Alterations in Lipoprotein Metabolism in Peroxisome Proliferator-activated Receptor α-deficient Mice*

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The peroxisome proliferator-activated receptor-α (PPARα) controls gene expression in response to a di-
verse class of compounds collectively referred to as per-
oxisome proliferators. Whereas most known peroxisome proliferators are of exogenous origin and include hypo-
lipidemic drugs and other industrial chemicals, several endogenous PPARα activators have been identified such as fatty acids and steroids. The latter finding and the fact that PPARα modulates target genes encoding enzymes involved in lipid metabolism suggest a role for PPARα in lipid metabolism. This was investigated in the PPARα-deficient mouse model. Basal levels of total se-
rum cholesterol, high density lipoprotein cholesterol, hepatic apolipoprotein A-I mRNA, and serum apol-
ipoprotein A-I in PPARα-deficient mice are significantly higher compared with wild-type controls. Treatment
with the fibrate Wy 14,643 decreased apoA-I serum lev-
els and hepatic mRNA levels in wild-type mice, whereas no effect was detected in the PPARα-deficient mice. Ad-
ministration of the fibrate Wy 14,643 to wild-type mice
results in marked depression of hepatic apolipoprotein
C-III mRNA and serum triglycerides compared with un-
treated controls. In contrast, PPARα-deficient mice were unaffected by Wy 14,643 treatment. These studies
demonstrate that PPARα modulates basal levels of se-
rum cholesterol, in particular high density lipoprotein
cholesterol, and establish that fibrate-induced modula-
tion in hepatic apolipoprotein A-I, C-III mRNA, and se-
rum triglycerides observed in wild-type mice is medi-
ated by PPARα.

Peroxisome proliferator-activated receptors (PPARs)1 are a
subfamily of the nuclear hormone receptor gene family. There
are three distinct PPARs, termed α, δ (also called β, NUC-1), and γ, each encoded by a separate gene and showing a distinct
tissue distribution (for review, see Refs. 1–5). Activated PPARs
erodimerize with another nuclear receptor, RXR and alter
transcription of target genes after binding to specific response elements or PPREs. PPREs consist of a direct repeat of the
nuclear receptor hexameric DNA recognition motif spaced by
one nucleotide. Numerous PPAR target genes have been iden-
tified (for review, see Ref. 4). Since they are activated by vari-
ous fatty acid metabolites as well as several drugs used in the
treatment of metabolic disorders, PPARs can be considered as
key messengers that modulate nutritional, pharmacological,
and metabolic stimuli into changes in gene expression. PPARs
were initially considered orphan receptors, since no direct bind-
ing of the various activators to the receptors could be demon-
strated. However, PPARα has recently been shown to bind and
be activated by leukotriene B4 and fibrates (6). In addition,
prostaglandin J2 derivatives and the antidiabetic thiazo-
linediones have been shown to be natural and synthetic li-
gands for PPARγ, respectively (7–9).

PPARα was the first PPAR to be identified (10), and it is ex-
pressed primarily in tissues that have a high level of fatty
acid catabolism such as liver (11). In the liver, PPARα modu-
lates oxidation of fatty acids and detoxification of several xe-
nobiotic compounds. Numerous studies have demonstrated
that several genes encoding enzymes involved in metabolic
pathways, such as β- and ω-oxidation, contain a PPRE in their
promoter region and are under transcriptional control of
PPARα (reviewed in Ref. 4). Consistent with this observation,
PPARα knockout mice, which are apparently healthy under
basal conditions, are not able to induce genes involved in β-
and ω-oxidation when treated with compounds that activate
PPARα (12). Fatty acid oxidation pathways have diverse roles
in physiology, extending from a role in lipid metabolism strictu
sensu to a role in the metabolism of various lipid mediators and
signaling factors (6).

In addition to its role in ω- and β-oxidation pathways,
PPARα was suggested to be important in the control of extra-
cellular lipid metabolism (for review, see Refs. 1–5). For example,
PPARα activators such as fibrates have an important role
in the control of HDL cholesterol levels. Gene expression of
apoA-I and apoA-II, the major apolipoproteins in HDL, is con-
trolled by PPARα. Thus, these protagonists of reverse choles-
terol transport (13), a protective pathway against coronary
artery disease (14), can be modulated by PPARα (reviewed in
Refs. 3–5). Besides the role of PPARα in HDL metabolism, they
also affect metabolism of triglyceride-rich lipoprotein particles
(reviewed in Refs. 3–5). In fact, activation of either PPARα
and/or PPARγ has pronounced triglyceride-lowering effects in
animals and man due to effects on both clearance and produc-
tion rates of triglyceride-rich lipoproteins.

Given the effects of treatment with PPARα activators on
lipoprotein metabolism, the goal of this study was to analyze in
a more direct fashion whether PPARα is involved in the regu-
lation of lipoprotein metabolism. Thus, we examined lipopro-

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1 The abbreviations used are: PPAR, peroxisome proliferator-activat-
ed receptor; PPRE, peroxisome proliferator-response element; HDL,
high density lipoprotein; VLDL, very low density lipoprotein.
tein metabolism in PPARα-deficient mice. These data unequivocally demonstrate that PPARα has an important regulatory role in lipid metabolism.

EXPERIMENTAL PROCEDURES

Materials—(4-chloro-6-(2,3-xylydine)-pyrimidinylthioacetic acid (Wy 14,643), a powerful PPARα activator and ligand (6), was purchased commercially (ChemSyn Science Laboratories, Lenexa, KS). Pelleted mouse chow was commercially prepared containing either 0.0 (control) or 0.1% Wy 14,643 (BioServ, Frenchtown, NJ).

Animals—For all experiments, we used male mice, 10–16 weeks of age, 20–35 g. F4 C57BL/6N X Sv129 homozygous wild-type (+) or knockout (−/−) for PPARα (12). Mice from both genotypes were fed either the control or Wy 14,643 diet ad libitum for 14 days. At the end of the experiments, animals were weighed and euthanized by overexposure to carbon dioxide. Blood was collected, and serum was separated and used within 1 week for analysis of lipids, lipoproteins, and apolipoproteins. The liver was removed immediately, weighed, and frozen in liquid nitrogen and stored at −80 °C until further analysis.

Lipid, Apolipoprotein, and Lipoprotein Measurements—Serum lipids (cholesterol and triglycerides) and HDL cholesterol were determined by enzymatic assay adapted to microtiter plates using commercially available reagents (Boehringer Mannheim). Serum HDL cholesterol content was determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid/mg (Boehringer Mannheim). Serum levels of mouse apoA-I and apoA-II were measured by an immunonephelometric assay using specific polyclonal antibodies.

The distribution of lipoproteins in serum from mice was analyzed by nondenaturing discontinuous gradient polyacrylamide gel electrophoresis (Lipofilm kit, Sebia, Issy-les-Moulineaux, France) according to the manufacturer’s instructions.

HDL lipoprotein fraction (d = 1.063–1.21 g/ml) was isolated by sequential ultracentrifugation as described (15). The HDL fraction was assayed for its protein (16) and lipid (cholesterol, triglyceride, and phospholipid) content. HDL size was determined on polycrylamide gels (23, 24). Novex, San Diego, CA. 10 μg of HDL protein were loaded, and the electrophoresis was performed in a Novex apparatus at 125 V for 13 h in 0.025 M Tris, 0.192 M glycine, pH 8.3. Gels were stained with Coomassie Brilliant Blue R-250. Proteins in the high molecular mass calibration mixture (Pharmacia Biotech Inc.) were used as calibration proteins on these gels.

Lipoprotein cholesterol profiles were obtained by fast protein liquid chromatography as described (17). This system allows separation of the three major lipoprotein classes; VLDL, low density lipoproteins, and apolipoproteins. The liver was removed immediately, weighed, and frozen in liquid nitrogen and stored at −80 °C until further analysis.

RNA Analysis—RNA was isolated from liver by the acid guanidinium thiocyanate/phenol/chloroform method (18). Northern and dot blot analysis of total cellular RNA was performed as described (19). Rat apoA-I, apoA-II, apoA-IV, and apoC-III cDNAs were used as probes (20, 21). GAPDH and 36B4 (22, 23) (encoding the human acidic ribosomal phosphoprotein PO (23)) were used as control probes. All probes were labeled by random primed labeling (Boehringer Mannheim). Filters were hybridized with 1.5 × 10⁶ cpm/ml of each probe as described (21). Autoradiograms were analyzed by quantitative scanning densitometry (Bio-Rad GS670 Densitometer) as described (21). RNA expression data of the various apolipoproteins were corrected for the expression of a control probe. An arbitrary value of 100 was assigned to the average of the wild-type (+/+ ) untreated animals for each experiment.

RESULTS

PPARα (−/−) Mice Have Elevated Levels of HDL Particles—Compared with control (+/+) mice, serum total and HDL cholesterol concentrations were significantly higher in PPARα (−/−) mice (Table I). Total serum cholesterol and serum HDL cholesterol levels in the PPARα (−/−) mice were 64 and 63% higher, respectively, than values in control PPARα (+/+ ) mice. Plasma triglyceride values were not significantly different between the two groups.

Separation of the different lipoprotein fractions by electrophoresis of lipostained samples from PPARα (−/−) and (+/+ ) mice confirmed a robust increase in HDL concentrations in PPARα (−/−) serum (data not shown). When staining intensity of 100% was arbitrarily attributed to the HDL band of the PPARα (+/+) animals, the relative staining intensity of the HDL from the PPARα (−/−) animals was 197%.

Serum Lipoproteins in PPARα (−/−) Mice—To analyze in more detail the distribution of lipoproteins in plasma from the PPARα (−/−) and (+/+ ) mice, aliquots from pooled serum from these animals were subjected to gel filtration chromatography. The cholesterol profiles showed a striking increase in HDL levels as depicted in Fig. 1. This increase is consistent with the increase in HDL cholesterol levels measured by the phosphotungstic acid/mg precipitation technique (Table I) and by lipofilm electrophoresis. Cholesterol concentrations from each of the lipoprotein fractions were further measured. As expected, almost all of the cholesterol was distributed in HDL in both genotypes of mice (85.9 ± 3.5% versus 88.9 ± 0.6% in PPARα (+/+ ) and (−/−), respectively). In addition, the data confirmed a large increase in HDL cholesterol concentrations in PPARα (−/−) mice compared with PPARα (+/+ ) mice (137.1 ± 9.7 versus 78.9 ± 10.2 mg/dl, respectively).

HDL from pooled serum samples from PPARα (−/−) and (+/+ ) mice was isolated by density equilibrium ultracentrifugation, and the composition was determined. HDL particles from PPARα (+/+ ) were not different in their composition compared with PPARα (−/−) mice (Table II). Since the particle size distribution of HDL was comparable and showed a homogeneous population of HDL particles in both groups of animals (Fig. 2), we can conclude that the increase in HDL cholesterol is essentially due to an increase in the amount of circulating HDL lipoproteins.

The Increase in HDL Is Due to an Increase in the HDL Apolipoprotein apoA-I—To verify whether the observed increase in HDL particle number and HDL cholesterol was associated with a concomitant increase in the major HDL apoliproteins, we determined serum apoA-I and apoA-II concentrations in PPARα (−/−) and (+/+ ) mice by a nephelometric assay (Fig. 3, A and B). Serum apoA-I was significantly greater in the PPARα (−/−) mice compared with (+/+ ) mice (136 ± 46 versus 96 ± 19 mg/dl; p < 0.01). The increase in serum apoA-I levels was due to an increase in hepatic apoA-I mRNA levels observed in PPARα (−/−) mice compared with control (+/+) mice (Figs. 3A and 4). ApoA-II concentrations tended also to be higher in PPARα (−/−) mice relative to the (+/+) controls, but the difference was not statistically significant (64 ± 27 versus 54 ± 15 mg/dl) (Fig. 3B). Similarly, hepatic apoA-II mRNA had a tendency to be more elevated in the (−/−) animals (Figs. 3B and 4).

ApoA-I Levels Are Not Controlled by Fibrates in PPARα (−/−) mice—Previous work from our laboratories and others has shown that rodent serum apoA-I and hepatic mRNA levels are down-regulated by treatment with fibrate hypolipidemic drugs. Since fibrates are potent activators and ligands of PPARα, we examined this regulation in PPARα (−/−) mice. A significant decrease in both hepatic apoA-I mRNA levels (p < 0.05) and serum apoA-I (p < 0.01) was observed in (+/+) mice after treatment with Wy 14,643. However, administration of Wy 14,643 to PPARα (−/−) animals resulted in no significant change in hepatic apolipoprotein A-I mRNA and serum apoA-I levels (Figs. 3A and 4). Absence of a regulatory effect of fibrates on apoA-I protein and mRNA levels in Wy 14,643 fed PPARα

### Table I

| PPARα (+/+) | 85 ± 15 | 14 ± 41 | 59 ± 10 |
|------------|---------|---------|---------|
| PPARα (−/−) | 139 ± 27* | 157 ± 36 | 96 ± 24* |

* p < 0.0001 by Mann-Whitney test.

Lipoproteins in PPARα (−/−) and (+/+ ) mice (Table I). Total serum cholesterol and serum HDL cholesterol levels in the PPARα (−/−) mice were 64 and 63% higher, respectively, than values in control PPARα (+/+ ) mice. Plasma triglyceride values were not significantly different between the two groups.
Mass composition of HDL particles isolated by equilibrium ultracentrifugation from pooled serum from PPARα (−/−) and (+/+) mice

Results are expressed as mass percentage of HDL. Values correspond to one HDL preparation isolated from a pooled sample of 3 mice in each group.

|         | Cholesterol | Triglycerides | Phospholipids | Protein |
|---------|-------------|---------------|---------------|---------|
| PPARα (+/+)| 25.9        | 3.3           | 12.6          | 58.2    |
| PPARα (−/−)| 24.5        | 2.0           | 15.1          | 58.4    |

(-/-) mice suggests and implies PPARα unequivocally as a major determinant of HDL metabolism. ApoA-II serum levels increased after fibrate treatment in PPARα (+/+) mice (p < 0.01) whereas they decreased in PPARα (−/−) mice (p < 0.01) (Fig. 3B). The increase in apoA-II levels was accompanied by an increase in hepatic apoA-II mRNA levels in PPARα (+/+) mice (Figs. 3B and 4). No change in hepatic apoA-II mRNA levels was present in PPARα (−/−) mice (Figs. 3B and 4).

Fibrate Treatment Reveals Abnormalities in Triglyceride and ApoC-III Metabolism in PPARα (−/−) Mice—Although no major difference in basal serum triglyceride levels between PPARα (+/+) and (−/−) mice was detected (Table I), previous studies have shown that administration of fibrates to both rodents and humans results in lower triglyceride concentrations and lower hepatic apoC-III mRNA (21, 24, 25). In addition, the apoC-III gene has a PPRE and plays a critical role in the control of triglyceride metabolism. Combined, these observations suggest that fibrate regulation of triglyceride metabolism occurs through a PPARα-dependent pathway. Consistent with this idea, PPARα (+/+) mice fed the Wy 14,643 diet had significantly lower serum triglyceride concentration compared with untreated control (+/+) mice (p < 0.001) (Fig. 5A). In contrast, the characteristic lowering of triglyceride levels in response to fibrate administration was not observed in the PPARα (−/−) mice fed the Wy 14,643 diet compared with controls (Fig. 5A).

Consistent with the hypothesis that PPARα has a major role in regulating apoC-III levels accompanying major changes in triglyceride metabolism, we observed a decrease in liver apoC-III mRNA levels in PPARα (+/+) mice fed the Wy 14,643 diet (p < 0.001) (Fig. 5, A and B). In contrast, liver apoC-III mRNA levels were not affected in the PPARα (−/−) mice fed the Wy 14,643 diet compared with untreated controls (Fig. 5, A and B).

DISCUSSION

Since peroxisome proliferators induce altered expression of genes encoding peroxisomal β-oxidation enzymes, microsomal ω-oxidation enzymes, and apolipoproteins, a role in lipid homeostasis for these compounds can be hypothesized. This idea is further supported by the known triglyceride-lowering effects of fibrates, a commonly used class of hypolipidemic agents. Recently it was shown that several peroxisome proliferators including fibrates and a fatty acid derivative are capable of activating PPARα. To better delineate a role for PPARα in lipid homeostasis, we examined lipid and lipoprotein metabolism in PPARα-deficient mice.
Lipoproteins in PPARα-deficient Mice

An active area of research. There is some confusion in this area that stems from differences between human and rodent apoA-I gene regulation by fibrates. In rats, serum HDL cholesterol and hepatic apoA-I mRNA levels are typically down-regulated by fibrates (20, 26), whereas these parameters increase to a variable extent in humans (26, 29, 30). The mechanisms by which fibrates exert an overall negative effect on the minimal promoter of the mouse apoA-I gene are as yet not defined. The present results, which demonstrate an increase in liver apoA-I mRNA levels under basal conditions in PPARα (−/−) mice and show no regulatory effect of fibrates in these mice, are consistent with a negative regulatory effect of PPAR activators on rodent apoA-I expression (20, 26). This indicates that PPARα is one of the key players in determining liver apoA-I expression.

Human liver apoA-II expression is stimulated by PPARα. In fact, PPAR induces apoA-II expression through interaction with a PPRE located in the apoA-II J site (27). The absence in PPARα (−/−) mice of increased apoA-II mRNA and serum levels, typically observed in PPARα (+/+) mice after fibrate treatment, indicates that regulatory mechanisms similar to those for the human apoA-II gene must exist in mice. The regulatory function of PPARα on gene expression of these two major HDL apolipoproteins may have important clinical implications in man. Since apoA-I and apoA-II are major determinants of HDL metabolism, alterations in their gene expression could significantly affect reverse cholesterol transport pathway, which seems to protect against coronary atherosclerosis.

PPARα (+/+) mice fed the 0.1% Wy 14,643 diet have significantly lower hepatic apoC-III mRNA levels and triglyceride concentration compared with control untreated (+/+) mice. This is consistent with previous reports showing a similar reduction in apoC-III levels in rats after treatment with hypolipidemic fibrates (21, 24, 25). In contrast, PPARα (−/−) mice fed Wy 14,643 did not exhibit the prototypical response to a peroxisome proliferator since hepatic apoC-III mRNA and triglyceride levels were not different compared with control (+/+) mice. These observations demonstrate that Wy 14,643-induced reductions in hepatic apoC-III mRNA and triglyceride metabolism are mediated by PPARα and hence establish a role for PPARα in the control of VLDL and triglyceride metabolism in addition to its role in HDL metabolism described above.

Our observations are also consistent with previous studies, which suggested that activation of PPARα results in lower
serum triglyceride levels in animals and man. Steady state triglyceride levels are dependent on two pathways, endogenous synthesis and tissue clearance. Production of triglycerides in the liver is controlled in large part by substrate (fatty acids) availability, whereas tissue clearance of triglycerides is dependent on lipoprotein lipase activity and apolipoprotein C-III. Fibrates can affect both of these processes (reviewed in Ref. 4). For example, fibrates have been shown to stimulate lipolysis and clearance of triglycerides due to decreased transcription and production of apoC-III (21, 24, 25), an apolipoprotein that limits tissue clearance of triglyceride. The effect of peroxisome proliferators on apoC-III expression may involve competition by PPAR for binding to a cis-acting sequence on the apoC-III promoter as well as a direct repression of hepatic nuclear factor-4 expression (25). In addition to apoC-III-mediated effects on the clearance of triglyceride-rich lipoproteins induced by PPARα, this receptor also influences production rates of these particles. Fibrate activation of PPARα in the liver stimulates fatty acid uptake and conversion to acyl-CoA derivatives by the induction of the genes coding for the fatty acid transporter protein (31–33). The resulting acyl-CoA derivatives in hepatocytes are then more efficiently oxidized by induction of fatty acid β-oxidation pathways in peroxisomes and mitochondria (see above). Besides modulating β-oxidation of fatty acids, PPARα activation can also inhibit de novo fatty acid synthesis that contributes to decreased triglyceride synthesis and VLDL production (reviewed in Ref. 4). Hence, both enhanced catabolism of triglyceride-rich particles as well as reduced secretion of VLDL particles are mechanisms that contribute to the hypolipidemic effect of PPARα activation.

Our results clearly show that PPARα modulates lipid metabolism in a homeostatic mechanism since mice lacking functional PPARα have higher levels of serum HDL. In addition, PPARα has an important role in mediating the effects of fibrates on apolipoprotein, HDL, and triglyceride metabolism. Our observations in the PPARα-deficient mouse warrant further studies to delineate the role of normal and abnormal PPAR activity in man. It may be especially interesting to evaluate genetic linkage in the PPARα gene from patients with abnormalities in lipid metabolism such as hypoalphaproteinemia, hypertriglyceridemia, or combined hyperlipidemia.

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