Fibrate treatment in mice is known to modulate high density lipoprotein (HDL) metabolism by regulating apolipoprotein (apo)AI and apoAII gene expression. In addition to alterations in plasma HDL levels, fibrates induce the emergence of large, cholesteryl ester-rich HDL in treated transgenic mice expressing human apoAI (HuA-ITg). The mechanisms of these changes may not be restricted to the modulation of apolipoprotein gene expression, and the aim of the present study was to determine whether the expression of factors known to affect HDL metabolism (i.e. phospholipid transfer protein (PLTP), lecithin:cholesterol acyltransferase, and hepatic lipase) are modified in fenofibrate-treated mice. Significant rises in plasma PLTP activity were observed after 2 weeks of fenofibrate treatment in both wild-type and HuA-ITg mice. Simultaneously, hepatic PLTP mRNA levels increased in a dose-dependent fashion. In contrast to PLTP, lecithin:cholesterol acyltransferase mRNA levels in HuA-ITg mice were not significantly modified by fenofibrate despite a significant decrease in plasma cholesterol esterification activity. Fenofibrate did not induce any change in hepatic lipase activity. Feno-
cause PPARα has been shown to mediate the effects of fenofibrate on several genes, we sought to test the hypothesis that PPARα is involved in the observed changes using PPARα-deficient mice.

**Materials and Methods**

**Animals**

Mice used in this study were nontransgenic C57BL/6 mice, heterozygous HuAI-Tg mice, PLTP-deficient mice (PLTP−/−) and their wild-type controls (PLTP+/+), and homozygous PPARα-deficient mice (PPARα−/−) and their wild-type controls (PPARα+/+). HuAI-Tg mice contained 21 copies of an 11-kilobase pair human apoAI gene promoter enhancer of the human apoAI gene and the liver-specific enhancer of the human apoAI gene promoter necessary to drive hepatic apoAI expression (18). PLTP−/− were obtained by deletion of part of the PLTP gene containing exon 2. Because this exon contains the translation initiation codon and the signal peptide, mice were null PLTP animals (19). PPARα−/− mice were obtained by disrupting the ligand-binding domain of the PPARα by homologous recombination (20, 21). 10–12-week-old mice were caged in an animal room with alternating 12-h light (7 a.m. to 7 p.m.) and dark (7 p.m. to 7 a.m.) cycles and were fed either the control or fenofibrate (Sigma) diet (Sigma) ad libitum for 2 weeks. Fenofibrate was mixed in mouse chow at 0.02 and 0.2% (w/diet w), two dosages commonly used in mice (16). Body weight and food intake of the mice were regularly checked during the treatment period. No toxic side effect of fenofibrate treatment as checked by alterations in body weight was observed.

**Enzymatic Assays of PLTP, LCAT, and HL Activities**

**Plasma PLTP Activity**—PLTP activity was measured in total plasma as the transfer of radiolabeled phosphatidylcholine from [14C]phosphatidylcholine liposomes to an excess of exogenous human HDL, (22). Briefly, 10 μmol of [14C]a-phosphatidylcholine (PerkinElmer Life Sciences) and 0.1 μmol of butylated hydroxytoluene were mixed, and the lipids were dried under nitrogen, suspended in 1 ml of 150 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 7.4), sonicated, and centrifuged to remove lipid aggregates and metallic debris. Plasma samples were incubated for 1 h at 37 °C with radiolabeled liposomes (50 nmol of phospholipid) and isolated human HDL, (250 μg of protein) in a final volume of 400 μl with 150 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 7.4). At the end of the incubation, liposomes were precipitated with dextran sulfate. The supernatant (0.5 ml) was used for radioactivity determinations. Plasma volumes were chosen to keep PLTP activity in the linear range of the assay. PLTP activity was expressed as micromoles of phosphatidylcholine transferred from liposomes to exogenous human HDL, per milliliter of plasma.

**LCAT Activity**—LCAT activity was determined using an exogenous substrate according to the method described by Chen and Albers (23). Mouse plasma (50 μl) was incubated for 1 h at 37 °C with a proteoliposome substrate containing apoAI, [14C]cholesterol, and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. After incubation, lipids were extracted with chloroform/methanol (2:1, v/v) and separated by thin-layer chromatography on silica gel plates using a solvent system composed of petroleum ether/diethyl ether/acetic acid (90:10:5, v/v/v). Cholesterol ester and free cholesterol radioactivity were measured using a Packard liquid scintillation counter. LCAT activity was expressed as the percentage of free cholesterol esterified during incubation.

**Hepatic Lipase Activity**—HL activity was measured according to the method described by Henderson et al. (24) in postheparin plasma. Postheparin plasma was obtained 5 min after an intravenous bolus injection of 60 IU of sodium heparin/kg of body weight. Blood was immediately centrifuged at 4 °C and frozen at −80 °C until assay. The assay mixture for HL was prepared with 5 μCi of tri-[1-14C]oleoylglycerol, 5.68 ml of 90 g/liter Arabic gum in 0.2 M Tris-HCl, pH 8.5, 100 mg of unlabeled triolein, and 2.75 ml of 200 g/liter bovine serum albumin. After sonication, 2.58 ml of 3.24 mM NaCl in 0.2 M Tris buffer, pH 8.5, was added to the mixture. Triglyceride hydrolysis was started by adding postheparin plasma and allowed to proceed for 1 h at 37 °C. Triglyceride hydrolysis was stopped by adding 3.25 ml of chloroform/methanol/heptane (50:56:40, v/v/v) and 1 ml of 0.1 M carbonate borate buffer, pH 10.5. Reaction tubes were shaken for 5 min and centrifuged. A 0.5-ml aliquot of the upper phase was removed and counted in a scintillation counter. After correction for recovery, results were expressed in micromoles of fatty acids liberated per milliliter of plasma per hour.

**RNA Analysis**

Total cellular RNA was extracted using the guanidinium thiocyanate/phenol/chloroform method (25). Northern blot hybridizations were performed as previously described (26) using the cDNA fragments of LCAT (27) and PLTP and 28 S rRNA as probes. PLTP cDNA was amplified by reverse transcription-polymerase chain reaction using the following primers: 5′-TCG GCG GAG GGT GTG TCC AT-3′ and 5′-CAT

**Fig. 1.** Size distribution of HDL in HuAI-Tg mice before and after treatment with fenofibrate. Animals were treated for 15 days with fenofibrate (0.02 and 0.2%), and plasma was collected. Lipoproteins were isolated by sequential ultracentrifugation and separated by polyacrylamide gradient gel electrophoresis (4–20%). Gels were stained with Coomassie Brilliant Blue R250 and destained with a solution of methanol/acetic acid/water (30:58:12, v/v/v). The gels were then scanned with a densitometer (GS-300 scanning densitometer, Hoefer Scientific Instruments, San Francisco, CA). The size of lipoproteins was determined from a curve built from standards of known size (high molecular weight standards, Amersham Pharmacia Biotech) that were migrated simultaneously.
GGC AGA GTC AAA GAA GA-3' (fragment size 477 base pairs). Quantification was performed by phosphorimaging with the Molecular Analyst™ software (Bio-Rad). The relative levels of PLTP and LCAT were determined by normalizing to the level of 28S rRNA.

RESULTS

Effect of Fenofibrate on HDL Size Distribution in HuAITg Mice—As expected (16), fenofibrate treatment induced marked alterations in the plasma HDL profile in HuAITg mice. As measured by native polyacrylamide gradient gel electrophoresis, the mean apparent diameter of the most prominent HDL subpopulation rose from 11.7 nm in untreated HuAITg mice toward 15.0 and 18.0 nm in animals treated with 0.02 and 0.2% fenofibrate, respectively (Fig. 1).

PLTP, LCAT, and HL Activities in HuAITg Mice—To determine whether the modifications observed in HDL physicochemical characteristics induced by fenofibrate were linked to those of activities of lipoprotein modifying enzymes, we measured PLTP, LCAT, and HL in the plasma of HuAITg mice. PLTP activity measured in treated and nontreated mice is shown in Fig. 2a. Fenofibrate significantly increased PLTP activity in a dose-dependent fashion from 7 μmol/ml/h in nontreated mice to 22 and 37 μmol/ml/h in mice fed with 0.02 and 0.2% fenofibrate, respectively (Fig. 1).

LCAT activity was measured in the same animals using an exogenous substrate. The low dose of fenofibrate did not significantly modify LCAT activity as compared with controls, whereas enzyme activity was 3-fold lower with the high dose of fenofibrate as compared with the nontreated animals (Fig. 2a).

HL activity measured in postheparin plasma was not modified by fenofibrate treatment (results not shown).

Effect of Fenofibrate on PLTP and LCAT mRNA Levels in Livers of Human ApoAI Transgenic Mice—Phospholipid transfer and cholesterol esterification activities measured in the present study reflect plasma PLTP and LCAT concentrations, respectively. We thus checked whether the modifications of the enzyme plasma levels induced by fenofibrate were due to changes in their gene expression levels. As shown in Fig. 2b, the levels of hepatic PLTP mRNA significantly rose in a dose-dependent manner in fenofibrate-treated HuAITg mice with approximately 4- and 10-fold increases in PLTP mRNA levels observed in animals treated with 0.02 or 0.2% fenofibrate, respectively. Unlike for the PLTP gene, fenofibrate did not produce significant changes in the levels of LCAT mRNA in the livers of HuAITg treated mice (Fig. 2b).

Effect of Fenofibrate on Plasma Lipoproteins in Nontransgenic Mice—Given that the expression of both human apoAI and murine PLTP genes are increased concomitantly in HuAITg animals, the contribution of PLTP induction to the fenofibrate-induced changes in HDL size was assessed using nontransgenic mice. In nontreated wild-type C57BL/6 mice, the analysis of HDL size showed a monodisperse population of HDL with a mean apparent diameter of 11.0 nm (Fig. 3). As observed in HuAITg mice (see above), fenofibrate treatment of wild-type mice produced a shift of plasma HDL toward the large size range. One new discrete subfraction of 12.9-nm diameter appeared in mice treated with 0.02% fenofibrate, and
Fenofibrate Induces PLTP Gene Expression in Mouse

**Graphical Abstract**

**Effect of Fenofibrate on HDL Size Distribution in PLTP-deficient Mice**—Because fenofibrate exerts a marked effect on the expression of PLTP in both HuAITg and wild-type mice, the relative contribution of PLTP to the observed alterations in HDL structure was determined by comparing the effect of fenofibrate treatment on HDL size distribution in wild-type and PLTP-deficient mice. In agreement with data presented above, treatment of wild-type mice with 0.2% fenofibrate produced a marked 2-fold rise in plasma PLTP activity (data not shown). As expected (19), PLTP activity was undetectable in homozygous PLTP-deficient mice whether they were treated or not with fenofibrate (data not shown). HDL size was measured in both wild-type and PLTP-deficient mice (n = 4/group). A representative profile of an animal of each group is shown in Fig. 5. In treated wild-type mice, two types of HDL of 12.8 ± 0.5 and 11.4 ± 0.1 nm were observed, whereas HDL was monodisperse in nontreated wild-type mice (11.0 ± 0.5 nm). In contrast, fenofibrate treatment did not produce any detectable effect on the size distribution of plasma HDL (9.7 ± 0.6 and 9.3 ± 0.4 nm in nontreated versus treated PLTP-deficient mice).

**DISCUSSION**

HDL structure and metabolism are modulated by fibric acid derivatives in humans as well as in rodents. Whereas most attention has been paid to the effects of fibrates on apoAI and apoAII, their effects on the expression of other proteins involved in HDL metabolism have not been studied previously. LCAT is associated with HDL in that it generates cholesteryl esters and lysophospholipids with phospholipids and free cholesterol as substrates, driving as such the further influx of free cholesterol into HDL. As a result of the LCAT-mediated influx of core lipids, HDL tends to enlarge (28). Conversely, shrinkage of HDL as the consequence of phospholipid and triglyceride hydrolysis by HL leads to the emergence of small HDL (29). PLTP by facilitating phospholipid transfer between lipoproteins plays an important role in HDL remodeling, favoring the appearance of both large and lipid-poor apoAI-containing particles (9). Thus, plasma HDL metabolism in mice (i.e. a cholesteryl ester transfer protein-deficient species) is mainly dependent on LCAT, HL, and PLTP activities. Given that HDL was previously shown to undergo significant structural changes as the result of fenofibrate treatment in HuAITg mice (16), we postulated that one or several enzyme/transfer activities involved in HDL metabolism are modulated by fenofibrate. Therefore, the aim of the present study was to search for potential alterations in LCAT, PLTP, and HL in several mouse lines treated or not with fenofibrate.

The most striking finding of the present study is the marked dose-dependent increase in PLTP activity and hepatic mRNA levels in PPARα-deficient mice (0.2%). Treatment of PPARα−/− mice with fenofibrate increased PLTP activity from 4 to 13 μmol/ml/h, whereas no increase in PLTP activity was observed in treated PPARα−/− mice (Fig. 6a). As shown in Fig. 6b, basal PLTP mRNA levels were comparable in wild-type and PPARα−/− mice. By contrast, fenofibrate treatment induced a significant 4-fold increase in PLTP mRNA levels in livers of wild-type mice, whereas no changes in PLTP mRNA levels were observed in PPARα−/− animals.

**Table**

| Treatment | HDL Size (nm) | PLTP Activity (μmol/ml/h) |
|-----------|---------------|--------------------------|
| FF 0%     | 11.0          | 4                        |
| FF 0.02%  | 12.9          | 13                       |
| FF 0.2%   | 13.1          | 11.5                     |

**Figure 3. Size distribution of HDL in C57BL/6 mice before and after treatment with fenofibrate.** The methods were identical with those described in the legend of Fig. 1. AU, absorbance units; FF, fenofibrate.
FIG. 4. Effect of fenofibrate on plasma activities (a) and liver mRNA (b) of PLTP and LCAT in C57BL/6 mice. The methods were identical with those described in the legend of Fig. 2. Bars represent the mean ± S.D. of five animals (*, \( p < 0.05; **, \( p < 0.01; ***, \( p < 0.001 \)). R.A.U., mRNA arbitrary unit.

FIG. 5. Size distribution of lipoproteins in PLTP-deficient mice before and after treatment with fenofibrate. The methods were identical with those described in the legend of Fig. 1. AU, absorbance units; FF, fenofibrate.
wild-type mice, fenofibrate led to weaker changes in HDL size after receiving an adenovirus infection (32). In nontransgenic described in HuAITg mice strongly expressing human PLTP human LDL size, was observed much similar to those recently with the high dose of fenofibrate, very large HDL, almost of clear differences in HDL size distribution appeared between 

In conclusion, results of the present study established for the first time that the HDL size redistribution induced by fenofibrate is completely abolished in homozygous PLTP-deficient animals. Although fibric acid derivatives are known to affect mouse lipoprotein metabolism in several distinct ways, in particular through the regulation of the expression of a number of proteins, the lack of HDL size redistribution in fenofibrate-treated PLTP-deficient mice clearly indicates that PLTP overexpression is the most important if not the sole mechanism that accounts for the observed HDL enlargement. Nevertheless and as demonstrated in the present studies in HuAITg mice, the impact of PLTP on HDL size can be markedly modulated by the level of apolipoprotein gene expression.

Because fibrates exert their effects through activation of the transcription factor PPARα (15), it has been postulated that the increase in hepatic PLTP mRNA was mediated through a PPARα-dependent mechanism. It can be concluded from the present study that fenofibrate affects PLTP gene expression via the activation of the nuclear receptor PPARα. Several other genes involved in lipoprotein metabolism are transcriptionally induced by PPARα activation. Indeed, fibrates activate PPARα, which after heterodimerization with another nuclear receptor, the retinoid X receptor, interacts with specific response elements termed peroxisome proliferator response elements in the regulatory sequences of target genes. The binding of PPARα-retinoid X receptor heterodimer to a peroxisome proliferator response element leads to altered gene expression (37). It can, therefore, be suggested that the promoter of the mouse hepatic PLTP gene contains a functional peroxisome proliferator response element, but a direct demonstration of its presence remains to be done.

In conclusion, results of the present study established for the first time that fenofibrate induces the expression of murine PLTP in liver through a PPARα-dependent mechanism. The
resulting rise in plasma PLTP activity was shown to account for the HDL size enlargement in plasma from treated animals. Finally, the absence of HDL size changes in PLTP-deficient mice clearly demonstrated that PLTP is a key factor in determining the size distribution of αHDL in vivo.

Acknowledgments—We thank Drs. Jeffrey M. Peters and Frank J. Gonzalez from the Laboratory of Molecular Carcinogenesis, NCI, National Institutes of Health, Bethesda, MD for PPAR genomic mining the size distribution of mice.

REFERENCES
1. Cheung, M. C., Brown, B. G., Wolf, A. C., and Albers, J. J. (1991) J. Lipid Res. 32, 383–394
2. Daerr, W. H., and Greten, H. (1982) Biochim. Biophys. Acta 710, 128–133
3. Newnham, H. H., Hopkins, G. J., Devlin, S., and Barter, P. J. (1998) Atherosclerosis 130, 257–262
4. Hopkins, G. J., Chang, L. B., and Barter, P. J. (1985) J. Clin. Invest. 86, 379–384
5. Lagrost, L., Desrumaux, C., Masson, D., Deckert, V., and Gambert, P. (1998) Curr. Opin. Lipidol. 9, 203–209
6. Tu, A. Y., Nishida, H. I., and Nishida, T. (1993) J. Biol. Chem. 268, 23098–23105
7. Jauhiainen, M., Metso, J., Pahlman, R., Blomqvist, S., Van Tol, A., and Ehnholm, C. (1993) J. Biol. Chem. 268, 4032–4036
8. Albers, J. J., Wolfbauer, G., Cheung, M. C., Day, J. R., Ching, A. F. T., Lok, S., and Tu, A. Y. (1995) Biochim. Biophys. Acta 1258, 27–34
9. Von Eckardstein, A., Jauhiainen, M., Huang, Y., Metso, J., Langer, C., Pussinen, P., Wu, S., Ehnholm, C., and Assmann, G. (1996) Biochim. Biophys. Acta 1301, 255–262
10. Jiang, X. C., Bruce, C., Mar, J., Lin, M., Ji, Y., Francone, O. L., and Tall, A. R. (1997) J. Clin. Invest. 99, 2373–2380
11. Jiang, X., Francone, O. L., Bruce, C., Milne, R., Mar, J., Walsh, A., Breslow, J. L., and Tall, A. R. (1996) J. Lipid Res. 37, 2373–2380
12. Salter, A. M., Bugos, M., Saxton, J., Fisher, S. C., and Brindley, D. N. (1987) Biochem. J. 247, 79–84
13. Shepherd, J. (1994) Atherosclerosis 110, 555–583
14. Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E., and Fruchart, J. C. (1998) Circulation 98, 2088–2095
15. Schoonjans, K., Staels, B., and Auwerx, J. (1996) Biochim. Biophys. Acta 1302, 93–109
16. Berthou, L., Duverger, N., Emmanuel, F., Langouet, S., Auwerx, J., Guillouzo, A., Fruchart, J. C., Rubin, E., Denefle, P., Staels, B., and Branellec, D. (1996) J. Clin. Invest. 97, 2408–2416
17. Vuduc, N., Schoonjans, K., Kreykh, V., Dallongeville, J., Fruchart, J. C., Staels, B., and Auwerx, J. (1995) J. Clin. Invest. 96, 741–750
18. Rubin, E. M., Ishida, B. Y., Clift, S. M., and Krauss, R. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 434–438
19. Jiang, X. C., Bruce, C., Mar, J., Lin, M., Ji, Y., Francone, O. L., and Tall, A. R. (1999) J. Clin. Invest. 103, 907–914
20. Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salgueiro, P. M., Westphal, H., and Gonzalez, F. J. (1995) Mol. Cell. Biol. 15, 3012–3022
21. Peters, J. M., Hennuyer, N., Staels, B., Fruchart, J. C., Fievet, C., Gonzalez, F. J., and Auwerx, J. (1997) J. Biol. Chem. 272, 27307–27312
22. Damen, J., Regta, J., and Scherphof, G. (1982) Biochim. Biophys. Acta 712, 444–452
23. Chen, C., and Albers, J. J. (1982) J. Lipid Res. 23, 680–691
24. Henderson, A. D., Richmond, W., and Elkeles, R. S. (1993) Clin. Chem. 39, 218–223
25. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
26. Staels, B., Van Tol, A., Andreu, T., and Auwerx, J. (1992) Arterioscler. Thromb. 12, 286–294
27. Staels, B., Van Tol, A., Skretting, G., and Auwerx, J. (1992) J. Lipid Res. 33, 727–735
28. Hamilton, R. L., Williams, M. C., Fielding, C. J., and Havel, R. J. (1976) J. Clin. Invest. 58, 667–680
29. Deckelbaum, R. J., Eisenberg, S., Oschry, Y., Butbul, E., Sharon, I., and Oliverona, T. (1982) J. Biol. Chem. 257, 6509–6517
30. Albers, J. J., Pitman, W., Wollbauer, G., Cheung, M. C., Kennedy, H., Tu, A. Y., Marcevina, S. M., and Paigen, B. (1999) J. Lipid Res. 40, 295–301
31. Foger, B., Santamariafinao, S., Shamburek, R. D., Parrot, C. L., Talley, G. D., and Brewer, H. B. (1997) J. Biol. Chem. 272, 27393–27400
32. Ehnholm, S., Vandijk, K. W., Vanthof, B., Vanderze, A., Olkkonen, V. M., Jauhiainen, M., Holker, M., Havekes, L., and Ehnholm, C. (1998) J. Lipid Res. 39, 1248–1253
33. Albers, J. J., Tu, A. Y., Paigen, B., Chen, H., Cheung, M. C., and Marcevina, S. M. (1996) Int. J. Clin. Lab. Res. 26, 262–267
34. Marquesvidal, P., Jauhiainen, M., Metso, J., and Ehnholm, C. (1997) Atherosclerosis 134, 87–95
35. Speijer, H., Groener, J. E., van Ramshorst, E., and Van Tol, A. (1991) Atherosclerosis 90, 159–168
36. Franccone, O. L., Boyer, L., and Haghpassand, M. (1996) J. Lipid Res. 37, 1268–1277
37. Fruchart, J. C., Duriez, P., and Staels, B. (1999) Curr. Opin. Lipidol. 10, 245–257
