hypercholesterolemia were excluded by appropriate genetic analysis (17). No difference in the above biological and clinical characteristics was observed among patient groups. Micronized fenofibrate (200 mg at bedtime) was initiated in all patients; after 3 months of active treatment, a second blood analysis was performed. Compliance with treatment and diet was assessed as described previously (18).

Ninety-eight age- and sex-matched apparently healthy normolipidemic subjects were selected from individuals receiving a medical check-up at our hospital and served as controls. The study was approved by the Ethics Committee of the University Hospital of Ioannina.

Subfractionation of plasma lipoproteins

Lipoproteins were fractionated by isopycnic density gradient ultracentrifugation as previously described (20). Total plasma and the HDL-containing supernatant, after treatment of plasma with magnesium chloride/dextran sulfate (to precipitate all apoB-containing lipoproteins), were separately subjected to ultracentrifugation. After ultracentrifugation, 30 fractions of 0.4 ml each were collected and analyzed for their protein content. When plasma was used, equal volumes of gradient fractions 1 to 12 were pooled to constitute the following apoB-containing subfractions: fractions 1 and 2 (VLDL+IDL; d < 1.019 g/ml); 3 and 4 (LDL-1; d = 1.019–1.023 g/ml); 5 and 6 (LDL-2; d = 1.023–1.029 g/ml); 7 and 8 (LDL-3; d = 1.029–1.039 g/ml); 9 and 10 (LDL-4; d = 1.039–1.050 g/ml); 11 and 12 (LDL-5; d = 1.050–1.063 g/ml). When the HDL-containing supernatant was used, equal volumes of gradient fractions 13 to 23 were pooled to constitute the following apoA-I-containing subfractions: fractions 13 to 15 (HDL-2b; d = 1.063–1.091 g/ml); 16 and 17 (HDL-2a; d = 1.091–1.100 g/ml); 18 and 19 (HDL-3a; d = 1.100–1.133 g/ml); 20 and 21 (HDL-3b; d = 1.133–1.156 g/ml); 22 and 23 (HDL-3c; d = 1.156–1.179 g/ml) (21). It must be noted that PON1 activities were also determined in HDL subfractions. In these experiments, serum was used instead of plasma.

Effect of fenofibrate on PAF-AH production and secretion by human monocyte/macrophages

Peripheral blood monocytes from patients (before the initiation of therapy with fenofibrate) as well as from healthy volunteers were isolated and cultured as previously described (21). After 6 days of culture, the cells were treated either with fenofibrate or with fenofibric acid (dissolved in DMSO) at final concentrations ranging from 10 μM to 300 μM. Treatments were performed for 24 h and 48 h in RPMI medium containing 10% human serum in which endogenous PAF-AH was completely and irreversibly inactivated by preincubation with 1 mM Pefabloc for 30 min. After treatment, PAF-AH activity was determined in supernatants and cell lysates prepared as previously described (18).

Measurement of PAF-AH and PON1 activities

PAF-AH activity in plasma, lipoprotein subfractions, cell lysates, and supernatants was measured by the trichloroacetic acid precipitation procedure using [³H]PAF (100 mM final concen-

tration) as a substrate, whereas PON1 activities in serum and lipoprotein subfractions were determined using paraoxon and phenyl acetate as substrates (18).

Analytical methods

Serum total cholesterol, triglycerides, HDL-C, apoB, apoA-I, and apoE were determined as previously described (18). Serum LDL-C was calculated using the Friedewald formula (provided that triglyceride levels were lower than 350 mg/dl. In 15 patients with high triglyceride values, LDL-C was not determined). The total cholesterol, triglyceride, and phospholipid content in each HDL subfraction were measured enzymatically using the Bio-Merieux kit (20), whereas the protein content of the lipoprotein subfractions was measured by the BCA method (Pierce). The lipoprotein mass of each subfraction was calculated as the sum of the mass of the individual lipid and protein components (20).

Statistical analysis

Data were expressed as mean \pm SD. Statistical analysis was performed using paired Student's *t*-test for comparisons between baseline and posttreatment values, while one-way ANOVA followed by the LSD test was used for comparisons between individual groups. Correlations between PAF-AH activity and lipid parameters were estimated using linear regression analysis.

RESULTS

Effect of fenofibrate therapy on plasma lipid profile in phenotypes IIA, IIB, and IV

Fenofibrate significantly decreased serum total cholesterol and triglyceride levels as well as serum apoE levels in all patient groups (Table 1). A significant reduction in serum apoB levels in all groups was also observed, whereas LDL-C levels were reduced in Type IIA and IIB patients, but not in Type IV patients; this finding suggests that the decrease in serum total cholesterol levels in Type IV patients was mainly due to reduction in VLDL-C levels. Most importantly, fenofibrate induced significant elevation in both serum HDL-C and apoA-I levels in Type IIB and IV dyslipidemic patients, but not in the Type IIA patient group (Table 1).

Plasma PAF-AH activity

Total plasma PAF-AH activity at baseline was higher in all patient groups as compared with controls. Furthermore, baseline values of enzyme activity in dyslipidemic Type IIB patients were significantly higher compared ei-

ther to those in Type IIA or to Type IV patients (Table 2). HDL-associated PAF-AH activity (HDL-PAF-AH) in dyslipidemic Type IIB and Type IV patients was significantly lower compared either to normolipidemic controls or to Type IIA patients. Furthermore, Type IV patients exhibited significantly lower HDL-PAF-AH activity as compared with Type IIB patients (Table 2). In all patient groups, HDL-PAF-AH activity was negatively correlated to plasma triglyceride levels ($r = -0.29$, $P < 0.02$). It is important to note that the ratio of HDL-PAF-AH to LDL-C levels before treatment was significantly lower in all patient groups compared with controls, whereas no difference in this ratio was observed among the patient groups (Table 2).

Fenofibrate treatment led to a reduction in total plasma PAF-AH activity in all patient groups. Enzyme activity in Type IIA and Type IV patients was decreased by 28% and 22%, respectively, to reach control values. A decrease (27%) in enzyme activity was also observed in Type IIB patients, although it remained significantly elevated as compared with controls, even after fenofibrate therapy (Table 2). The reduction in plasma PAF-AH activity in Type IIA and Type IIB patients, but not in Type IV patients, was positively correlated with a reduction in LDL-C levels ($r = 0.45$, $P < 0.005$ for Type IIA and $r = 0.53$, $P < 0.01$ for Type IIB). In contrast, the reduction in enzyme activity in Type IV patients was positively correlated with reduction in plasma apoE levels ($r = 0.45$, $P < 0.05$). Importantly, fenofibrate treatment significantly increased HDL-PAF-AH in Type IIB and Type IV patients, although posttreatment values remained lower than levels in controls. By contrast, no change was observed in HDL-PAF-AH in Type IIA patients after fenofibrate administration (Table 2). Furthermore, the ratio of HDL-PAF-AH to LDL-C levels significantly increased in all patient groups after fenofibrate treatment (50% in Type IIA and Type IIB, and 43% in Type IV), although it still remained lower as compared with the control group (Table 2).

Serum PON1 activities

No difference was observed in baseline values of serum PON1 activity toward paraoxon in any of the groups studied. Equally, no difference was found in PON1 activity toward phenyl acetate between Type IIA or Type IV dyslipidemic patients and controls, whereas Type IIB patients exhibited lower enzyme activity compared with controls

TABLE 1. Effect of fenofibrate on plasma lipid and lipoprotein levels in Type IIA, Type IIB, and Type IV dyslipidemias

	Type IIA (n = 18)				Type IIB (n = 23)				Type IV (n = 30)			
	Before	After	Change	<i>P</i>	Before	After	Change	<i>P</i>	Before	After	Change	<i>P</i>
			%				%				%	
Cholesterol	271.4 \pm 32.6	217.8 \pm 36.3	-19.4	0.000	296.3 \pm 31.9	237.7 \pm 34.5	-19.4	0.000	237.1 \pm 38.8	219.6 \pm 36.7	-6.7	<0.01
Triglycerides	151.1 \pm 34.6	116.1 \pm 41.1	-22.8	0.000	318.3 \pm 92.7	177.5 \pm 70.6	-43.6	0.000	395.8 \pm 179.3	219.7 \pm 104.7	-41.7	0.000
LDL-cholesterol	193.7 \pm 31.8	149.9 \pm 34.3	-22.2	0.000	193.5 \pm 27.8	157.4 \pm 32.3	-18.1	0.000	127.8 \pm 28.5	136.3 \pm 34.6	+7.9	NS
HDL-cholesterol	47.5 \pm 12.8	46.6 \pm 11.7	-5.0	NS	39.2 \pm 5.4	44.8 \pm 8.2	+14.6	<0.001	32.5 \pm 5.3	38.8 \pm 10.6	+22.1	<0.01
ApoB	139.6 \pm 27.0	107 \pm 21.7	-22.4	0.000	156.3 \pm 20.0	126.6 \pm 23.2	-19.0	0.000	127.1 \pm 19.3	117 \pm 26.9	-8.1	<0.05
ApoA-I	142.3 \pm 26.2	137.2 \pm 19.3	-1.7	NS	144.5 \pm 18.0	159.4 \pm 24.2	+10.4	<0.005	127.3 \pm 20.7	139.3 \pm 21.6	+11.8	<0.05
ApoE	4.0 \pm 0.9	3.3 \pm 0.9	-15.9	<0.05	6.1 \pm 2.6	3.8 \pm 1.0	-31.5	0.000	6.4 \pm 2.6	4.6 \pm 1.6	-24.2	0.000

Values represent the mean \pm SD and are expressed as mg/dl. Paired Student's *t*-test was used for comparisons between baseline and posttreatment values. A *P* value < 0.05 was considered significant.

TABLE 2. Effect of fenofibrate on plasma platelet activating factor acetylhydrolase and plasma paraoxonase 1 activities in Type IIA, Type IIB, and Type IV dyslipidemias

	Controls	Type IIA		Type IIB		Type IV	
		Before	After	Before	After	Before	After
Plasma PAF-AH activity, (nmol/ml/min)	48.8 ± 13.3	63.63 ± 23.8 ^d	45.9 ± 12.52 ^b	78.1 ± 19.5 ^{d,e}	56.7 ± 18.4 ^{b,c,e}	66.13 ± 25.16 ^{d,g}	51.36 ± 21.5 ^b
HDL-PAF-AH activity, (nmol/ml/min)	3.3 ± 1.3	3.22 ± 0.89	3.13 ± 0.92	2.37 ± 0.79 ^{d,e}	2.71 ± 0.76 ^{a,c}	1.78 ± 0.43 ^{d,f,g}	2.48 ± 0.7 ^{b,d}
Ratio HDL PAF-AH/ LDL-C (nmol/mg/min)	2.6 ± 1.5	1.4 ± 0.6 ^c	2.1 ± 0.8 ^{a,c}	1.2 ± 0.4 ^c	1.8 ± 0.8 ^{b,c}	1.4 ± 0.5 ^d	2.0 ± 0.7 ^{b,c}
PON1 activity (paraoxon) (U/l)	75.1 ± 45.7	48.6 ± 28.4	53 ± 32.8	70.8 ± 40.6	69.2 ± 41.3	67.4 ± 58.9	74 ± 68.3
PON1 activity (phenylacetate) (U/ml)	63.2 ± 20.6	72.3 ± 41.9	61.7 ± 31	56.6 ± 22.7	58.2 ± 22.8	71.3 ± 36	74.3 ± 38

PAF-AH, platelet-activating factor-acetylhydrolase; PON1, paraoxonase 1. Values represent the mean ± SD. Paired Student's *t*-test was used for comparisons between baseline and posttreatment values while one-way ANOVA followed by LSD test was used for comparisons between individual groups. A *P* value < 0.05 was considered to be significant.

^a *P* < 0.05 compared with baseline values.

^b *P* < 0.001 compared with baseline values.

^c *P* < 0.05 compared with controls.

^d *P* < 0.001 compared with controls.

^e *P* < 0.05 compared with Type IIA at the same time (baseline or posttreatment).

^f *P* < 0.001 compared with Type IIA at the same time (baseline or posttreatment).

^g *P* < 0.05 compared with Type IIB (baseline or posttreatment).

(although it did not reach statistical significance). Fenofibrate therapy did not affect enzyme activity in any patient group (Table 2).

PAF-AH activity in plasma lipoprotein subspecies

To further investigate the effect of fenofibrate on the PAF-AH activity associated with apoB- and apoA-I-containing plasma lipoprotein subspecies, we fractionated plasma lipoproteins before and after fenofibrate therapy. To study the effect of fenofibrate on enzyme activity associated with apoB-lipoprotein subspecies, total plasma was subjected to ultracentrifugation. As previously reported, a proportion of LDL-bound PAF-AH dissociates and is redistributed to HDL during ultracentrifugation of total plasma (3). Thus, to avoid any contamination of HDL-PAF-AH activity with the LDL-associated enzyme during ultracentrifugation, we studied the effect of fenofibrate on apoA-I-associated PAF-AH activity using plasma depleted of apoB-lipoproteins (see Methods).

Among the apoB-containing lipoproteins, PAF-AH activity was preferentially associated with the dense LDL-5 subfraction in all patient groups, as well as in normolipidemic controls (Table 3). Dyslipidemic Type IIB and Type IV patients exhibited significantly higher enzyme activity associated with the VLDL+IDL subfraction compared with controls, a phenomenon not observed in Type IIA patients. Furthermore, the Type IIB patient group exhibited significantly higher enzyme activity associated with this subfraction compared with Type IIA, but significantly lower enzyme activity compared with dyslipidemic Type IV patients (Table 3). In Type IIA and Type IIB patient groups, PAF-AH activity associated with the dense LDL-5 subfraction was higher compared either with controls or with Type IV patients, the Type IIB group displaying higher enzyme activity compared with Type IIA patients (Table 3). Furthermore, the Type IIA and Type IIB patient

groups exhibited significantly higher enzyme activity associated with the LDL-4 subfraction compared either to controls or to Type IV patients, a phenomenon not observed for the other LDL subfractions.

Among the apoA-I-containing lipoproteins, PAF-AH activity in all patient groups as well as in controls was preferentially associated with the HDL-3c subfraction, which corresponds to the VHDL-1 subfraction in our previous study (20). A representative profile of the distribution of PAF-AH activity among the HDL subfractions in Type IIB patients is shown in Fig. 1A. Dyslipidemic patients of Type IIB and Type IV exhibited significantly lower enzyme activity associated with this subfraction compared either with controls (unpublished observations) or with Type IIA patients (Fig. 1B), a phenomenon not observed in the other HDL subfractions (data not shown). Furthermore, Type IV patients had significantly lower enzyme activity in HDL-3c

TABLE 3. Comparison of baseline PAF-AH activity associated with apoB-containing lipoprotein subfractions in Type IIA, IIB, and IV dyslipidemias with normolipidemic controls

	Controls	Type IIA	Type IIB	Type IV	<i>P</i>
	<i>nmol/ml/min</i>				
VLDL+IDL	0.3 ± 0.2	0.4 ± 0.2	1.0 ± 0.5 ^{a,b}	1.6 ± 1.2 ^{a,b,c}	<0.05
LDL-1	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3	0.1 ± 0.1	NS
LDL-2	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	NS
LDL-3	0.5 ± 0.4	0.5 ± 0.1	0.9 ± 0.6	0.5 ± 0.4	NS
LDL-4	1.5 ± 1.1	3.9 ± 1.5 ^a	3.8 ± 2.4 ^a	1.4 ± 2.4 ^{b,c}	NS
LDL-5	5.0 ± 2.8	7.0 ± 1.2 ^a	10.2 ± 3.6 ^{a,b}	4.9 ± 2.5 ^{b,c}	<0.05

Values represent the mean ± SD. ANOVA followed by LSD test was used for comparisons between individual groups. A *P* value < 0.05 was considered significant.

^a *P* < 0.05 compared with controls.

^b *P* < 0.05 compared with Type IIA at the same time (baseline or posttreatment).

^c *P* < 0.05 compared with Type IIB (baseline or posttreatment).

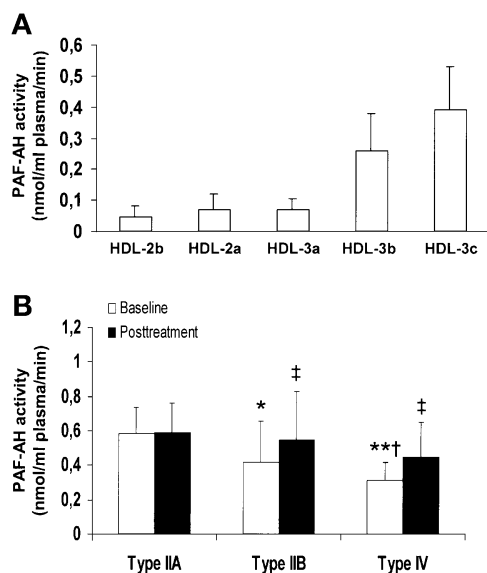


Fig. 1. A: Representative profile of the distribution of platelet-activating factor-acetylhydrolase (PAF-AH) activity among the HDL subfractions in dyslipidemic patients of Type IIB. B: Effect of fenofibrate therapy on PAF-AH activity associated with the HDL-3c subfraction in patients with dyslipidemias of Type IIA, Type IIB, and Type IV. HDL subfractions were isolated by isopycnic gradient ultracentrifugation of the HDL-containing supernatant after treatment of plasma with magnesium chloride-dextran sulfate (to precipitate all apoB-containing lipoproteins). Enzymatic activity was determined by the trichloroacetic acid (TCA) precipitation procedure. Values represent the mean \pm SD. * $P < 0.05$ and ** $P < 0.01$ as compared with IIA patients; $^{\dagger}P < 0.05$ as compared with IIB patients; and $^{\ddagger}P < 0.01$ as compared with baseline values.

compared with Type IIB, whereas no difference was observed between Type IIA patients and controls (unpublished observations). Interestingly, fenofibrate therapy in Type IIB and Type IV patients significantly increased PAF-AH activity associated with the HDL-3c subfraction, a phenomenon that was not observed in Type IIA patients (Fig. 1B). Drug administration did not affect enzyme activity associated with the other HDL subfractions in either patient group (unpublished observations).

To further investigate the relationship between the increase in plasma HDL-C levels and HDL-associated PAF-AH

induced by fenofibrate in Type IIB and Type IV patient groups, we determined the lipoprotein mass in each HDL subfraction. As shown in Table 4, baseline values of HDL-2a and HDL-2b mass in Type IIB and Type IV patient groups were significantly lower compared with those of Type IIA, the Type IV having lower values compared with Type IIB. No difference was observed in the mass of any HDL subfraction between Type IIA patients and controls (data not shown). Fenofibrate therapy significantly increased the mass of HDL-2a and HDL-3a subfractions in Type IIB and Type IV patient groups, whereas it did not affect the mass of the other HDL subfractions in these patient groups as well as the mass of all HDL subfractions in Type IIA patient group.

In contrast to the increase in HDL-PAF-AH activity, fenofibrate treatment significantly reduced PAF-AH activity associated with VLDL+IDL subfraction in Type IIB and Type IV patient groups, a phenomenon not observed in Type IIA patients (Fig. 2A). It must be noted that the above reduction was not observed when enzyme activity was expressed per milligram of VLDL+IDL subfraction (data not shown). Furthermore, a reduction in enzyme activity associated with LDL-4 and LDL-5 subfractions was observed after fenofibrate treatment in Type IIA and Type IIB patients. (Fig. 2B). Fenofibrate treatment in Type IV patients reduced enzyme activity associated only with the LDL-5 subfraction (Fig. 2B). Finally, in all patient groups, fenofibrate did not affect PAF-AH activity associated with intermediate and large LDL particles (data not shown).

PON1 activities in lipoprotein subspecies

PON1 activities toward paraoxon and phenyl acetate were determined in lipoprotein subspecies separated by ultracentrifugation of serum depleted of apoB-lipoproteins (see Methods). In all patient groups as well as in controls, both enzyme activities were preferentially associated with the dense HDL subfraction, HDL-3c. No differences were observed for the PON1 activities associated with each HDL subfraction among all studied groups. Fenofibrate therapy did not affect PON1 activity associated with any HDL subfraction in all patient groups. Figure 3 illustrates the PON1 activities toward paraoxon (Fig. 3A) and toward phenyl acetate (Fig. 3B) associated with HDL subfractions before and after fenofibrate therapy in dyslipidemic Type IIB patients.

TABLE 4. Effect of fenofibrate on the mass of HDL subfractions in Type IIA, Type IIB, and Type IV dyslipidemias

	Type IIA		Type IIB		Type IV	
	Before	After	Before	After	Before	After
HDL-2b	55.1 \pm 7.1	53.2 \pm 9.4	48.9 \pm 8.2	47.4 \pm 10.1	47.7 \pm 11.2	45.2 \pm 8.9
HDL-2a	68.9 \pm 12.4	69.7 \pm 14.2	58.7 \pm 10.4 ^a	47.4 \pm 10.1	51.0 \pm 12.5 ^{b,c}	64.2 \pm 8.4 ^d
HDL-3a	72.1 \pm 14.7	74.4 \pm 10.9	59.9 \pm 12.1 ^a	73.8 \pm 13.2 ^d	52.9 \pm 7.9 ^{b,c}	68.1 \pm 10.8 ^d
HDL-3b	30.0 \pm 8.8	32.3 \pm 11.3	31.7 \pm 7.5	33.6 \pm 10.4	30.6 \pm 6.8	34.1 \pm 9.4
HDL-3c	29.9 \pm 6.9	31.4 \pm 9.3	31.8 \pm 6.3	29.2 \pm 8.0	29.8 \pm 9.2	28.4 \pm 10.6

Values represent the mean \pm SD and are expressed as mg/dl. Paired Student's *t*-test was used for comparisons between baseline and posttreatment values while one-way ANOVA followed by LSD test was used for comparisons between individual groups. A *P* value of < 0.05 was considered significant.

^a $P < 0.03$ compared with baseline values of Type IIA.

^b $P < 0.02$ compared with baseline values of Type IIA.

^c $P < 0.05$ compared with baseline values of Type IIB.

^d $P < 0.03$ compared with baseline values of the same patient group.

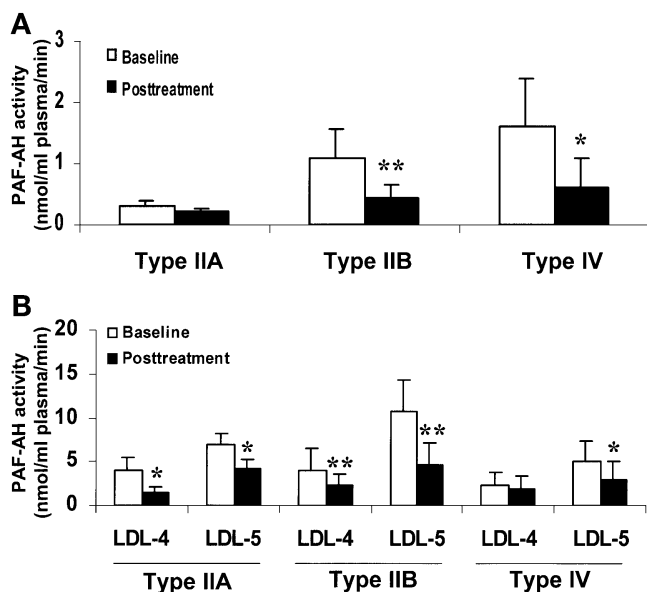


Fig. 2. Effect of fenofibrate therapy on PAF-AH activity associated with VLDL+IDL subfraction (A) and the dense LDL subfractions (LDL-4, LDL-5) (B) in patients with dyslipidemias of Type IIA, Type IIB, and Type IV. Lipoprotein subfractions were isolated by isopycnic gradient ultracentrifugation of fasting plasma obtained before fenofibrate administration and 3 months afterwards. PAF-AH activity was determined by the TCA precipitation procedure. Values represent the mean \pm SD. * $P < 0.01$ and ** $P < 0.001$ as compared with pretreatment values.

Effect of fenofibrate on PAF-AH secretion from macrophages

To explore the possibility that the effects of fenofibrate on LDL- and HDL-associated PAF-AH might be due to drug effect on enzyme secretion, we studied the effect of fenofibrate on PAF-AH production and secretion by peripheral blood monocyte-derived macrophages. We used

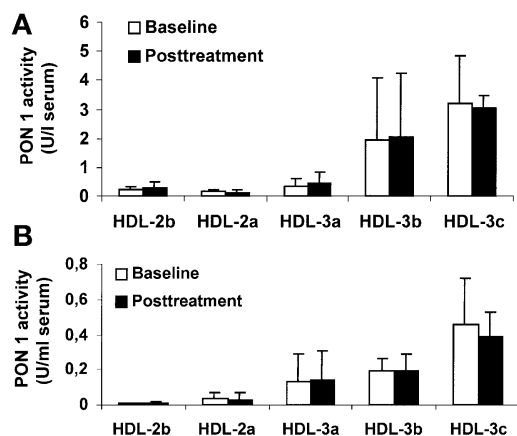


Fig. 3. Distribution of paraoxonase activities toward paraoxon (A) and phenyl acetate (B) among HDL subfractions obtained before and 3 months after fenofibrate therapy in patients with dyslipidemias of Type IIA, Type IIB, and Type IV. HDL subfractions were isolated by isopycnic gradient ultracentrifugation of the HDL-containing supernatant after treatment of serum with magnesium chloride-dextran sulfate (to precipitate all apoB-containing lipoproteins). Values represent the mean \pm SD.

macrophages since these cells represent a major source of plasma PAF-AH (22). Cells isolated from six subjects of each group were used in these studies. Incubations were performed for 24 h and 48 h, with various concentrations of fenofibrate or fenofibric acid ranging from 10 μ M to 300 μ M. As expected, there was a steady increase in both the secreted and the cell-associated PAF-AH activity in untreated cells (incubated with DMSO) of normolipidemic controls, attaining 178 ± 46 and 51 ± 18 nmol/mg DNA/h, respectively, at 24 h as well as 323 ± 97 and 84 ± 36 nmol/mg DNA/h, respectively, at 48 h of culture. Similar results were obtained for untreated macrophages from each of the three groups of dyslipidemic patients studied (data not shown). Cell treatment with fenofibrate or fenofibric acid at concentrations studied up to 300 μ M did not significantly alter the secreted or the cell-associated enzyme activity measured in untreated cells (data not shown). It must be noted that in enzyme assays performed in the presence of 50 μ M or 300 μ M of either fenofibrate or fenofibric acid using macrophage supernatant as the source of the enzyme (containing 5.1 nmol/ml/min of PAF-AH activity), no effect of both substances on PAF-AH activity was observed.

DISCUSSION

In the present study, we show for the first time that fenofibrate therapy in patients with primary hypercholesterolemia (IIA), combined hyperlipidemia (IIB), and primary hypertriglyceridemia (IV) significantly reduces PAF-AH activity associated with atherogenic apoB-containing lipoproteins. Most importantly, fenofibrate therapy in dyslipidemic patients of Type IIB and Type IV significantly increased the HDL-associated PAF-AH activity due to the increase in enzyme activity associated with HDL-3c subfraction. In contrast, fenofibrate did not affect HDL-associated PON1 activities toward paraoxon and phenyl acetate in either patient group.

The decrease in plasma LDL-C levels induced by fenofibrate could represent the major mechanism accounting for the drug-induced reduction in total plasma PAF-AH activity in hyperlipidemic patients of Type IIA and Type IIB. This is supported by the strong positive correlation observed between the decrease in enzyme activity and in plasma LDL-C levels. Fenofibrate preferentially reduced PAF-AH activity associated with the dense LDL particles, i.e., those particles carrying the majority of LDL-associated enzyme activity (20). We may suggest that such a reduction could primarily be due to the well-known drug effect on the transformation of small, dense LDL particles to the large buoyant LDL, i.e., particles that exhibit a higher rate of clearance from the circulation compared with small, dense ones (23, 24). Furthermore, the drug-induced reduction in enzymatic activity associated with the VLDL+IDL subspecies may play a contributory role in the reduction of plasma PAF-AH activity that was observed in Type IIB patients.

Our results show for the first time that patients with pri-

mary hypertriglyceridemia exhibit significantly higher plasma PAF-AH activity than controls. However, in contrast to Type IIA and IIB patients, a key role in this elevation may be played by the increased levels of triglyceride-rich lipoproteins. Indeed, unlike in dense LDL-5 subfraction, baseline PAF-AH activity associated with the VLDL+IDL subfraction in Type IV patients was significantly higher compared with all other patient groups. The above hypothesis is further supported by the finding that, although fenofibrate treatment did not affect LDL-C levels in this group, it significantly reduced the total plasma-associated and the VLDL+IDL-associated PAF-AH activity. Moreover, unlike other patient groups, the reduction in plasma enzyme activity in Type IV patients is positively correlated with the reduction in plasma levels of apoE, which is primarily associated with the VLDL+IDL subspecies. Overall, we may suggest that, in primary hypertriglyceridemia, the fenofibrate-induced reduction in PAF-AH activity depends on the catabolism and the rate of clearance of the triglyceride-rich lipoproteins from the circulation.

An important observation of our studies is that baseline values of HDL-PAF-AH in dyslipidemic Type IIB and Type IV patient groups were significantly lower compared with controls or with Type IIA patients. It is well known that both Type IIB and Type IV dyslipidemic patients are characterized by abnormal catabolism of triglyceride-rich lipoproteins. This metabolic defect significantly influences the plasma HDL levels (19), and the results of the present study are in accordance to this observation. Therefore, we may suggest that the low HDL-PAF-AH observed in these patient groups reflects this metabolic defect. This hypothesis is supported by the negative correlation between baseline plasma triglyceride levels and baseline HDL-PAF-AH activities in these patient groups. Accordingly, baseline HDL-PAF-AH in normotriglyceridemic Type IIA patients was similar to controls and was not modified by fenofibrate therapy. It is well established that the elevation in plasma HDL-C levels induced by fenofibrate arises primarily from synthesis of new HDL particles, as well as from the production of HDL due to liberation of surface fragments during the enhanced catabolism of triglyceride-rich lipoprotein particles (25). Consequently, increase in HDL-PAF-AH induced by fenofibrate might be due to the drug action on HDL production. The possibility that the drug-induced increase in HDL-PAF-AH in Type IIB and Type IV patients is due to the synthesis of new PAF-AH containing HDL particles in the liver is unlikely, since early studies failed to detect PAF-AH mRNA in these cells (26). We also ruled out the possibility that the elevation in HDL-PAF-AH induced by fenofibrate is attributed to enhanced PAF-AH secretion by macrophages, since neither fenofibrate nor fenofibric acid affected PAF-AH production and secretion by these cells. Thus, it is possible that the increase in HDL-PAF-AH in patients with abnormal catabolism of triglyceride-rich lipoproteins (Type IV and Type IIB) could be due to enzyme transfer from triglyceride-rich apoB-containing lipoproteins to HDL during their enhanced lipolysis by lipoprotein lipase induced by fenofibrate (25). This hypothesis may also explain the low baseline HDL-PAF-AH

in these patient groups. The observation that the elevation in HDL-PAF-AH concerns primarily the HDL-3c subfraction (the plasma levels of which remained unaffected by fenofibrate therapy) may suggest that this subfraction represents a better acceptor of PAF-AH from the triglyceride-rich apoB-containing lipoproteins during their degradation. Finally, the possibility that PAF-AH can also be transferred from other HDL subfractions to HDL-3c cannot be excluded.

An enzyme that is exclusively transported in human plasma by HDL is PON1 (15). In accordance with previously published results (27), PON1 activities in dyslipidemic Type IIB or Type IV patient groups were not different from those observed in the control group; neither are they affected by fenofibrate therapy. Considering that, unlike PAF-AH, PON1 is not associated with the apoB-containing lipoproteins, the differential effect of fenofibrate on HDL-PAF-AH versus HDL-PON1 further supports the hypothesis for the PAF-AH transfer from triglyceride-rich apoB-containing lipoproteins to HDL during fenofibrate therapy in dyslipidemic Type IIB or Type IV patients. Although the results of the present study seem to support the above hypothesis, the relatively small size of population participating in this study represents a limiting factor for extrapolation of the above suggestion to other population groups.

Several recently published studies revealed that HDL-PAF-AH plays an important contributory role in HDL antioxidant and antiatherogenic effects (28), and that among HDL subfractions, HDL-3 exerts the most powerful antioxidant properties (29). Thus, the fenofibrate-induced increase in PAF-AH activity associated with this subfraction in Type IIB and Type IV patients, as well as the increase in the ratio of HDL-PAF-AH to plasma LDL-C levels in all patient groups, may represent an important new antiatherogenic effect of this potent lipid-modulating agent. ■

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