Altered Constitutive Expression of Fatty Acid-metabolizing Enzymes in Mice Lacking the Peroxisome Proliferator-activated Receptor α (PPARα)*

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Peroxisome proliferator-activated receptor α (PPARα) is a member of the steroid/nuclear receptor superfamily and mediates the biological and toxicological effects of peroxisome proliferators. To determine the physiological role of PPARα in fatty acid metabolism, levels of peroxisomal and mitochondrial fatty acid metabolizing enzymes were determined in the PPARα null mouse. Constitutive liver β-oxidation of the long chain fatty acid, palmitic acid, was lower in the PPARα null mice as compared with wild type mice, indicating defective mitochondrial fatty acid catabolism. In contrast, constitutive oxidation of the very long chain fatty acid, lignoceric acid, was not different between wild type and PPARα null mice, suggesting that constitutive expression of enzymes involved in peroxisomal β-oxidation is independent of PPARα. Indeed, the PPARα null mice had normal levels of the peroxisomal acyl-CoA oxidase, bifunctional protein (hydratase + 3-hydroxyacyl-CoA dehydrogenase), and thiolase but lower constitutive expression of the D-type bifunctional protein (hydratase + 3-hydroxyacyl-CoA dehydrogenase). Several mitochondrial fatty acid metabolizing enzymes including very long chain acyl-CoA dehydrogenase, long chain acyl-CoA dehydrogenase, short chain-specific 3-ketoacyl-CoA thiolase, and long chain acyl-CoA synthetase are also expressed at lower levels in the untreated PPARα null mice, whereas other fatty acid metabolizing enzymes were not different between the untreated null mice and wild type mice. A lower constitutive expression of mRNAs encoding these enzymes was also found, suggesting that the effect was due to altered gene expression. In wild type mice, both peroxisomal and mitochondrial enzymes were induced by the peroxisome proliferator Wy-14,643; induction was not observed in the PPARα null animals. These data indicate that PPARα modulates constitutive expression of genes encoding several mitochondrial fatty acid-catabolizing enzymes in addition to mediating inducible mitochondrial and peroxisomal fatty acid β-oxidation, thus establishing a role for the receptor in fatty acid homeostasis.

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Peroxisomes are single membrane-bound subcellular organelles that contain a variety of enzymes involved in a number of metabolic processes (1). The most well characterized reactions carried out by peroxisomes are those that catalyze in fatty acid β-oxidation. Since plants lack mitochondria, peroxisomes are solely responsible for their fatty acid β-oxidation. The peroxisomal fatty acid β-oxidation pathway produces hydrogen peroxide through the activity of acyl-CoA oxidase, thus historically accounting for the name "peroxisomes." Typically, H₂O₂ is decomposed to molecular oxygen and water by catalase and glutathione peroxidase. Human genetic deficiencies in peroxisome biogenesis and individual peroxisomal enzymes have been described that result in accumulation of long chain fatty acids (2). The most severe of the peroxisome deficiencies causes neurological and anatomical abnormalities.

In addition to fatty acid oxidation, peroxisomes also carry out β-oxidation of the cholesterol side chain during the synthesis of bile acids and participate in the biosynthesis of cholesterol (3), ether glycolipids, and dolichols. Catabolism of purines, polyamines, glyoxylate and certain amino acids have been attributed to peroxisome-localized enzymes. Thus, peroxisomes are essential organelles for maintaining cellular and organismal homeostasis.

The number of peroxisomes is increased in rodents by treatment with high fat diets, cold temperature, starvation, ACTH, and certain chemicals generically termed peroxisome proliferators (1). Peroxisome proliferators include a structurally diverse group of chemicals that include 1) hypolipidemic drugs (clofibrate, gemfibrozil, fenofibrate, bezafibrate, etofibrate, and Wy-14,643), 2) the azole antifungal compounds such as bifonazole, 3) leukotriene D₄ antagonists, 4) herbicides, 5) pesticides, 6) phthalate esters used in the plastics industry (di-2-ethylhexyl phthalate), 7) simple solvents including trichloroethylene, and 8) natural chemicals such as phenyl acetate and the steroid dehydroepiandrosterone sulfate. Among them, the most potent peroxisome proliferator is Wy-14,643.

Peroxisome proliferation is most pronounced in liver, kidney, and heart. In liver, the number of peroxisomes increases from about 500–600/cell to >5,000/cell after exposure to peroxisome proliferators (1). This is accompanied by an increase in cell volume and cell number, resulting in hepatomegaly. Coincident with an increase in the number of peroxisomes, several peroxisomal enzymes are induced by transcriptional activation (4). Transcription of genes encoding the key β-oxidation enzymes acyl-CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (bifunctional enzyme), and thiolase are markedly elevated as a result of treatment with peroxisome proliferators.
is highly expressed in the central nervous system (7). The role of PPARα in tissue and spleen. PPARs are abundant in the liver, kidney, and heart, all of which display peroxisome proliferation in response to PPARα activators and have high rates of lipid metabolism (7). Expression of PPARδ is ubiquitous and is highly expressed in the central nervous system (7). The role of PPARδ is not known. PPARγ and PPAR-2, resulting from differential mRNA splicing, are present predominantly in adipose tissue and spleen. PPARγ is responsible in part for adipocyte differentiation and regulation of adipocyte-specific genes (8). It is also the target for the thiazolidinedione drugs that increase insulin sensitivity of target tissues (9).

To determine the function of PPARα and its role in peroxisome proliferation and hepatocarcinogenesis, a PPARα null mouse was generated (10). These animals exhibit a normal phenotype and normal basal levels of hepatic peroxisomes. However, the PPARα null mouse is nonresponsive to peroxisome proliferation. Compared with wild type mice, administration of peroxisome proliferators to PPARα null mice does not cause an increase in the number of peroxisomes, hepatomegaly, or increases in mRNA encoded by target genes. Furthermore, these mice do not display physiological, toxicological, or carcinogenic responses induced by peroxisome proliferators (11-13).

Interestingly, abnormal hepatic lipid accumulation was initially reported in the PPARα null mice, suggesting an alteration in lipid metabolism (10). To investigate the biochemical basis for altered lipid metabolism, constitutive levels of peroxisomal and mitochondrial fatty acid-metabolizing enzymes were examined in the PPARα null mouse.

EXPERIMENTAL PROCEDURES

Materials—Sodium 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA) was purchased from Byk Gulden Pharmazeutika (Konstanz, Germany). [1-14C]Lauric acid (55 mCi/mmol), [1-14C]Palmitic acid (54 mCi/mmol), and [1-14C]Lignoceric acid (47 mCi/mmol) were from American Radiolabeled Chemicals (St. Louis, MO).

Animals and Wy-14,643 Treatment—PPARα null mice on an Sv/129 genetic background were produced as described (10). Wild type Sv/129 mice were used as controls in all experiments. Mice were fed either a control diet or one containing 0.1% Wy-14,643 for 2 weeks. Mice were counted. In some experiments, 20 μM POCA or 2 mM potassium cyanide was added to the incubation mixture to inhibit mitochondrial β-oxidation activity. Fatty acid β-oxidation activity was measured as nmol/min/μl.

Analysis of Fatty Acid Synthesizing Enzymes—Fatty acid synthetase (15), malic enzyme (ME) (16), ATP-citrate lyase (17), acetyl-CoA carboxylase (18), and glucose-6-phosphate dehydrogenase (19) were measured as described previously.

Analysis of Fatty Acid β-Oxidizing Enzymes—Liver extracts were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with the primary antibody followed by alkaline phosphatase-conjugated goat anti-rabbit IgG. Immunoblotting was performed using rabbit polyclonal antibodies against rat acyl-CoA oxidase (AOX) (20), short chain-specific 3-ketoacyl-CoA thiolase (T1) (21), acetoacetyl-CoA thiolase (T2) (22, 23), cytosolic thioesterase (CET) II (24), short chain acyl-CoA dehydrogenase (SCAD) (25), medium chain acyl-CoA oxidase (MCAD) (25), long chain acyl-CoA dehydrogenase (LCAD) (25), very long chain acyl-CoA dehydrogenase (VLCD) (26), long chain acyl-CoA synthetase (LACS) (27), very long chain acyl-CoA synthetase (VLACS) (28), peroxisomal thiolase (PT) (21), carnitine palmitoyl-CoA transferase (CPT II) (29), short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) (30), peroxisomal bifunctional protein (PH) (30), mitochondrial short chain specific hydrolase (MEI) (31), mitochondrial trifunctional protein α and β subunit (TPα and TPβ) (32), mitochondrial thioesterase I (MTEI) (33), and peroxisomal D-type bifunctional protein (DBF) (34).

mRNA Analysis—mRNA analysis was performed by Northern blotting. Total liver RNA was extracted, electrophoresed on 1.1% formaldehyde containing 1% agarose gels, and transferred to nylon membranes (23). The membranes were incubated with 32P-labeled cDNA probes and analyzed on a Fuji system analyzer (Fuji Photo Film Co., Tokyo, Japan). The cDNA probes used were for VLCD (35), LCAD (36), LACS (37), ME (38), and SCHAD (39).

RESULTS

Analysis of Fatty Acid-Metabolizing Enzymes—To identify specific fatty acid-metabolizing enzymes that were influenced by PPARα, antibodies were used to measure protein levels on immunoblots (Fig. 1, Table I). Constitutive expression of several enzymes (VLCD, LCAD, LACS, and T1) were lower by 30–60% in untreated PPARα null mice as compared with untreated wild type mice. Curiously, constitutive expression of the SCHAD was higher by about 4-fold in the PPARα null mouse liver as compared with wild type mice. Other mitochondrial enzymes examined were expressed at similar levels in untreated PPARα null and wild type mice. The expression of all mitochondrial, microsomal, and cytosolic fatty acid-metabolizing enzymes except for ME were increased in wild type mice fed Wy-14,643 compared with controls, with levels of induction ranging from 1.7-fold for T2 to 4.7-fold for SCHAD. The expression of CET II was totally dependent on Wy-14,643 treatment in wild type mice. In the PPARα null animals, there was no increase in expression of these enzymes after feeding Wy-14,643 for 2 weeks (Fig. 1, Table I).

Constitutive levels of hepatic peroxisomal fatty acid β-oxidation enzymes were expressed at 3–7-fold in the wild type mice, and this effect was not observed in the PPARα null mice. Basal activities of cytosolic enzymes involved in fatty acid synthesis including fatty acid synthetase, acetyl-CoA carboxylase, glucose-6-phosphate dehydrogenase, and ATP-citrate

1 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; VLCD, very long chain acyl-CoA dehydrogenase; LCAD, long chain acyl-CoA dehydrogenase; MCAD, medium chain acyl-CoA dehydrogenase; SCAD, short chain acyl-CoA dehydrogenase; Tα, trifunctional protein α subunit (long chain-specific hydrolase + long chain-specific 3-hydroxyacyl-CoA dehydrogenase); Tβ, trifunctional protein β subunit (long chain-specific 3-ketoacyl-CoA thiolase); ME, mitochondrial short chain-specific hydrolase; MEI, short chain 3-hydroxyacyl-CoA dehydrogenase; T1, short chain-specific 3-ketoacyl-CoA thiolase; T2, acetoacetyl-CoA thiolase; LACS, long chain acyl-CoA synthetase; PH, carnitine palmitoyl-CoA transferase; MTEI, mitochondrial thioesterase I; CETE II, cytosolic thioesterase II; AOX, acyl-CoA oxidase; DBF, D-type (peroxisomal) bifunctional protein (hydratase + 3-hydroxyacyl-CoA dehydrogenase) and key enzyme of bile acid synthesis from cholesterol; PT, peroxisomal thiolase; VLACS, very long chain acyl-CoA synthetase; ME, malic enzyme; POCA, sodium 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate.

PH 7.2, 0.1 mM EDTA, 1 mM potassium phosphate buffer, pH 7.2, 5 mM Tris malonate, 10 mM magnesium chloride, 1 mM carnitine, 0.15% bovine serum albumin, 5 mM ATP, and 50 μM each fatty acid (5 × 10^4 CPM of radioactive substrate). The reaction was run for 30 min at 25 °C and stopped by the addition of 0.2 ml of 0.6 N perchloric acid. The mixture was centrifuged at 2,000 × g for 10 min, and the unreacted fatty acid in the supernatant was removed with 2 ml of n-hexane using three extractions. Radioactive degradation products in the water phase were counted. In some experiments, 20 μM POCA or 2 mM potassium cyanide was added to the incubation mixture to inhibit mitochondrial β-oxidation activity. Fatty acid β-oxidation activity was measured as nmol/min/μl.
lyase were similar in the untreated wild type and PPARα null mice except for ME, which was present at only 50% of the level in the PPARα null mouse (Table II). ME and glucose-6-phosphate dehydrogenase were marginally induced (4-fold and 2-fold, respectively) by Wy-14,643 feeding in the wild type mice, and these effects were not observed in the PPARα null mouse treated with Wy-14,643.

Expression of mRNAs—To determine whether the lower expression of fatty acid-metabolizing enzymes and ME is due to altered gene expression, hepatic mRNA levels were analyzed by Northern blots (Fig. 2). Constitutive levels of VLCAD, LCAD, ME, and LACS mRNA were lower in the PPARα null mice compared with controls, consistent with the protein measurements. It is noteworthy that hepatic levels of mRNA for SCHAD were not different between untreated PPARα null and wild type mice even though the protein levels were increased by 4-fold in the null mouse. Levels of the mRNAs encoding, LCAD, and ME were significantly induced in wild type mice by Wy-14,643. These data are also consistent with the Western blot analysis. In contrast, the mRNAs encoding LACS and SCHAD were not significantly increased by the drug. In the PPARα null mice, there was no difference in mRNA levels for any of the enzymes after treatment with Wy-14,643.

Analysis of Overall Fatty Acid β-Oxidation Activity—As shown in Table I, six enzymes involved in fatty acid β-oxidation had lower constitutive expression in the PPARα null mice. Four of the six (VLCAD, LCAD, LACS, and DBF) have highest catalytic activities with long chain fatty acid substrates (25–27, 34), whereas the other two (SCHAD and T1) are more active with short and medium chain fatty acids (21, 30). To evaluate the significance of the altered fatty acid β-oxidation enzymes, total hepatic β-oxidation was measured using lauric acid (C-12), palmitic acid (C-16), and lignoceric acid (C-24). Compared with wild type controls, the PPARα null mice basal levels of total fatty acid β-oxidation was lower with palmitic acid as a substrate; there was no difference in metabolism of lauric acid and lignoceric acid (Fig. 3). Wy-14,643 feeding caused a significant increase in metabolism of all three fatty acids in wild type animals. No induction was observed in PPARα null mice, consistent with the results found with the enzymes levels (Fig. 1 and Table I). Results were identical whether the data were calculated per liver protein or per liver. These data provide evidence that the lower constitutive expression of several long chain-specific fatty acid β-oxidation enzymes in the PPARα null mice compared with wild type mice (Table I) significantly

![Immunoblot analysis of selected fatty acid-metabolizing enzymes. Three lanes form a group of mice. Diet, age, and genotype are indicated. Liver cell lysate (8 µg) was subjected to electrophoresis and Western immunoblotting. The blots were stained with antibodies against VLCAD (panel A), LCAD (panel B), SCHAD (panel C), T1 (panel D), LACS (panel E), DBF (panel F), and CTE II (panel G).](image)

**Table I**

Western immunoblot quantitation of hepatic fatty acid-metabolizing enzymes in wild type (+/+ ) and PPARα-null (−/−) mice

| Enzyme | (+/+ ) control | (−/−) control | p value | (+/+ ) Wy-14,643 | (−/−) Wy-14,643 | P value |
|--------|----------------|---------------|---------|-----------------|-----------------|---------|
| VLCAD  | 1.00           | 0.32 ± 0.04   | 0.000   | 1.59 ± 0.40     | 0.41 ± 0.08     | 0.011   |
| LCAD   | 1.00           | 0.40 ± 0.21   | 0.039   | 3.44 ± 1.49     | 0.37 ± 0.14     | 0.047   |
| MCAD   | 1.00           | 1.05 ± 0.14   | 0.652   | 3.30 ± 0.28     | 0.86 ± 0.04     | <0.001 |
| SCAD   | 1.00           | 0.57 ± 0.14   | 0.248   | 4.23 ± 0.40     | 0.58 ± 0.07     | <0.001 |
| TFFα   | 1.00           | 0.98 ± 0.09   | 0.810   | 3.49 ± 0.29     | 0.84 ± 0.17     | <0.001 |
| TFFβ   | 1.00           | 1.03 ± 0.07   | 1.000   | 3.88 ± 0.43     | 1.15 ± 0.15     | <0.001 |
| MH     | 1.00           | 1.12 ± 0.12   | 0.423   | 0.88 ± 0.04     | 0.96 ± 0.08     | 0.042   |
| SCHAD  | 1.00           | 3.90 ± 1.02   | 0.008   | 4.71 ± 1.05     | 3.43 ± 1.06     | 0.004   |
| T1     | 1.00           | 0.59 ± 0.02   | 0.001   | 1.88 ± 0.18     | 0.61 ± 0.05     | 0.001   |
| T2     | 1.00           | 0.98 ± 0.29   | 0.912   | 1.67 ± 0.35     | 0.86 ± 0.14     | 0.020   |
| LACS   | 1.00           | 0.42 ± 0.06   | 0.001   | 2.09 ± 0.64     | 0.37 ± 0.02     | 0.032   |
| CPT II | 1.00           | 0.90 ± 0.18   | 0.574   | 3.42 ± 0.10     | 0.96 ± 0.04     | <0.001 |
| MTE I  | 1.00           | 0.95 ± 0.07   | 0.567   | 3.45 ± 0.12     | 1.11 ± 0.06     | <0.001 |
| CTE II | ND             | ND            | ND      | 1.00            | ND              | ND      |
| AOX    | 1.00           | 0.98 ± 0.14   | 0.850   | 6.59 ± 0.48     | 1.08 ± 0.12     | <0.001 |
| PH     | 1.00           | 0.93 ± 0.20   | 0.653   | 7.25 ± 0.60     | 0.87 ± 0.04     | <0.001 |
| DBF    | 1.00           | 0.64 ± 0.01   | 0.001   | 6.55 ± 0.19     | 0.61 ± 0.03     | <0.001 |
| PT     | 1.00           | 1.06 ± 0.03   | 0.196   | 6.77 ± 0.41     | 1.23 ± 0.09     | <0.001 |
| VLACS  | 1.00           | 0.98 ± 0.07   | 0.087   | 3.40 ± 0.28     | 0.99 ± 0.10     | <0.001 |

a Comparing (+/+ ) control with (−/−) control.

b Comparing (+/+ ) control with (+/+ ) Wy-14,643.
affects long chain fatty acid oxidation. The lack of a difference in constitutive oxidation of the very long chain-specific fatty acid, lignoceric acid, carried out by peroxisomal enzymes supports the finding of no effect of PPARα on expression of these enzymes except DBF in untreated null mice (Table I).

To confirm that palmitic acid is preferentially metabolized by mitochondrial enzymes, the inhibitors KCN, an inhibitor of the mitochondrial respiratory chain, and POCA, a potent inhibitor of carnitine palmitoyl-CoA transferase I, were employed (Table III). Both compounds inhibited palmitic acid oxidation by 70–90% in either mouse line, irrespective of Wy-14,643 administration. These data confirm that the contribution of peroxisomal enzymes to palmitic acid β-oxidation was minimal.

**Time Course of Induction**—The kinetics of VLCAD and ME mRNA and protein expression were determined after administration of Wy-14,643. VLCAD mRNA and protein were rapidly induced within 1 day after Wy-14,643 treatment in wild type mice (Fig. 4A). No induction of VLCAD was found in PPARα null mice after 14 days of feeding Wy-14,643. Levels of VLCAD mRNA and protein decreased to about 2-fold after 5 days of feeding yet remained elevated up to 14 days of feeding. A similar time course of induction was also observed in protein levels of several mitochondrial fatty acid β-oxidation enzymes (LCAD, SCAD, TPα, and TPβ). Induction of ME mRNA and enzyme activity reached maximal levels of 4-fold after 7 days of Wy-14,643 feeding in the wild type mice; neither mRNA nor activity were induced in the PPARα null animal (Fig. 4B). Similar time course of induction was observed in protein levels of several other enzymes (LACS, AOX, PH, PT, DBF, VLACS, MCAD, and CTE II). Thus, the kinetics of the increase in ME expression is slower than that of VLCAD. The reason for this differential induction is not presently known.

**DISCUSSION**

Constitutive expression of VLCAD, LCAD, SCHAD, T1, LACS, DBF, and ME is regulated by PPARα since their abundance was significantly altered in the absence of PPARα as compared with wild type controls. With the exception of SCHAD, which was up-regulated in the PPARα null mice, all of these proteins were found at lower levels in the PPARα null mice. This shows that PPARα has an important role in regulating basal levels of these enzymes involved in fatty acid β-oxidation and ME that participates in fatty acid synthesis. Although the mechanism for this peroxisome proliferator-independent mechanism is not known, it may be a result of altered gene expression since PPARα is known to control transcription through interaction with peroxisome proliferator response elements (4). The down-regulation is selective since constitutive expression of other enzymes including MCAD, SCAD, TPα, TPβ, MH, T2, CPT II, and MTE I appear to be unaffected by loss of the receptor. This is similar to the peroxisomal enzymes AOX, PH, PT, and VLACS, where there was no difference in expression levels between the untreated wild type and PPARα null mice. The data on enzyme levels are supported by the results of total fatty acid metabolism in liver where oxidation of long chain fatty acid, palmitate, which is reflective of mitochondrial metabolism, was lower in the PPARα null mice, whereas oxidation of the very long chain fatty acid, lignocerate, was not different between the wild type and PPARα null mice. The lack of difference in metabolism of this very long chain fatty acid is almost certainly due to similar levels of the peroxisomal enzymes in the two genotypes. Lower constitutive expression of fatty acid-metabolizing enzymes and mitochondrial palmitic acid β-oxidation suggests that PPARα controls gene expression in the absence of exogenous ligands for the receptor, and mice lacking PPARα have an impaired ability to metabolize lipids.

To further elucidate the role of PPARα in lipid metabolism, the effect of the prototypical peroxisome proliferator Wy-14,643 in PPARα null mice was investigated. Indeed, PPARα was shown to transactivate genes in the presence of peroxisome proliferators (40–42). In addition to the peroxisomal β-oxidation enzymes and microsomal fatty acid hydroxylase P450, liver fatty acid binding protein and the genes encoding MCAD (43), 3-hydroxy-3-methylglutaryl-CoA synthase (44), and ME (45) are also activated by PPARα as indicated by transactivation.

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**TABLE II**

Activities of enzymes involved in fatty acid synthesis in wild type (+/+ ) and PPARα-null (−/− ) mice

| Enzyme                  | (+/+ ) control | (−/− ) control | p value | (+/+ ) Wy-14,643 | (−/− ) Wy-14,643 | P value |
|-------------------------|----------------|----------------|---------|-----------------|-----------------|---------|
| Fatty acid synthetase   | 0.16 ± 0.02    | 0.11 ± 0.02    | 0.048   | 0.17 ± 0.02     | 0.14 ± 0.01     | 0.572   |
| Acetyl-CoA carboxylase  | 0.63 ± 0.08    | 0.65 ± 0.07    | 0.564   | 0.86 ± 0.05     | 0.92 ± 0.09     | 0.023   |
| ME                      | 57.9 ± 6.1     | 25.4 ± 6.0     | 0.003   | 227.5 ± 27.3    | 26.7 ± 5.7      | <0.001  |
| Glucose-6-phosphate dehydrogenase | 4.4 ± 0.7 | 5.0 ± 0.7 | 0.353 | 7.1 ± 0.6 | 4.8 ± 0.6 | 0.007 |
| ATP-citrate lyase       | 3.8 ± 0.4      | 3.1 ± 0.3      | 0.724   | 4.5 ± 0.4       | 2.7 ± 0.3       | 0.079   |

*a* Comparing (+/+ ) control with (−/− ) control.

*b* Comparing (+/+ ) control with (+/+ ) Wy-14,643.

**Fig. 2. Northern analysis of hepatic mRNAs.** Representative samples from three separate mice were used. Diet and genotype are indicated. Total RNA (5.4 μg) from three representative mice from each group were electrophoresed on a denaturing gel and probed using cDNAs for VLCAD (panel A), LCAD (panel B), ME (panel C), LACS (panel D), and SCHAD (panel E). The blots were exposed to autoradiographic film for 5 days (panels A–D) and 1 day (panel E).
assays. The present study extends these observations by confirming that expression of VLCAD, LCAD, SCAD, TPα, TPβ, MTE I, CTE II, SCHAD, T1, T2, LACS, and CPT II are all higher as a result of Wy-14,643 feeding in wild type mice but not in PPARα null mice. Northern analysis of mRNA encoding some of these enzymes revealed that induction is most likely due to increases in mRNA. These observations demonstrate that changes in gene expression of proteins involved in lipid metabolism are mediated by PPARα after exposure to peroxisome proliferators. Indeed, peroxisome proliferator response elements have been found and shown to be functionally active in the MCAD (43) and LACS (46) genes. A peroxisome proliferator response element has not been found in the LCAD (47), even though it is induced in wild type mice by Wy-14,643 feeding. Expression of ME is also elevated at the mRNA and protein level in agreement with a role of PPARαs in its regulation (45).

The fibrate class of drugs can also lead to suppression of gene expression of numerous proteins involved in lipid metabolism (4). Levels of apolipoprotein A-I, apolipoprotein C-III, apolipoprotein A-IV, hepatic lipase, and lecithin-cholesterol acyltransferase are all lowered by treatment of mice with fibrate drugs (4, 48, 49). The alterations of these proteins in addition to altered gene expression of peroxisomal fatty acid β-oxidizing enzymes are thought to contribute to the lipid-lowering effects of hypolipidemic drugs (4). In addition, it was recently shown that the down-regulation of apolipoprotein C-III mRNA and protein that contributes to the triglyceride-lowering effect of Wy-14,643 is mediated by PPARα (50). The results presented here extend these observations by demonstrating that the peroxisome proliferator Wy-14,643 induces significant changes in many fatty acid β-oxidizing enzymes and total hepatic β-oxidation, which in turn are likely to further contribute to the triglyceride-lowering effect of the fibrate class of hypolipidemic drugs. Combined, these results establish that PPARα functions in the control of lipid homeostasis in mice by regulating constitutive and inducible expression of fatty acid catabolism. Since nuclear receptors usually require a ligand for gene activation, the constitutive control of the enzymes would suggest that an endogenous ligand exists in liver.

The lower expression of mRNAs encoding several fatty acid metabolizing enzymes in PPARα null mice suggests either the presence of an endogenous ligand that preferentially controls genes encoding fatty acid-metabolizing enzymes and ME or that a ligand-independent mechanism is involved. Indeed, phosphorylation of a Ser-112 in PPARγ has been shown to modulate its activity (51, 52). This kinase recognition site is conserved between PPARγ and PPARα. Irrespective of the mechanism of PPARα activation, these results suggest that it differentially activates genes in the absence of exogenous ligands.

The identification of endogenous ligands has recently been addressed. It was shown that PPARα participates in the control of the inflammatory response involving leukotriene B4 (53). These studies also established that leukotriene B4 can directly bind to recombinant PPARα. Possible direct binding of peroxisome proliferators was shown by induced conformational changes as detected by protease sensitivity of in vitro translated PPARα (54). Other indirect transactivation experiments suggest that PPARα may mediate the action of 8(S)-hydroxyeicosatetraenoic acid (55). It is likely that other ligands for PPARα exist, including fatty acid metabolites, as first suggested by the ability of fatty acids to transactivate the receptor (56, 57). Evidence exists for the presence of endogenous PPARα activators in cultured cells used for transactivation studies. High background levels of reporter gene activation are usually
Role of PPARα in Constitutive Gene Expression

Among the important issues that need to be addressed is the species differences in response to peroxisome proliferators (60). Mice and rats are highly susceptible to peroxisome proliferation and hepatocarcinogenesis, whereas nonhuman primates and humans appear to be resistant. The mechanism of this species differences is not presently known, but it might be due to lower hepatic levels of PPARα (61, 62). Despite the lack of demonstratable peroxisome proliferation, the fibrate drugs are highly effective lipid-lowering agents in humans (63). Thus, PPARα may differentially regulate expression of genes that cause peroxisome proliferation and genes encoding enzymes that are responsible for fatty acid mobilization, transport, and catabolism. For example, high cellular levels of receptor may activate genes encoding peroxisomal, mitochondrial, and microsomal fatty acid metabolism in addition to genes that directly or indirectly control the cell cycle and peroxisome proliferation.

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