A novel compound that elevates high density lipoprotein and activates the peroxisome proliferator activated receptor

Charles L. Bisgaier,[1,*] Arnold D. Essenburg,[*] Blake C. Barnett,[*] Bruce J. Auerbach,[*] Sabine Haubenwallner,[†] Todd Leff,[†] Andrew D. White,[§] Paul Creger,[§] Michael E. Pape,[**] Thomas J. Rea,[**] and Roger S. Newton[*]

Departments of Vascular and Cardiac Diseases,* Cell Biology,† Medicinal Chemistry,§ and Molecular Biology,** Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105

Abstract In the current studies we describe the effects of PD 72953 and related compounds on lipoprotein levels in chow-fed male rats. After 2 weeks, 10 mg/kg of PD 72953 daily was as effective as 100 mg/kg gemfibrozil for elevating HDL-cholesterol. At 100 mg/kg, PD 72953 further elevated HDL-cholesterol to 232% of control levels, and was associated with increased HDL size and plasma apoE (169% of control), despite no change in hepatic apoE mRNA. ApoA-I rose transiently (at 1 week), but by 2 weeks only apoE remained elevated. PD 72953 dose-dependently reduced plasma apoB, VLDL-cholesterol, LDL-cholesterol, and triglyceride. Hepatic apoC-III mRNA reduction parallelled triglyceride lowering. After 1 week, 30 and 100 mg/kg per day PD 72953 reduced plasma apoC-III levels by 30 and 34%, and triglycerides by 60 and 83%, respectively. PD 72953 treatment had no effect on triglyceride production rates; however, ^{125}I-labeled VLDL apoB disappearance was enhanced. We compared PD 72953 to a structurally similary diacid, PD 69405, that also reduced VLDL and LDL, but had no effect on HDL elevation. Compared to PD 72953, PD 69405 further accelerated ^{125}I-labeled VLDL apoB disappearance, decreased triglyceride production, and elevated the ratio of post-heparin hepatic to lipoprotein lipase activity. Whole animal studies, transient transfection studies in HepG2 cells, and chimeric receptor studies in kidney 293 cells suggest that PD 72953 is a ligand for the peroxisomal proliferation activated receptor alpha (PPARα), and PPARγ. Overall, PD 72953 may act through a peroxisomal proliferation activated receptor and result in plasma triglycerides and apoB-containing lipoprotein reduction, while also raising HDL cholesterol. Reduced apoC-III may allow triglyceride-rich remnants to more efficiently bind and present substrate to peripheral tissue lipoprotein lipase, and therefore allow enhanced shedding of remnant phospholipid surface for HDL production.—**Bisgaier, C. L., A. D. Essenburg, B. C. Barnett, B. J. Auerbach, S. Haubenwallner, T. Leff, A. D. White, P. Creger, M. E. Pape, T. J. Rea, and R. S. Newton.** A novel compound that elevates high density lipoprotein and activates the peroxisome proliferator activated receptor. *J. Lipid Res.* 1998. **39:** 17–30.

Supplementary key words PD72953 • apoC-III • triglyceride • gemfibrozil • HepG2 cells • fibrates • lipoproteins

Fibric acids are generally indicated for the treatment of hypertriglyceridemia (1–3); however, these compounds have variable effects on HDL apolipoprotein and cholesterol elevation. These compounds may mimic the natural ligands for nuclear receptors that act through peroxisomal proliferation response elements (PPREs) that are present on a variety of genes associated with peroxisomal enzymes, lipoprotein metabolizing enzymes, and apolipoproteins, including apoC-III which appears to be downregulated by fibrates (4–6) and MEDICA 16 (7). In comparative studies of select fibrates in chow-fed (5, 8) and cholesterol-fed (8, 9) male rats, it was previously observed that gemfibrozil, unlike the other fibrates tested at similar doses, caused an elevation of HDL cholesterol and particle size and was associated with apoE elevation. The plasma elevation of apoE occurred despite no change in its hepatic mRNA levels (5, 10), which suggested increased synthesis at extrahepatic sites or a decreased catabolism of this protein from plasma.

Abbreviations: ACO, acyl CoA oxidase; ANOVA, analysis of variance; apo, apolipoprotein; CMC/Tween, 1.5% carboxymethylcellulose plus 0.2% Tween-20; CMV, cytomegalovirus; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; gal, β-galactosidase; HDL, high density lipoprotein; HL, hepatic lipase; HPGC, high performance gel filtration chromatography; HSV, herpes simplex virus; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; MEM, minimum essenial media; PHLA, post-heparin lipolytic activity; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisomal proleferation response elements; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

The finding that HDL could be specifically elevated with gemfibrozil, despite no increase in mRNA levels for hepatic apoA-I and apoE (5, 10), strengthened the idea that a considerable portion of HDL is derived as a catabolic product of triglyceride-rich lipoproteins (11–15). Although existing evidence suggests that HDL are derived directly from liver (16, 17) and intestine (18–20), the importance of phospholipid–apolipoprotein surface generated during triglyceride-rich lipoprotein lipolysis cannot be underestimated as a major source of these particles. The enhanced catabolism of triglyceride-rich lipoproteins by lipoprotein lipase is a potential mechanism by which increased phospholipid may form nascent HDL or be transferred to existing HDL pools (13). Possibly the differential effects of fibrates on HDL elevation may be related to a balance between lipolytic activity and remnant clearance.

In the current report we describe a series of ether diacids and one alkyl diacid. One ether diacid studied extensively, PD 72953, down-regulates apoC-III expression and results in reduced plasma triglycerides. However, these compounds are distinctive in their effects on the amount and type of HDL formed, which may, in part, be related to their differential effects on VLDL clearance.

MATERIALS AND METHODS

Animals, diets, and treatments

Male Sprague-Dawley rats (100–200 g) were obtained from Charles River Laboratories. All animals were allowed normal rat chow (Ralston-Purina) and water ad libitum in temperature-controlled rooms, under a 12-h light, 12-h dark cycle beginning with lights on at 6 AM. Rats were dosed daily between 6 and 10 AM by oral gavage using a suspension vehicle of 1.5% carboxymethylcellulose plus 0.2% Tween-20 (CMC/Tween). Control animals received vehicle alone. Vehicle volume represented 0.25% of body weight. Compounds were administered at up to 100 mg/kg per day for up to 2 weeks. Lipid, lipoprotein, and apolipoprotein analysis were performed on plasma obtained from non-fasted rats anesthetized by ether inhalation 10–12 h post-dosing and bled by cardiac puncture. Liver weights were determined and select samples were taken for RNA extraction or for determination of peroxisomal enzyme activity. Triglyceride production rates and ^{125}I-labeled VLDL-apoB clearance studies were carried out in animals fasted from midnight to 8 AM. These animals were also dosed on the day of the experiment. Post-heparin plasma lipolytic activities were measured 4 h post-dosing (9 AM–1 PM) in non-fasted animals. Blood was transferred to ethylenediaminetetraacetic acid-containing Vacutainer tubes for plasma isolation. A liver sample was taken for total RNA isolation.

Synthesis of diacids

6,6′-Oxybis(2,2-dimethylhexanoic acid) PD 72953. Isobutyric acid (52.9 g, 0.6 mol) was added to a stirred solution of sodium hydride (28 g, 0.7 mol of 60% mineral oil dispersion) and diisopropyl anime (61 g, 0.6 mol) in dry tetrahydrofuran (600 mL). The reaction mixture was stirred at room temperature (30 min) then cooled to 0°C with an ice/acetone bath. n-Butyl lithium (286 mL 2.1 M in hexane, 0.6 mol) was added and the mixture was stirred at 0°C (30 min), then 4-4′-dichlorobutyl ether (59.7 g, 0.297 mol) was added dropwise over 15 min. The mixture was warmed to room temperature and stirred for 48 h, then quenched with water (600 mL). the aqueous layer was separated, washed with ether (200 mL), acidified to pH 5.0 with 6 M HCl (150 mL), and extracted with ether (3 × 300 mL). The combined ether layers were washed with brine, dried over $MgSO_4$, and the solvent was removed in vacuo. The oil was distilled at 160°C (3 mm Hg) to yield 66.7 g of the title compound (**Fig. 1**): mp 49–51°C; 400 MHz 1H NMR (DMSO) 12.02 (br s, 2H), 3.31 (t, J = 6.4Hz, 4H), 1.44 (m, 8H), 1.21 (m, 4H), 1.06 (s, 12H); CIMS m/z 303 (MH^+). Anal. ($C_{16}H_{30}O_5$) C, H.

Utilizing the appropriate dihalide in the above procedure, the following compounds were synthesized: PD 105726, PD 105752, PD 72548, PD 72660.

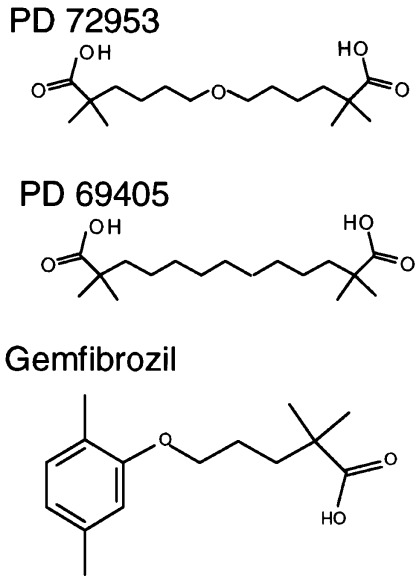

Fig. 1. Chemical structure of PD 72953 (6,6′-oxybis(2,2-dimethylhexanoic acid)), PD 69405, 2,2,12,12-tetramethyltridecanoic acid and gemfibrozil.

2,2,12,12-Tetramethyltridecanoic acid PD 69405. n-Butyl lithium (338 mL 2.1 M in hexane, 0.71 mol) was added dropwise to disopropylamine (71.8 g, 0.71 mol) at 0°C and the mixture was stirred for 2 h. Isobutyric acid (31.3 g, 0.355 mol) was added dropwise over 30 min and the mixture was heated to 50°C for 1 h then allowed to cool to room temperature. 1,9-Dibromononane (50.0 g, 0.175 mol) was added and the mixture was stirred for 4 days then quenched with water (800 mL). The aqueous layer was washed with ether (2 × 100 mL), acidified to pH 5.0 with 6 M HCl, and extracted with ether (2 × 400 mL). The ether extracts were washed with brine, dried over $MgSO_4$, concentrated in vacuo, and recrystallized twice from acetonitrile to afford 25.3 g of the title compound (Fig. 1): 400 MHz 1H NMR ($CDCl_3$) 11.54 (br s, 2H), 1.52 (m, 4H), 1.26 (s, 14H), 1.18 (s, 12H); CIMS *m/z* 301 (MH^+). Anal. ($C_{17}H_{32}O_4$) C, H.

Triglyceride production rates

Triglyceride producation rates (i.e., secretion rate) were determined in rats fasted overnight (midnight to 8 AM) after treatment with compounds for 8 days. A baseline tail-vein blood sample was drawn 2 h post the last drug dose (on day 8) and rats were then administered intravenous Triton WR1339 (600 mg/kg in normal saline) as previously described (5, 21). The difference between the basal and the 2 h post-Triton WR 1339 samples was used to determine triglyceride production rates. For data analysis, plasma volume was assumed to be 3.86 ml/100 g body weight (21).

Post-heparin lipolytic activity (PHLA)

Ex vivo post-heparin lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in plasma from rats treated with compounds for 8 days. On the day of the experiments, animals received their eighth dose in the morning between 9 and 10 AM. Heparin (100 U/kg) was administered by intravascular injection 4 h after dosing, and blood was obtained 10 min post-heparin. PHLA, LPL and HL activities were then determined as described by Kuusi et al. (22). Under these assay conditions, excess substrate and added cofactors allow measurement of lipolytic activities under V_{max} conditions, therefore reflecting enzyme mass and not necessarily the true in vivo activity which would be influenced by the relative levels of apoC-II and apoC-III.

^{125}I-labeled VLDL-apoB clearance studies

Nascent VLDL was isolated from the plasma of donor rats pretreated with Triton WR-1339. VLDL was iodinated by the Bilheimer, Eisenberg, and Levy (23) modification of the iodine monochloride method of McFarlane (24).

Clearance studies were performed in rats that were orally dosed with 100 mg/kg gemfibrozil, PD 72953, PD 69405, or CMC/Tween vehicle for 8 days prior to intravenous administration of ^{125}I-labeled VLDL (~25 μCi/0.1 ml saline) in to the tail vein of conscious restrained rats fasted for 10 h (from midnight to 10 AM). The last drug dose was given at 8 AM on day 8. After ^{125}I-labeled VLDL administration, tail blood samples (~100 μl) were periodically taken at intervals up to 2 h. Radioactivity specifically associated with apoB was determined by the isopropanol precipitation method (25).

Analytical methods for plasma lipids and apolipoproteins

Plasma total cholesterol and triglycerides were determined enzymatically as previously described (5). Plasma lipoprotein cholesterol profiles and distribution among lipoproteins were determined by on-line post column analysis on Superose 6HR high performance gel filtration chromatography (HPGC) as previously described (5, 26). Apolipoprotein levels were determined as previously described (5).

Hepatic peroxisomal enzyme activity

Carnitine acyltransferase activity, an index of peroxisomal proliferation when acetyl-CoA is used as a substrate (i.e., carnitine acetyltransferase activity), was determined on frozen–thawed liver samples from control, gemfibrozil, and PD72953-treated rats as previously described (27). These (27) and other historical data (unpublished) of the assay determined on non-frozen liver samples for control and gemfibrozil-treated animals were comparable.

Hepatic mRNA analysis

Total hepatic RNA was isolated as previously described (5). Hepatic apoA-I, apoE, and mRNA were determined by an internal standard/RNAse protection assay as previously described (5, 28, 29). Hepatic apoC-III and actin mRNA were determined by Northern blot analysis as previously described (5).

Transient transfection in HepG2 cells

pLT-ACO contains the rat acyl CoA oxidase gene promoter (−1239 to −457) inserted upstream of the HSV thymidine kinase (TK) core promoter (−110 to +54) in the luciferase reporter vector pGL3-basic (Promega Corp., Madison, WI). CMV-hPPARα contains the full-length human peroxisomal proliferator activated receptor α inserted into pCDNA3 (Invitrogen Corp., San Diego, CA). HepG2 cells were maintained in MEM with 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 μg/ml). Transient transfec-

tions were performed in 30% confluent 24-well plates containing 1.6 μg of lipofectAMINE (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD) and 0.8 μg of DNA (0.58 μg pLT-ACO-luciferase, 0.12 μg CMV-hPPARα and 0.1 μg of a β-galactosidase reference plasmid) per well. Transfections were carried out for 4 h in serum-free medium. After transfection, cells were washed once and transferred to MEM plus FBS containing PD 72953, WY14643, or dimethylsulfoxide vehicle alone. Treatment was for 48 h. Cells were washed twice with phosphated-buffered saline and lysed in 100 μl lysis buffer (Promega Corp.), and 20-μl aliquots of lysate were used in luciferase (Promega Corp.) and β-galactosidase (Tropix, Inc., Bedford, MA) chemiluminescence assays. Transcriptional activity was expressed as ratio of luciferase activity divided by β-galactosidase activity.

Chimeric receptor assay

The chimeric receptor clones contain amino acids 205–506 for mouse PPARγ_2, 208–468 for mouse PPARα, and 127–440 for mouse PPARδ linked to the DNA binding domain of the *E. coli* tetracyclin repressor (amino acids 1–214) cloned into the expression plasmid pCDNA3 (Invitrogen). The luciferase reporter vector contains a Hind III–Xho I fragment of the plasmid pTET-tTAK (GIBCO-BRL) that contains the TET repressor binding site imbedded in a minimal hCMV promoter, inserted into the luciferase vector pGL3-basic (Promega). Transfections into human kidney 293 cells were carried out by electroporation in a GIBCO-BRL electroporator according to the manufacturer's recommendations. After transfection, cells were plated into 96-well plates and treated immediately with vehicle or drug as indicated in the legend to Fig. 11. Reference agents used in the assays included WY 14653 and eicosapentaenoic acid (EPA), which were purchased from Biomol Research Laboratories, Plymouth Meeting, PA, and BRL 49653, which was synthesized at Parke-Davis, Warner-Lambert.

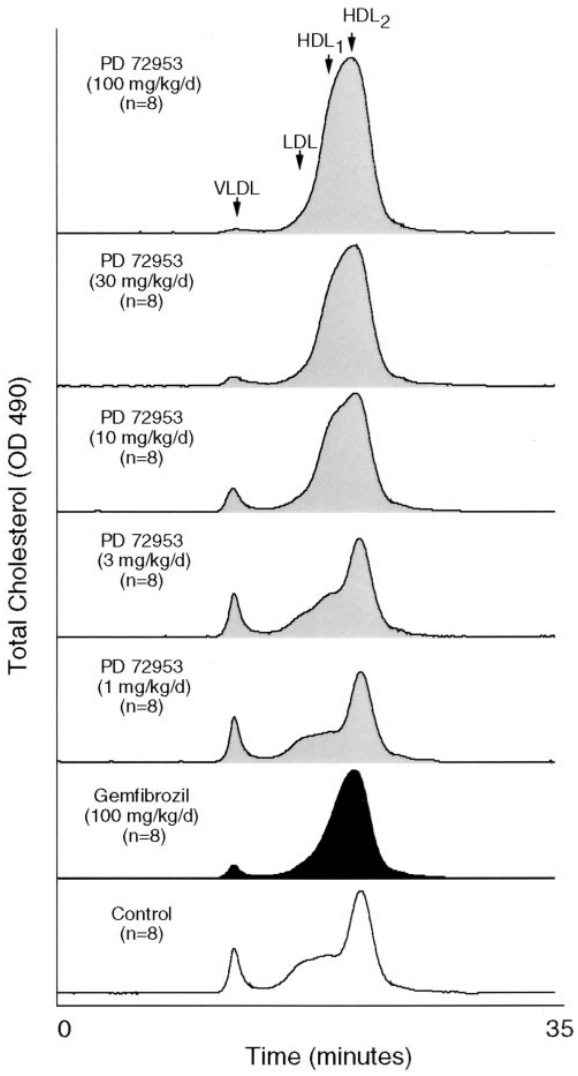

Fig. 2. Lipoprotein cholesterol profiles in PD 72953 and gemfibrozil-treated male Sprague-Dawley rats. Animals (n = 8/group) were dosed by oral gavage with compounds at the indicated daily dose for 2 weeks. Control animals were dosed with vehicle alone. Plasma cholesterol distribution was determined by Superose HR HPGC as described in Materials and Methods. Profiles were obtained from individual animals from each group. Shown are the pooled average profiles from each group and are typical of the individual profiles of the groups.

RESULTS

The effects of PD 72953 and gemfibrozil on lipoprotein cholesterol modification were assessed in male chow-fed Sprague-Dawley rats. In a 2-week study, HPGC cholesterol profiles demonstrated that 10 mg/kg per day of PD 72953 was as effective as a 100 mg/kg per day gemfibrozil dose for elevating HDL-cholesterol (**Fig. 2**) At 30 and 100 mg/kg per day, PD 72953 further elevated HDL-cholesterol and reached 232% of control levels at the higher dose (Fig. 2 and **Fig. 3A**). Gemfibrozil (100 mg/kg/d) reduced both VLDL and LDL cholesterol (Figs. 2 and 3A). Similarly, PD 72953 dose dependently reduced cholesterol in both VLDL and LDL (Figs. 2 and 3A). However, PD 72953 increased total cholesterol as a result of an increased size and cholesterol content of HDL (Figs. 2 and 3A). The overall effect of PD 72953 on plasma lipoproteins can be appreciated as the HDL to VLDL plus LDL cholesterol ratio (Fig. 3B). To confirm the HPGC cholesterol profile results, plasmas from PD 72953-treated rats were subjected to lipoprotein electrophoresis and oil red O

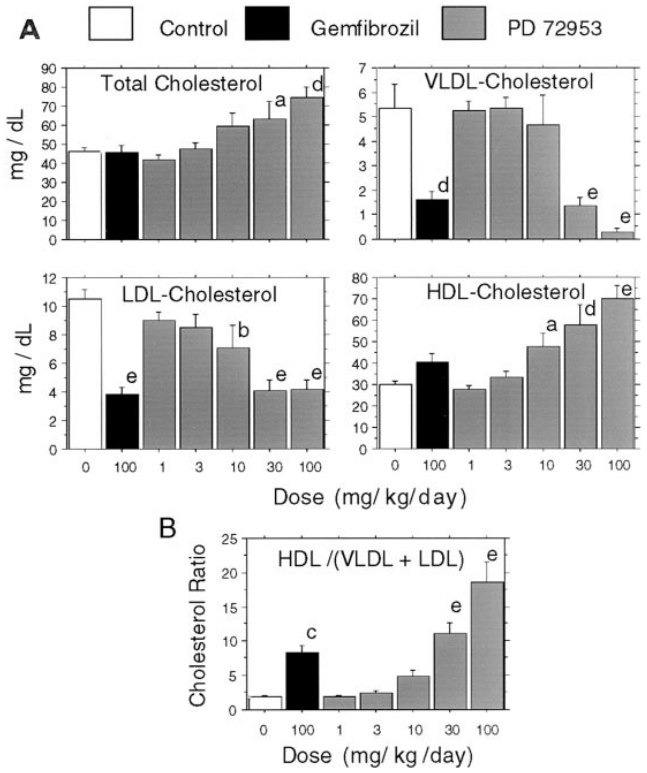

Fig. 3. (A) Lipoprotein cholesterol from PD 72953 and gemfibrozil-treated male Sprague-Dawley rats. Animals (n = 8/group) were dosed by oral gavage with compounds at the indicated daily dose for 2 weeks. Control animals were dosed with vehicle alone. (B) Ratio of HDL to VLDL plus LDL cholesterol. Data represent mean ± SEM for n = 8 rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.005$; d, $P < 0.0005$; e, $P < 0.0001$.

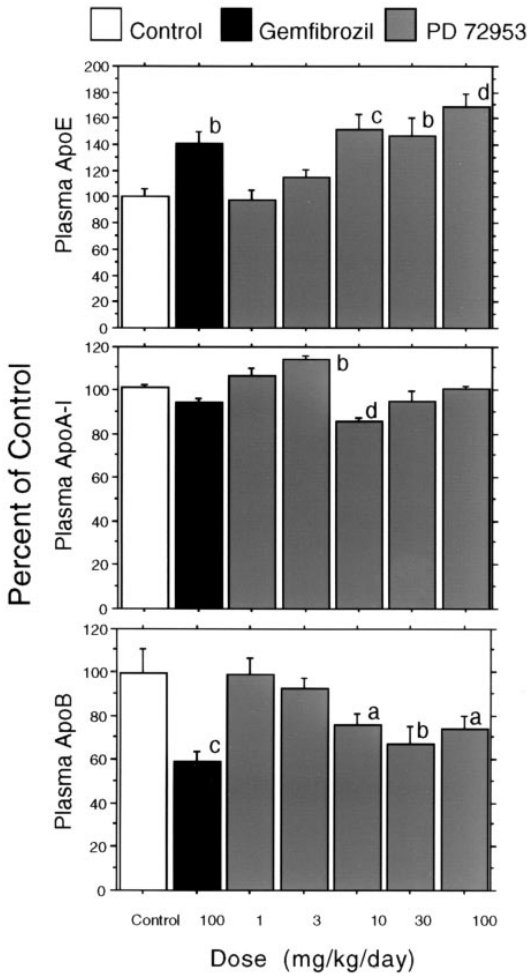

Fig. 4. Plasma apolipoproteins from PD 72953 and gemfibrozil-treated male Sprague-Dawley rats. See Figs. 2 and 3 for details. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls. a, $P < 0.05$; b, $P < 0.005$; c, $P < 0.0005$; d, $P < 0.0001$.

staining. The plasma from the PD 72953-treated rats showed a more intense alpha (HDL) migrating band and near complete disappearance of the pre-beta (VLDL) and beta (LDL) bands compared to controls (data not shown). PD 72953-induced HDL elevation was associated with a 69% increase in apoE at a dose of 100 mg/kg (**Fig. 4).** Despite the marked elevation in plasma apoE, hepatic levels of apoE mRNA were not affected by treatment (**Fig. 5**). Similarly, hepatic mRNA for apoA-I and HL were not elevated when compared to control levels (Fig. 5). PD 72953 reduced plasma apoB, but had no consistent effect on plasma apoA-I levels in the chow-fed rat at 2 weeks (Fig. 4). Dose-related reductions of plasma triglyceride (**Fig. 6**) were also observed with PD 72953 and the reduction of hepatic apoC-III mRNA parallelled triglyceride lowering (Fig. 6).

In a separate 1-week study (**Table 1**), PD 72953 was shown to reduce plasma apoC-III levels by 30 and 34% and plasma triglycerides by 60 and 83%, at 30 and 100 mg/kg per day doses, respectively. In this same study, (Table 1) PD 72953 caused reduction in plasma apoC-II and apoB, as well as an elevation of apoE. Unlike the 2-week study above where only apoE is elevated, at 1 week both apoA-I and apoE are elevated and this may represent a transition period for HDL-associated apolipoproteins. PD 69405, which is structurally similar to PD 72953, reduced triglycerides, apoB, apoC-II, and apoC-III. However, PD 69405 had no effect on elevating apoA-I or apoE. Drug effects on plasma apoC-III levels were directly correlated with plasma triglycerides (**Fig. 7A**). We had previously noted the marked inverse correlation between plasma triglycerides and the ratio of apoE to apoC-II plus C-III in fibrate-treated chow-fed rats (5), and find a similar relation for both PD 72953 and PD69405 in this 1-week study (Fig. 7B).

After 1 week of 100 mg/kg gemfibrozil or 3, 10, 30, or 100 mg/kg PD 72953 treatment in the chow-fed rat,

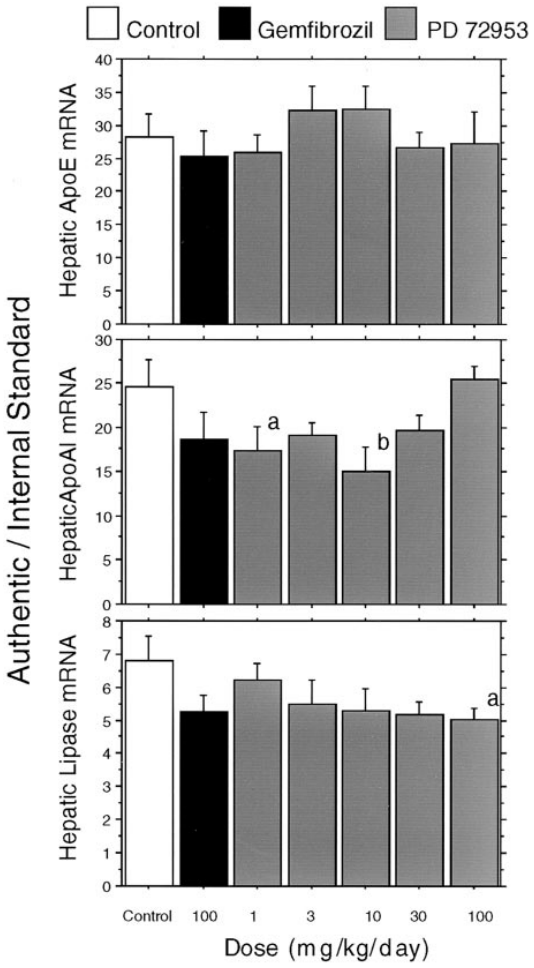

Fig. 5. Hepatic mRNA levels. Male Sprague-Dawley rats were treated for 2 weeks with compounds or vehicle as described in Figs 2 and 3. Hepatic total RNA was isolated and assessed for apoE, apoA-I, and hepatic lipase mRNA by an internal standard/RNAse protection assay as described in Materials and Methods. Data represent mean ± SEM for n = 8 rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls. a, $P < 0.05$; b, $P < 0.01$.

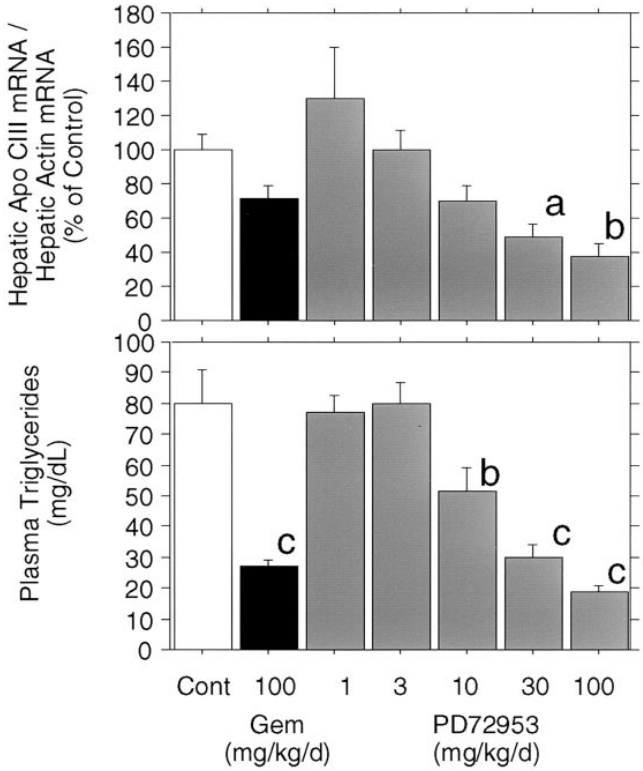

Fig. 6. Plasma triglycerides and hepatic apoC-III mRNA/actin mRNA. See Figs. 2 and 3 for experimental details. Data represent mean ± SEM for n = 8 rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls. a, $P < 0.05$; b, $P < 0.005$; c, $P < 0.0001$.

triglyceride production rates were determined in vivo using the Triton WR 1339 method (**Fig. 8A**). For all treatments the triglyceride secretion rates were normal when compared to vehicle treated controls. In a second experiment (Fig. 8B), triglyceride production rates were shown to be reduced by 34% ($P = 0.001$) with PD 69405 (110 mg/kg/day) but not with a slightly higher dose of PD 72953 (120 mg/kg/day). VLDL clearance as measure by ^{125}I-labeled VLDL apoB disappearance from plasma was markedly enhanced in gemfibrozil-, PD 72953-, and PD 69405-treated rats (each compound dosed at 100 mg/kg/day for 8 days) (**Fig. 9**).

Post-heparin plasma LPL and HL activities were determined after 8 days of vehicle (controls), gemfibrozil, PD 72953, or PD 69405 treatment (compounds dosed at 100 mg/kg/day). As lipolytic activity in rats shows a diurnal cycle (30), activities were determined at the same time of day for all rats. Activities are shown in **Table 2.** For all treatments, HL activity levels were similar; however, LPL activity was most markedly suppressed by PD 69405 (−72%), followed by gemfibrozil (−49%) and PD 72953 (−12%). PD 72953 treatment therefore caused a maintenance of the HL to LPL ratio (ratio = 1.6) similar to control (ratio = 1.4) that was less than that observed with gemfibrozil (ratio = 2.7) or PD 69405 (ratio = 5.6).

PD 72953 appeared to be unique in its ability to elevate and enlarge HDL in the chow-fed rat compared to a series of structural related ethers (**Fig. 10A and 10B**). Only PD 72953 raised HDL at 30 mg/kg per day after 1 week. Lipoprotein cholesterol profiles of PD 69405 after 1 week of treatment (Fig. 10C) demonstrate reduction of VLDL and LDL cholesterol but little change in the amount or size of HDL (enumerated in Table 1 and shown in Fig. 10C). Notice that even though gemfibrozil, PD 72953, and PD 69405 markedly elevated ^{125}I-labeled VLDL apoB clearance compared to controls

TABLE 1. Plasma apolipoprotein and lipid levels in gemfibrozil-, PD 69405-, and PD 72953-treated male Sprague-Dawley rats

Plasma Determinant	Control (n = 5)	Gemfibrozil 100 mg/kg (n = 5)	PD 69405 30 mg/kg (n = 5)	PD 69405 100 mg/kg (n = 5)	PD 72953 30 mg/kg (n = 5)	PD 72953 100 mg/kg (n = 5)
			mg/dL			
Triglycerides	83 ± 10	43 ± 7^{d}	61 ± 6^{e}	39 ± 4^{e}	34 ± 5^{e}	15 ± 2^{e}
VLDL cholesterol	5.1 ± 0.8	2.7 ± 0.6^{c}	3.7 ± 0.6	1.9 ± 0.2^{e}	1.4 ± 0.2^{e}	0.5 ± 0.1^{e}
LDL cholesterol	10.6 ± 1.4	3.7 ± 0.6^{e}	5.3 ± 0.4^{e}	1.2 ± 0.2^{e}	3.5 ± 0.7^{e}	3.3 ± 1.0^{e}
HDL cholesterol	33 ± 2	39 ± 1	34 ± 2	34 ± 4	51 ± 6^{a}	61 ± 9^{d}
			percent of control			
ApoC-III	100 ± 7	77 ± 5^{b}	83 ± 5^{a}	58 ± 6^{e}	70 ± 6^{c}	66 ± 5^{d}
ApoC-I	100 ± 4	67 ± 7^{e}	69 ± 2^{e}	37 ± 3^{e}	40 ± 3^{e}	47 ± 3^{e}
ApoE	100 ± 1	109 ± 5	99 ± 3	108 ± 4	117 ± 8^{a}	128 ± 8^{c}
ApoA-I	100 ± 6	81 ± 4	83 ± 4	78 ± 3^{a}	146 ± 7^{e}	148 ± 12^{e}
ApoB	100 ± 8	49 ± 3^{e}	69 ± 5^{e}	30 ± 3^{e}	48 ± 6^{e}	44 ± 9^{e}

Animals were dosed by oral gavage with compounds at the indicated daily dose for 1 week. Plasma determinants were measured as described in Materials and Methods. Data represent mean ± SE of the mean for the indicated number of rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.005$; $^{d}P < 0.0005$; $^{e}P < 0.0001$.

(Fig. 9), compared to each other, ^{125}I-labeled apoB clearance with PD 72953 was slowest relative to gemfibrozil, followed by PD 69405. This order is related to the magnitude of change in HDL cholesterol content and size (Figs. 2 and 10C).

Previous studies have shown that certain hypolipidemic drugs, including fibrates and MEDICA 16, are direct activators of PPAR transcription factors (4, 6, 7, 31, 32). Increased liver to body weight (**Table 3**) as well as increased hepatic carnitine acetyltransferase activity (Table 3) suggested a mechanism of action of PD 72953 perhaps consistent with activation of the nuclear hormone receptor PPARα in rats. It has been proposed that these drugs modify lipoprotein levels by regulating

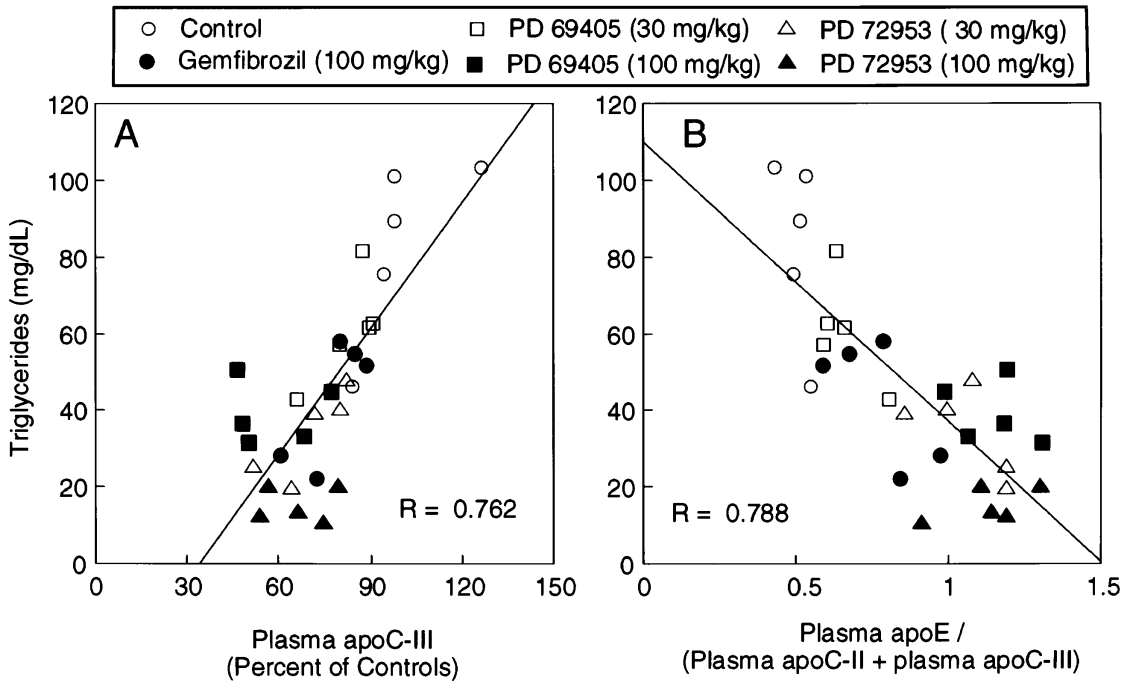

Fig. 7. Relation between plasma triglycerides and (A) apoC-III and (B) the ratio of plasma apoE to apoC-II plus apoC-III. Male Sprague-Dawley rats (n = 5/group) were dosed by oral gavage with compounds at the indicated daily dose for 1 week. Control animals were dosed with vehicle alone. Plasma triglycerides and apolipoproteins were determined as described in Materials and Methods and are enumerated in Table 1.

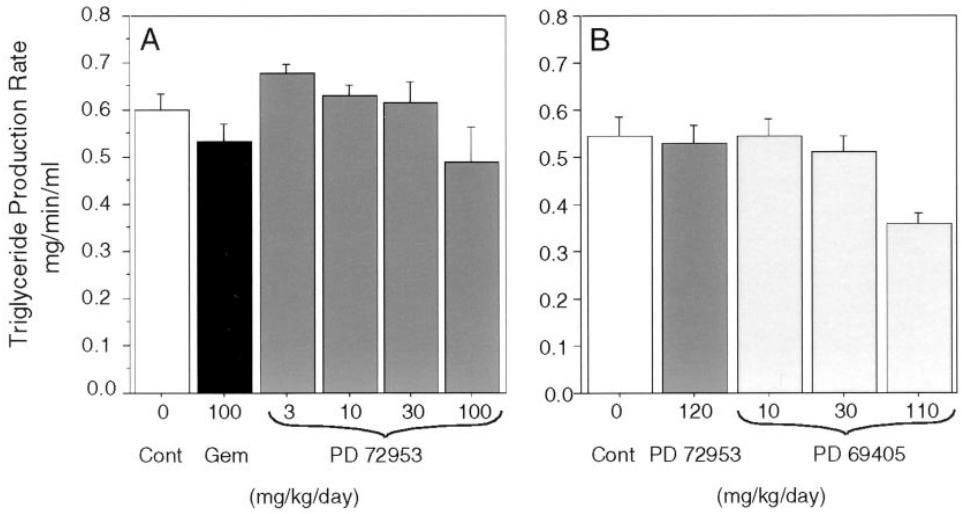

Fig. 8. Triglyceride production rates. (A) Rats were treated for vehicle alone (n = 10), 100 mg/kg gemfibrozil (n = 5), or 3 mg/kg (n = 5), 10 mg/kg (n = 5), 30 mg/kg (n = 5), or 100 mg/kg (n = 5) PD 72953 per day for 8 days. (B) Rats were treated for vehicle alone (n = 5), 120 mg/kg PD 72953 (n = 5), or 10 mg/kg (n = 5), 30 mg/kg (n = 4), or 110 mg/kg (n = 5) PD 69405 per day for 8 days. For both studies, rats were fasted overnight (midnight to 8 AM), then dosed with compound on day 8. Two hours post compound dosing, a basal tail-vein blood sample was drawn. Triton WR 1339 (600 mg/kg in normal saline) was then administered intravenously and additional blood samples were obtained at 2 h post Triton WR 1339 administration. The difference between the basal and 2 h post Triton measurements plus the blood volume (estimated from body weight) were used to calculate the triglyceride production rate. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls. a, $P = 0.001$.

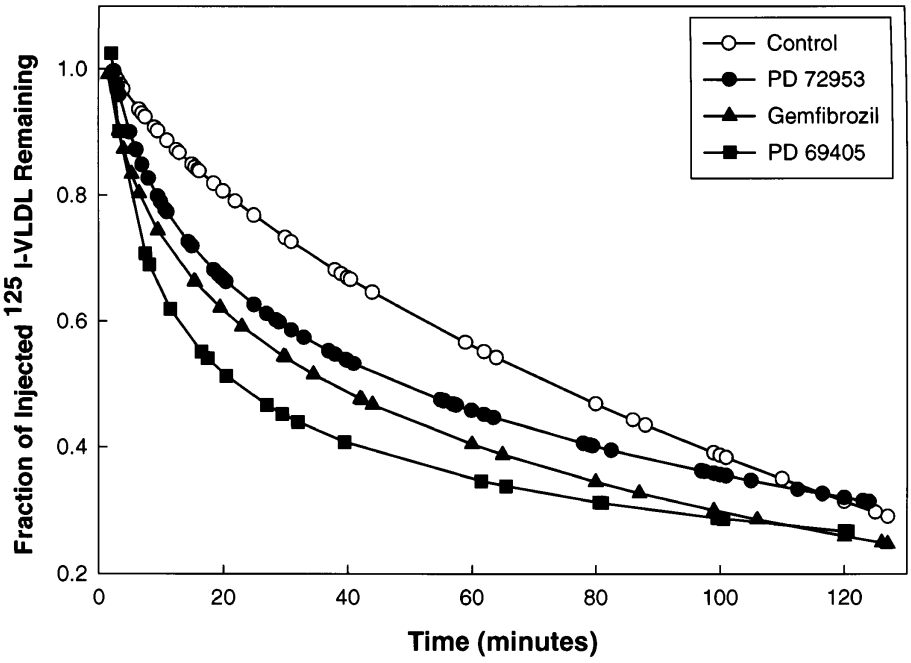

Fig. 9. ^{125}I-labeled VLDL apoB disappearance in control (n = 7), gemfibrozil- (n = 3), PD 72953- (n = 7), and PD 69405 (n = 2)-treated rats. Rats were dosed at 100 mg/kg per day for 8 days as described above in Fig. 7. Two hours past the last dose on day 8, rats were intravenously administered ^{125}I-labeled VLDL, and periodically bled over a 2-h period. ^{125}I-labeled VLDL apoB radioactivity was determined by isopropanol precipitation. Data curves are representative of a computer-generated fit (Sigmaplot 3.0, SPSS Inc., Chicago, IL) of the average of all data points within each group.

TABLE 2. Post-heparin lipolytic activities

Treatment	Post-Heparin Lipolytic Activity (nM FFA hydrolyzed/min/ml) PHLA	HL	LPL	Ratio (HL/LPL)
Control (n = 10)	688 ± 36	398 ± 33	289 ± 56	1.4
PD 72953 (n = 9)	672 ± 32	417 ± 21	255 ± 33	1.6
Gemfibrozil (n = 11)	543 ± 42[a]	400 ± 51	147 ± 29[b]	2.7
PD 69405 (n = 11)	533 ± 40[b]	454 ± 29	81 ± 22[c]	5.6

Male Sprague-Dawley rats were treated with the indicated drug or vehicle for 8 days. Animals were dosed between 8–10 AM on day 8 and injected with heparin 4 h later. Animals were bled 10 min post heparin injection for determination of total and hepatic lipoprotein lipase activities. Lipoprotein lipase activity was determined by difference. Data were derived from two separate experiments with 4–6 animals per group for each experiment. Data are the mean ± SE of the mean. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0005$.

the expression of genes involved in lipid metabolism by a PPAR-mediated mechanism.

To determine whether PD 72953 is a PPAR activator, the effect of the drug on transcription from the PPAR-dependent rat acyl-CoA oxidase (ACO) gene promoter was examined in transiently transfected HepG2 cells. The addition of PD 72953 to transfected cells induced a 4-fold increase in transcription from the ACO promoter (**Fig. 11A**) that was similar to the stimulation caused by the well-characterized PPARα ligand WY 14643 (33). In addition to the co-transfected PPARα, HepG2 cells also contain endogenous PPARγ. This makes it difficult to determine which PPAR isoform is responsible for the activation by PD72953. To determine the activity of PD72953 on each individual isoform, a chimeric receptor assay was utilized. Human kidney 293 cells were transfected with chimeric receptors composed of the ligand binding domain of a mouse PPAR receptor linked to the DNA binding domain of the bacterial tet repressor. These receptors were cotransfected with a luciferase reporter driven by a minimal promoter containing tet repressor binding site element. In the presence of this reporter and a single chimeric receptor, an increase in luciferase activity indicates the specific activation of the transfected re-

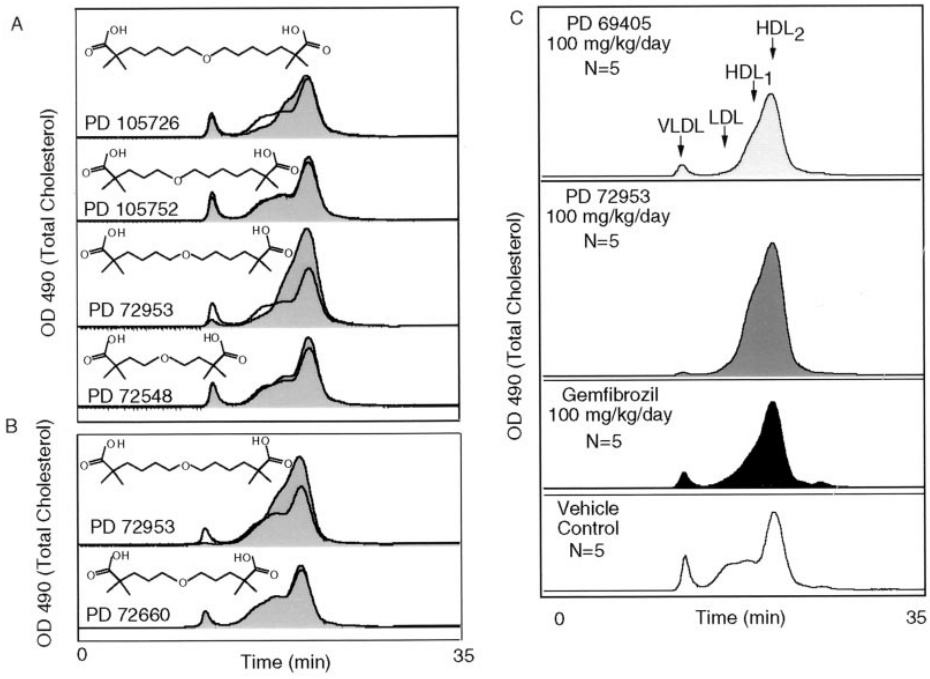

Fig. 10. Comparison of PD 72953 to structurally related carboxyalkylethers (A) PD 105726, PD 105752, or PD 72548 and (B) PD 72660 for lipoprotein cholesterol profiles in chow-fed male Sprague-Dawley rats. Animals were dosed for 1 week at 30 mg/kg per day. Profiles were obtained from individual animals from each group. Shown are the pooled average profiles from each group of 5 rats per group. Averaged profiles are typical of the individual profiles of the groups. Profiles in (A) and (B) are from separate experiments. For both experiments the open profiles from vehicle-treated animals are overlaid on the shaded profiles of the carboxyalkylether-treated animals. (C) Comparison of lipoprotein cholesterol profiles from male rats treated with PD 69405, PD 72953, gemfibrozil, or vehicle. Animals (n = 5/group) were dosed daily by oral gavage with compounds at 100 mg/kg for 1 week. Profiles were obtained from individual animals from each group. Shown are the pooled average profiles from a representative experiment. See Table 1 and Fig. 7 for additional data collected from this experiment.

TABLE 3. Hepatic peroxisomal proliferation indicators

Treatment (mg/kg)	n	Liver/Body Weight	*P* Value	Carnitine Acetyltransferase	*P* Value
		%		*nmol/min/mg*	
Duration, 2 weeks					
Control	8	4.50 ± 0.04		0.98 ± 0.12	
Gemfibrozil (100)	8	5.72 ± 0.19	<0.0001	6.90 ± 0.12	<0.0001
PD 72953 (1)	8	4.53 ± 0.10	ns	1.38 ± 0.10	ns
PD 72953(3)	8	4.81 ± 0.08	0.0394	2.27 ± 0.13	ns
PD 72953 (10)	8	5.66 ± 0.07	<0.0001	4.59 ± 0.27	<0.0001
PD 72953 (30)	8	6.51 ± 0.07	<0.0001	6.92 ± 0.98	<0.0001
PD 72953 (100)	8	6.87 ± 0.11	<0.0001	6.50 ± 0.43	<0.0001
Duration, 1 week					
Control	5	4.25 ± 0.10		nd	
Gemfibrozil (100)	5	5.26 ± 0.17	<0.0001	nd	
PD 69405 (30)	5	5.30 ± 0.17	<0.0001	nd	
PD 69405 (100)	5	5.87 ± 0.11	<0.0001	nd	
PD 72953 (30)	5	5.81 ± 0.07	<0.0001	nd	
PD 72953 (100)	5	6.39 ± 0.16	<0.0001	nd	

Liver to body weight and hepatic carnitine acetyltransferase activity determined in treated Sprague-Dawley male rats. Animals were dosed by oral gavage with compounds at the indicated daily dose for 1 or 2 weeks. Data represent mean ± SEM for the indicated number of rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls; nd, not determined; ns, not significant.

ceptor. When this experiment was performed with chimeric α, γ, and δ receptors (Fig. 11B) the results indicated that PD72953 was a mild activator of PPARγ (1.41-fold) and had no activity on the other two isoforms. In this assay, positive controls using WY 14643, BRL 49653, and EPA were each able to markedly activate the PPARα, PPARγ, and PPARδ receptors, respectively. The reduced effect of PD 72953 in the chimeric assay compared to the HepG2 cell assay (Fig. 11A) may be due to differences in cell type (liver vs. kidney) or species origin of the receptors (human native receptors vs. mouse chimeric receptors). However, taken together these results indicate that PD 72953 is a mild PPAR activator and suggest a possible mechanism for the lipid-lowering effect of the drug.

DISCUSSION

Elevated plasma triglycerides and low levels of HDL may each increase the risk of coronary and vascular disease (34–37). Although the fibrates are generally indicated for the treatment of hypertriglyceridemia, these compounds have variable effects on HDL apolipoproteins and cholesterol elevation in rats (5) and humans (38). For example, gemfibrozil was unique in its ability to elevate HDL-cholesterol and apoE compared to other fibrates at comparable doses in preclinical models (5, 8). Recently, the observation that fibrates down-regulate apoC-III gene expression suggested a potential mechanism for their hypolipidemic properties (4, 5, 8). As apolipoprotein C-III normally inhibits the clearance of triglyceride-rich lipoproteins, its down-regulation reduces this inhibition (39).

Differential regulation of apolipoproteins by fibrates may be a species-specific phenomenon. For example, Berthou et al. (40) have recently shown that the human apoA-I transgene in mice was activated by fenofibrate and, to a lesser extent, by gemfibrozil. In contrast, the endogenous mouse A-I gene was down-regulated by fenofibrate (40). Similarly, Staels et al. (10) have shown that fenofibrate, clofibrate, or gemfibrozil treatment markedly reduced hepatic apoA-I mRNA levels in male chow-fed rats. The marked up-regulation of the human apoA-I transgene in mice was also somewhat surprising as cotransfection experiments of human apoA-I promoter–CAT constructs in HepG2 cells demonstrated apoA-I down-regulation by fenofibrate (41). Although the cotransfection of mouse PPARα rescued the negative fenofibrate-induced response, apoA-I expression levels were not elevated above controls. These data led these investigators to conclude that the human apoA-I promotor contains a functional PPRE (41). Thus, the fenofibrate up-regulation of human apoA-I in these transgenic mice suggests that additional factors, possibly present in mouse liver or on the transgene, are necessary to elicit enhanced human apoA-I transcription.

Previous studies have shown that certain hypolipidemic drugs, including fibrates and MEDICA-16, are direct activators of PPAR transcription factors (4, 6, 7, 31, 32). Fibrates appear to act through these transcrip-

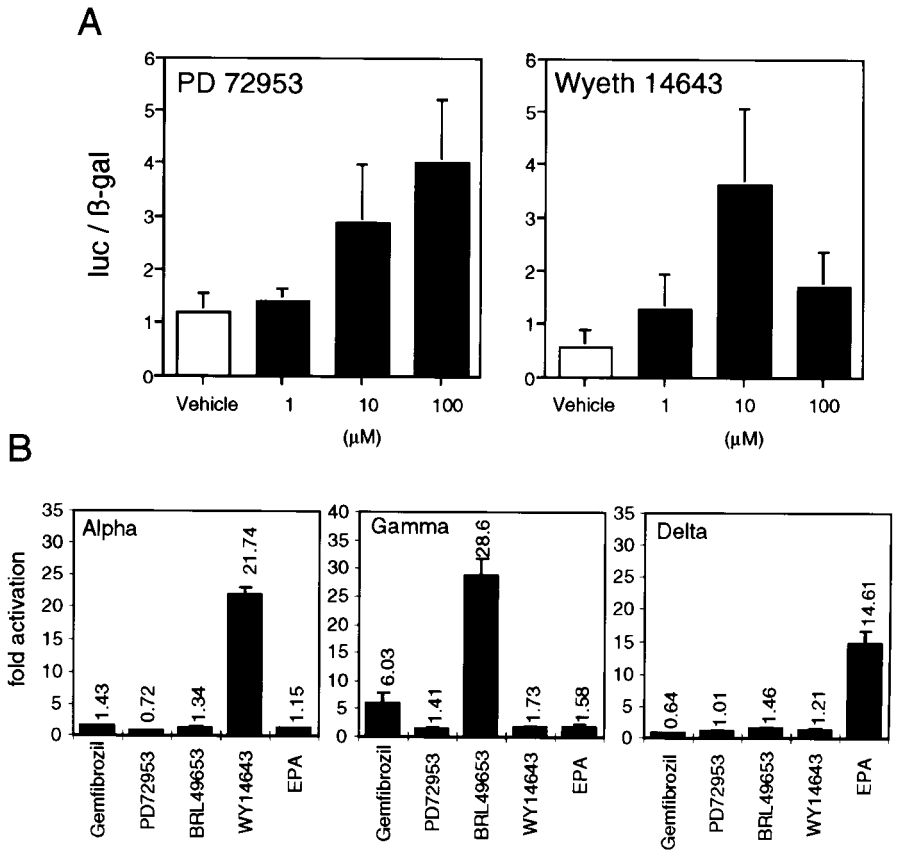

Fig. 11. PD 72953 activates PPAR-mediated transcription. (A) Transcription from a rat acylCoA oxidase promoter/luciferase reporter construction was activated by PD 72953 in transfected HepG2 cells. Cells were co-transfected with an hPPARα expressing plasmid and a CMV-βgal internal reference plasmid, and treated for 48 h with DMSO (vehicle) PD 72953 or the PPARα activator WY 14643. Data are expressed as a ratio of luciferase to βgal activity and are the means of three independent experiments. Error bars are standard deviation. (B) Activation of chimeric receptors containing the ligand binding domain of either PPARα, PPARγ, or PPARδ fused to the tet repressor DNA binding domain. Human kidney 293 cells were transfected with a chimeric PPAR receptor together with a luciferase reporter containing the tet repressor recognition element and a CMV-βgal internal reference plasmid. Cells were treated for 48 h with vehicle or drug (50 μm) as indicated. The effect of each drug is presented relative to the vehicle control and is the average of three experiments. Error bars are standard deviation.

tion factors at peroxisomal proliferation response elements (PPREs) that are present on a variety of genes associated with lipid metabolism enzymes (6). PPARs, which are comprised of three major subtypes (PPARα, PPARδ also known as PPARβ, and PPARγ) heterodimerize with other nuclear receptors (RXR) and bind PPRE within promotors (6, 32) of responsive genes. Therefore, fibrates may modify lipoprotein levels by regulating the expression of genes involved in lipid metabolism. Perhaps fibrates and PD 72953 mimic natural ligand(s), possibly diacids, fatty acids, prostaglandins, and leukotrienes that bind to specific nuclear receptors (31, 33, 42–46). Our results suggest that PD 72953 is a PPAR ligand and suggest a possible mechanism for the lipid-lowering effects of the drug.

In rodents, fibric acids are known to cause peroxisomal prolieferation, a phenomenon that is generally absent in non-human primates and humans (47–53). However, although high and chronic clofibrate or fenofibrate treatments have failed to demonstrate peroxisomal proliferation in non-human primates (47), a high dose of ciprofibrate will induce peroxisomal proliferation and therefore it has been suggested that fibrate activation of peroxisomal enzymes is a dose-dependent rather than a species-specific phenomenon (54). It should be noted that ciprofibrate in rats appears to be one of the most potent peroxisomal proliferators (27). Apolipoprotein C-III, whose gene contains a PPRE in its promoter, is down-regulated by fibrates. However, in contrast to the differential regula-

tion of peroxisomal enzymes, apoC-III appears to be commonly regulated by fibrates in primates and rodents (4). Whether these apparent differences in physiological response are due to divergence of the PPRE of apoC-III and peroxisomal enzymes, the nuclear receptors, or a 'factor' present in rats and mice but not in humans has not been determined. Interestingly, livers from athymic mice show a peroxisomal proliferative response to clofibrate; however, heterotransplantation of human hepatoma cell lines in these same mice failed to elicit a response to the drug (53). In similar experiments, transplantation of donor rat hepatocytes to recipient rats was responsive to ciprofibrate (52). Recently, Lee et al. (55) reported that fibrate-treated mice genetically deficient in PPARα are resistant to peroxisomal proliferation. Lastly, studies of Staels et al. (4) demonstrated that fenofibrate-treated cultured primary human hepatocytes displayed a divergence in apoC-III mRNA levels (lowered) from that of ACO mRNA levels (no change). Taken together, these data suggest that a species-specific template or transcription factor may be responsible for induction of peroxisomal genes to PPAR activators in rodents but are not responsible for repression of the apoC-III gene.

Studies with a compound structurally similar to PD 72953 demonstrated similar plasma triglyceride reduction; however, a differential effect on the amount and size of HDL was observed. Specifically, the alkyl compound (PD 69405) of similar length to that of PD 72953 caused no increase in HDL cholesterol content and no apparent change in particle size. Post-heparin lipase activitites (measured under V_{max} conditions in the presence of exogenously added apoC-II) were performed in rats treated with gemfibrozil, PD 72953, or PD 69405. Though hepatic lipase activities were similar for all treatment groups, LPL activity was markedly different among the groups, with PD 72953 having the highest LPL activity followed by gemfibrozil, then PD 69405. The increased HL to LPL ratio in PD 69405-treated rats may be also responsible for maintaining a smaller HDL population. In addition, the higher levels of HL to LPL in the PD 69405-treated rats may also enhance the clearance of remnants to liver (56). Interestingly, the disappearance of ^{125}I-lableled VLDL apoB in plasma was enhanced to a greater extent with PD 69405 compared to both PD 72953 and gemfibrozil. Perhaps the maintenance of a high LPL level in conjunction with a reduced apoC-III level permits a greater degree of peripheral tethering of remnants (to LPL), thereby generating increased amounts of phospholipid for generation of HDL. These data raise the possibility that differential effects of these compounds on VLDL catabolism can lead to alterations in the amount of HDL that persists in plasma. It is of interest that HDL levels are, in fact, markedly reduced when HL is overexpressed in the transgenic rabbits (56) or mice (57). In mice, HL overexpression has also been shown to reduce HDL size (57).

In our previous (5, 8) and current studies in male rats we observed that gemfibrozil and now PD 72953 cause a marked elevation and enlargement of HDL. These effects were not observed with other fibrates or compounds structurally similar to PD 72953 although triglyceride reduction was commonly observed with most of these compounds (5, 8). Because apoC-III inhibits lipoprotein lipase activity (58), we suspect that the reduced amount of apoC-III accelerates the lipolytic process and is likely a common effect of most of these compounds. A consequence would be the rapid formation of IDL (remnants) and reduced triglyceride levels. Perhaps the longer peripheral retention of IDL, as suggested by a slower ^{125}I-labeled apoB clearance with PD 72953 relative to PD 69405, combined with the enhanced lipolysis, leads to an increased generation of redundant remnant 'phospholipid' surface, which is then transferred to a pre-existing HDL pool. Alternatively, the generation of excess phospholipid may lead to the formation of nascent HDL. In either case, the phospholipid may act as a 'sink' that can accumulate unesterified cholesterol from both tissues and pre-existing lipoproteins.

Overall, our previous (5, 8) and current data suggest that fibrates and diacids may have some common structural and functional similarities, but also demonstrate distinct pharmacologic profiles. Likely, these compounds and/or their metabolites mimic ligands for the PPAR family of nuclear receptors, or possibly regulate intracellular levels of these receptors themselves (6). The effectiveness of the compounds on regulating metabolism will depend on a large variety of factors and include the ligands' PPAR specificity, tissue distribution, and metabolism. Furthermore, the species-specific diversity of the PPARs and their response elements will also dictate the physiological response to both natural and synthetic ligands.

The authors gratefully acknowledge the generosity of Dr. Patrick Tso of Lousiana State University Medical Center (Shreveport, LA) for providing us with goat antiserum to rat apoE, and to Dr. Peter J. Dolphin of Dalhousie University (Halifax, Nova Scotia, Canada) for providing goat antiserum to rat apoC-II and rat apoC-III. The authors thank Dr. Brian Krause of Parke-Davis/Warner-Lambert for valuable discussion. We also thank Ellen Urda, Tim Braden, and Dr. Donald Robertson, also from Parke-Davis/Warner-Lambert, for measurement of hepatic carnitine acetyltransferase activities.

Manuscript received 3 February 1997 and in revised form 25 July 1997.

REFERENCES

1. LaRosa, J. C. 1990. At what levels of total low- or high-density lipoprotein cholesterol should diet/drug therapy be initiated? United States Guidelines. *Am. J. Cardiol.* **65:** 7F–10F.
2. Gaw, A., and J. Shepherd. 1991. Fibric acid derivatives. *Curr. Opin. Lipidol.* **2:** 39–42.
3. Larsen, M. L., and D. R. Illingworth. 1993. Triglyceride-lowering agents: fibrates and nicotinic acid. *Curr. Opin. Lipidol.* **4:** 34–40.
4. Staels, B., N. Vu-Dac, V. A. Kosykh, R. Saladin, J-C. Fruchart, J. Dallongeville, and J. Auwerx. 1995. Fibrates down-regulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. *J. Clin. Invest.* **95:** 705–712.
5. Haubenwallner, S., A. D. Essenburg, B. C. Barnett, M. E. Pape, R. B. DeMattos, B. R. Krause, L. L. Minton, B. J. Auerbach, R. S. Newton, T. Leff, and C. L. Bisgaier. 1995. Hypolipidemic activity of select fibrates correlates to changes in hepatic apolipoprotein C-III expression: a potential physiologic basis for their mode of action. *J. Lipid Res.* **36:** 2541–2551.
6. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37:** 907–925.
7. Hertz, R., J. Bishara-Shieban, and J. Bar-Tana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs. Supression of apolipoprotein C-III. *J. Biol. Chem.* **270:** 13470–13475.
8. Krause, B. R., B. C. Barnett, A. D. Essenburg, K. A. Kieft, B. J. Auerbach, R. Bousley, R. Stanfield, R. S. Newton, and C. L. Bisgaier. 1996. Opposite effects of bezafibrate and gembifrozil in both normal and hypertriglyceridemic rats. *Atherosclerosis.* **127:** 91–101.
9. Krause, B. R., and R. S. Newton. 1985. Apolipoprotein changes assiciated with the plasma lipid-regulating activity of gemfibrozil in cholesterol-fed rats. *J. Lipid Res.* **26:** 940–949.
10. Staels, B., A. van Tol, T. Andreu, and J. Auwerx. 1992. Fibrates influence the expression of genes involved in lipoprotein metabolism in a tissue-selective manner in the rat. *Arterioscler. Thromb.* **12:** 286–294.
11. Eisenberg, S., and D. Rachmilewitz. 1975. Interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma. *J. Lipid Res.* **16:** 341–351.
12. Blanchette-Mackie, E. J., and R. O. Scow. 1976. Retention of lipolytic products in chylomicrons incubated with lipoprotein lipase: electron microscope study. *J. Lipid Res.* **17:** 57–67.
13. Tall, A. R., and D. M. Small. 1980. Body cholesterol removal: role of plasma high-density lipoproteins. *Adv. Lipid Res.* **17:** 1–51.
14. Tall, A. R., and P. H. R. Green. 1981. Incorportation of phosphatidylcholine into spherical and discoidal lipoproteins during incubation of egg phosphatidylcholine vesicles with isolated high density lipoproteins or with plasma. *J. Biol. Chem.* **256:** 2035–2044.
15. Tall, A. R., P. H. R. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apolipoproteins in the rat. *J. Clin. Invest.* **64:** 977–989.
16. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
17. Johnson, G. L., L. L. Swift, and L. L. Rudel. 1987. Nascent lipoproteins from recirculating and nonrecirculating liver perfusions and from the hepatic Golgi apparatus of African green monkeys. *J. Lipid Res.* **28:** 549–564.
18. Bearnot, H. R., R. M. Glickman, L. Weinberg, P. H. R. Green, and A. R. Tall. 1982. Effect of a biliary diversion on mesenteric lymph apolipoprotein A-I and high density lipoproteins. *J. Clin. Invest.* **69:** 210–217.
19. Forester, G. P., A. R. Tall, C. L. Bisgaier, and R. M. Glickman. 1983. Rat intestine secretes spherical high density lipoproteins. *J. Biol. Chem.* **258:** 5938–5943.
20. Bisgaier, C. L., and R. M. Glickman. 1983. Intestinal synthesis, secretion, and transport of lipoproteins. *Annu. Rev. Physiol.* **45:** 625–636.
21. Holt, P. R., and A. A. Dominguez. 1980. Triton-induced hyperlipidemia: a model for studies of intestinal lipoprotein production. *Am. J. Physiol.* **238:**G453–G457.
22. Kuusi, T., E. A. Nikkila, M-R. Taskinen, P. Somerharju, and C. Ehnholm. 1982. Human postheparin plasma hepatic lipase activity against triavylglycerol and phospholipid substrates. *Clin. Chim. Acta.* **122:** 39–45.
23. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
24. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature.* **182:** 53–57.
25. Egusa, G., D. W. Brady, S. M. Grundy, and B. V. Howard. 1983. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24:** 1261–1267.
26. Kieft, K. A., T. M. A. Bocan, and B. R. Krause. 1991. Rapid on-line determination of cholesterol distribution among plasma lipoproteins after high-performance gel-filtration chromatography. *J. Lipid Res.* **32:** 859–866.
27. Krause, B. R., R. Bousley, K. Kieft, D. Robertson, R. Stanfield, E. Urda, and R. S. Newton. 1994. Comparison of lifibrol to other lipid regulating agents in experimental animals. *Pharmacol. Res.* **29:** 345–357.
28. Pape, M. E., G. W. Melchior, and K. R. Marotti. 1991. mRNA quantitation by a simple and sensitive RNAse protection assay. *Genet. Anal.* **8:** 206–213.
29. Rea, T. J., R. B. Demattos, and M. E. Pape. 1993. Hepatic expression of genes regulating lipid metabolism in rabbits. *J. Lipid Res.* **34:** 1901–1910.
30. Marrino, P., D. Gavish, E. Shafrir, and S. Eisenberg. 1987. Diurnal variations of plasma lipids, tissue and plasma lipoprotein lipase, and VLDL secretion rates in the rat. A model for studies of VLDL metabolism. *Biochim. Biophys. Acta.* **920:** 277–284.
31. Auwerx, J. 1993. Regulation of gene expression by fatty acids and fibric acid derivatives: an integrative role for peroxisome proliferator activated receptors. *Horm. Res.* **38:** 269–277.
32. Schoonjans, K., J. Peinado-Onsurbe, A.-M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. 1996. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* **15:** 5336–5348.
33. Kliewer, S. A., J. M. Lenhard, T. M. Wilson, I. Patel, D. C. Morris, and J. Lehmann. 1995. A prostaglandin J2 metab-

olite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell.* **83:** 813–819.
34. Havel, R. 1994. McCollum Award Lecture, 1993: Triglyceride-rich lipoproteins and atherosclerosis—new perspectives. *Am. J. Clin. Nutr.* **59:** 795–799.
35. Lindenstrøm, E., G. Boysen, and J. Nyboe. 1994. Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerides on risk of cerebrovascular disease: the Copenhagen City Heart Study. *Br. Med. J.* **309:** 11–15.
36. Zilversmit, D. B. 1995. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin. Chem.* **41:** 153–158.
37. Consensus Development Panel. 1992. Triglyceride, high density lipoprotein, and coronary heart disease. *NIH Consensus Statement.* **10:** 1–28.
38. Frick, M. H., O. Elo, K. Haapa, O. P. Heinonen, P. Heinsalmi, P. Helo, J. K. Huttunen, P. Kaitaniemi, P. Koskinen, V. Manninen, H. Mäenpää, M. Mälkönen, M. Mänttäri, S. Norola, A. Pasternack, J. Pikkarainen, M. Romo, T. Sjöblom, and E. A. Nikkilä. 1987. Helsinki heart study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N. Engl. J. Med.* **317:** 1237–1245.
39. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* **255:** 8303–8307.
40. Berthou, L., N. Duverger, F. Emmanuel, S. Langouët, J. Auwerx, A. Guillouzo, J-C. Fruchart, E. Rubin, P. Denèfle, B. Staels, and D. Branellec. 1996. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J. Clin. Invest.* **97:** 2408–2416.
41. Vu-Dac, N., K. Schoonjans, B. Laine, J-C. Fruchart, J. Aureux, and B. Staels. 1994. Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. *J. Biol. Chem.* **269:** 31012–31018.
42. Ockner, R. K., R. M. Kaikaus, and N. M. Bass. 1993. Fatty-acid metabolism and the pathogenesis of hepatocellular carcinoma: review and hypothesis. *Hepatology.* **18:** 669–676.
43. Yu, K., W. Bayona, C. B. Kallen, H. P. Harding, C. P. Ravera, G. McMahon, M. Brown, and M. A. Lazar. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* **270:** 23975–23938.
44. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell.* **79:** 1147–1156.
45. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPAγ. *Cell.* **83:** 803–812.
46. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli. 1996. The PPARα-leutotiene B_4 pathway to inflammation control. *Nature.* **384:** 39–43.
47. Cohen, A. J., and P. Grasso. 1981. Review of the hepatic response to hypolipidaemic drugs in rodents and assessment of its toxicological significance to man. *Food Cosmet. Toxicol.* **19:** 585–605.
48. De La Iglesia, F. A., J. E. Lewis, R. A. Buchanan, E. L. Marcus, and G. McMahon. 1982. Light and electron microscopy of liver in hyperlipoproteinemic patients under long-term gemfibrozil treatment. *Atherosclerosis.* **43:** 19–37.
49. Hanefeld, M., C. Kemmer, and E. Kadner. 1983. Relationship between morphological changes and lipid-lowering action of *p*-chlorphenoxyisobutyric acid (CPIB) on hepatic mitochondria and peroxisomes in man. *Atherosclerosis.* **46:** 239–246.
50. Gariot, P., E. Barrat, L. Mejean, J. P. Pointel, P. Drouin, and G. Debry. 1983. Fenofibrate and human liver. Lack of proliferation of peroxisomes. *Arch. Toxicol.* **53:** 151–163.
51. Blümcke, S., W. Schwartzkopff, H. Lobeck, N. A. Edmondson, D. E. Prentice, and G. F. Blane. 1983. Influence of fenofibrate on cellular and subcellular liver structure in hyperlipidemic patients. *Atherosclerosis.* **46:** 105–116.
52. Reddy, J., R. L. Jirtle, T. K. Watanabe, N. K. Reddy, G. Michalopoulos, and S. A. Qureshi. 1984. Response of hepatocytes transplanted into syngeneic hosts and heterotransplanted into athymic nude mice to peroxisome proliferators. *Cancer Res.* **44:** 2582–2589.
53. Hertz, R., J. Arnon, A. Hoter, D. Shouval, and J. Bat-Tana. 1987. Clofibrate does not induce peroxisomal proliferation in human hepatoma cell lines PLC/PRF/5 and SK-HEP-1. *Cancer Lett.* **34:** 263–272.
54. Reddy, J. K., N. D. Lalwani, S. A. Qureshi, M. K. Reddy, and C. M. Moehle. 1984. Induction of hepatic peroxisomal proliferation in nonrodent species, including primates. *Am. J. Pathol.* **114:** 171–183.
55. Lee, S. S.-T., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal, and F. Gonzalez. 1995. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15:** 3012–3022.
56. Fan, J., J. Wang, A. Bensadoun, S. J. Lauer, Q. Dang, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **91:** 8724–8728.
57. Busch, S. J., R. L. Barnhart, G. A. Martin, M. C. Fitzgerald, M. T. Yates, S. J. T. Mao, C. E. Thomas, and R. L. Jackson. 1994. Human hepatic triglyceride lipase expression reduces high density lipoprotein and aortic cholesterol in cholesterol-fed transgenic mice. *J. Biol. Chem.* **269:** 16376–16382.
58. Wang, C.-S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75:** 384–390.

ERRATUM

In the article "A novel compound that elevates high density lipoprotein and activates the peroxisome proliferator activated receptor" by Bisgaier et al. which appeared in the January 1998 issue of the *Journal of Lipid Research* (**39:** 17–30), there was a typographical error in Table 1. In the first column, headed "Plasma Determinant," "ApoC-I" should be "ApoC-II." The corrected table is reproduced below.

TABLE 1. Plasma apolipoprotein and lipid levels in gemfibrozil-, PD 69405-, and PD 72953-treated male Sprague-Dawley rats

Plasma Determinant	Control (n = 5)	Gemfibrozil 100 mg/kg (n = 5)	PD 69405 30 mg/kg (n = 5)	PD 69405 100 mg/kg (n = 5)	PD 72953 30 mg/kg (n = 5)	PD 72953 100 mg/kg (n = 5)
			mg/dL			
Triglycerides	83 ± 10	43 ± 7^{d}	61 ± 6^{e}	39 ± 4^{e}	34 ± 5^{e}	15 ± 2^{e}
VLDL cholesterol	5.1 ± 0.8	2.7 ± 0.6^{c}	3.7 ± 0.6	1.9 ± 0.2^{e}	1.4 ± 0.2^{e}	0.5 ± 0.1^{e}
LDL cholesterol	10.6 ± 1.4	3.7 ± 0.6^{e}	5.3 ± 0.4^{e}	1.2 ± 0.2^{e}	3.5 ± 0.7^{e}	3.3 ± 1.0^{e}
HDL cholesterol	33 ± 2	39 ± 1	34 ± 2	34 ± 4	51 ± 6^{a}	61 ± 9^{d}
			percent of control			
ApoC-III	100 ± 7	77 ± 5^{b}	83 ± 5^{a}	58 ± 6^{e}	70 ± 6^{c}	66 ± 5^{d}
ApoC-II	100 ± 4	67 ± 7^{e}	69 ± 2^{e}	37 ± 3^{e}	40 ± 3^{e}	47 ± 3^{e}
ApoE	100 ± 1	109 ± 5	99 ± 3	108 ± 4	117 ± 8^{a}	128 ± 8^{c}
ApoA-I	100 ± 6	81 ± 4	83 ± 4	78 ± 3^{a}	146 ± 7^{e}	148 ± 12^{e}
ApoB	100 ± 8	49 ± 3^{e}	69 ± 5^{e}	30 ± 3^{e}	48 ± 6^{e}	44 ± 9^{e}

Animals were dosed by oral gavage with compounds at the indicated daily dose for 1 week. Plasma determinants were measured as described in Materials and Methods. Data represent mean ± SE of the mean for the indicated number of rats per group. ANOVA, Fisher PLSD post hoc analysis was used for determination of significant mean difference compared to controls: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.005$; $^{d}P < 0.0005$; $^{e}P < 0.0001$.