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Less Extrahepatic Induction of Fatty Acid β-Oxidation Enzymes by PPARα

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The peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor that transcriptionally regulates mitochondrial and peroxisomal fatty acid β-oxidation enzymes in the liver. Ligands include synthetic peroxisome proliferators and some fatty acids. PPARα activation leads to predictable pleiotropic responses in liver including peroxisome proliferation, increased fatty acid oxidation, and hepatocellular carcinoma. In the current study, the response to PPARα-activation was compared in the heart, kidney, and liver since the role of PPARα in extrahepatic fatty acid-oxidizing organs has not been fully explored. Basal expression of mitochondrial β-oxidation enzymes was comparable in the three tissues, but peroxisomal β-oxidation enzymes were most abundant in the liver and less so in the kidney and especially in the heart. After PPARα activation with ciprofibrate, both mitochondrial and peroxisomal β-oxidation enzymes were induced, with the strongest response seen in the liver, a moderate response in the kidney, and no significant response in the heart. PPARα mRNA analysis suggested that the differential response may be related to PPARα expression. © 2000 Academic Press

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Various xenobiotic compounds such as hypolipidemic drugs, plasticizers, herbicides, and others cause peroxisome proliferation in rodent livers. Therefore, they are collectively called peroxisome proliferators (1). When the presence of peroxisomal β-oxidation activity in the rat liver was first described, a marked increase in the overall activity of this system by the administration of the hypolipidemic drug clofibrate was noted (2). The increase in fatty acid oxidation activity was shown to be due to concomitant increases of the component enzymes of the peroxisomal β-oxidation system, including straight-chain fatty acyl-CoA oxidase (AOX), peroxisomal enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (L-PBE), and peroxisomal 3-ketoacyl-CoA thiolase (PTL) (3). These enzymes in rat liver were increased more than 20-fold by feeding a diet containing di-(2-ethylhexyl)phthalate, a peroxisome proliferator, reaching maximal levels after 2 weeks. The high levels were maintained during administration of the peroxisome proliferator, but these enzymes decreased to control levels 1 week after withdrawal. Change of the overall activity was closely related to enzyme quantity. Consequential to studies of the mechanism of the hypolipidemic effect of clofibrate, it was noticed that the administration of clofibrate changes the metabolism of both carbohydrates and fatty acids (4–6). Soon, it was elucidated that treatment of rats with peroxisome proliferators also results in an overall increase in the activity of the mitochondrial fatty acid oxidation system and some of its component enzymes (3, 7–9).

Pleiotropic responses to peroxisome proliferators including hepatic peroxisome proliferation, elevated levels of fatty acid oxidation enzymes, and hepatocellular carcinoma result from activation of the peroxisome proliferator-activated receptor α (PPARα) (10). In the presence of ligand, PPARα heterodimerizes with the retinoid-X receptor α (RXRα) and binds to DNA at the peroxisome proliferator-response element (PPRE) in the 5′ regulatory region of a responsive gene, activating transcription (11). The requirement of PPARα for transcriptional regulation of many fatty acid oxidation enzymes was confirmed *in vivo* by the use of PPARα-null (PPARα$^{-/-}$) mice (12, 13). Increases in peroxisomal and mitochondrial hepatic fatty acid oxidation enzymes were reported in AOX-null (AOX$^{-/-}$) mice similar to those seen in wild type mice treated with synthetic peroxisome proliferators (14). In AOX$^{-/-}$ mice endogenous PPARα ligands accumulate, resulting in constitutive activation of PPARα and spontaneous peroxisome proliferation. These endogenous ligands are

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presumably substrates of AOX (15). The induction seen in AOX$^{-/-}$ mice was found to be dependent upon PPARα, as demonstrated by the absence of a response in mice deficient of both AOX and PPARα (16).

Recent studies have further elucidated the critical role of PPARα in regulating the use of metabolic energy including the adaptive response to diabetes, fasting, and other nutritional changes (17–21). However, most studies of PPARα-mediated regulation of fatty acid oxidation have focused on the liver. Determination of the PPARα-mediated response to peroxisome proliferators in other major fat metabolizing organs such as heart and kidney is also important to understand the pathophysiological role of PPARα. This study deals with the changes in the amounts of peroxisomal and mitochondrial fatty acid oxidizing enzymes in the heart, kidney, and liver in response to ciprofibrate, a potent peroxisome proliferator. To account for the pharmacological distribution of ciprofibrate between organs, AOX$^{-/-}$ mice were also included in the study. Determination of basal protein levels established that mitochondrial enzymes are present in similar quantities in heart, kidney and liver, but that peroxisomal enzymes appeared more abundant in liver, moderately expressed in kidney, and low in heart. Both mitochondrial and peroxisomal enzymes responded strongly to PPARα activation in liver, moderately in kidney, and not significantly in heart. This pattern of induction may be due to higher basal PPARα mRNA expression observed in the liver, which was further induced by ciprofibrate treatment.

MATERIALS AND METHODS

Animals. Male wild-type (C57BL/6J) and AOX$^{-/-}$ mice (14) ages 2 to 3 months were used. Each group was subdivided and fed either a control diet or a diet containing 0.025% ciprofibrate, a potent peroxisome proliferator, for 2 weeks or more. All animal procedures used in this study were reviewed and pre-approved by the Institutional Review Boards for Animal Research of Northwestern University.

Quantification of enzyme proteins. Total protein concentrations of heart, kidney and liver homogenates were determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as a standard. Contents of β-oxidation enzymes and other proteins were determined by immunoblot analysis as described previously (16). CYP4A10 was detected with rabbit polyclonal antibodies against purified rat liver CYP4A1 (22). For detection of the CYP4A10, a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody was used with an enhanced chemifluorescent substrate kit (ECF, Amersham Pharmacia Biotech, Piscataway, NJ) and detected with a Molecular Dynamics Storm 860 in chemifluorescence mode (Amersham Pharmacia Biotech, Piscataway, NJ). For detection of the other enzymes, an alkaline phosphatase-conjugated second antibody was used for color development.

RNase protection assay. Total RNA was isolated from liver using the acid guanidinium thiocyanate-phenol-chloroform extraction method. RNase protection assay was performed using the following gene specific probes: PPARα, nucleotides 1186–1565 (GenBank Accession No. X57638) (10); PPARγ, nucleotides 1597–1914 (GenBank Accession No. U01841) (23); PPARδ, nucleotides 1004–1268 (GenBank Accession No. U10375) (24); and CYP4A10, nucleotides 1421–1555 (GenBank Accession No. ABO18421). Antisense RNA probes were transcribed in the presence of ^{32}P-UTP (20 mCi/ml, 800 Ci/mmol, (Amersham Pharmacia Biotech, Piscataway, NJ)) using the MAXIscript *in vitro* transcription kit (Ambion, Austin, TX). After transcription, the labeled riboprobes were purified in a 5% TBE-urea polyacrylamide Ready Gel (Bio-Rad, Hercules, CA). All seven probes were hybridized simultaneously with 8 μg of each total RNA sample overnight at 42°C and then digested for 30 min with RNase A/RNase T1 mix at 37°C. Protected fragments were separated on a 6% polyacrylamide sequencing gel 0.4 mm in thickness (Bio-Rad, Hercules, CA), and exposed to film or a phosphorimager plate (Molecular Dynamics, Amersham Pharmacia Biotech, Piscataway, NJ). Quantitation was with a Molecular Dynamics Storm 860 phosphorimager.

TABLE 1

Contents of Mitochondrial and Peroxisomal Fatty Acid Oxidation Enzymes in Liver, Kidney, and Heart of Wild-Type Mice

	Liver	Kidney	Heart
VLACS[a,c]	1.0	1.2	—
PCS[c]	1.0	0.23	0.67
Mitochondrial enzymes			
OCS	1.0	0.15	0.07
CPT II	1.0	0.47	0.67
CAT	1.0	1.5	2.3
VLCAD	1.0	0.80	1.3
LCAD	1.0	0.87	2.1
MCAD	1.0	1.1	1.1
SCAD	1.0	0.50	0.66
ETF	1.0	0.62	0.94
MH	1.0	0.74	0.80
HADH	1.0	0.80	0.80
MTL1	1.0	0.38	0.80
TFP	1.0	0.34	1.7
SCOT[b]	—	1.0	0.58
MTL2	1.0	0.31	0.46
Peroxisomal enzymes			
COT[a]	1.0	0.30	—
AOX[a]	1.0	0.33	0.07
L-PBE[a]	1.0	0.90	0.09
D-PBE	1.0	0.13	—
PTL	1.0	0.32	0.07

Note. The quantities (per mg proteins of the tissue) were estimated by immunoblot analysis using pooled samples of three mice. The values are expressed relative to liver.

[a] VLACS, COT, and D-PBE in heart were hardly detected.

[b] SCOT is not present in liver.

[c] VLACS is a bipartite (microsomal and peroxisomal) enzyme and PCS is a tripartite (microsomal, mitochondrial, and peroxisomal) enzyme (see ref. 25).

RESULTS AND DISCUSSION

Change of Enzyme Contents

Table 1 summarizes the basal quantities of selected enzymes in the heart and kidney relative to the liver. These data demonstrate that most of the mitochondrial enzymes are present in relatively similar amounts in the three tissues analyzed, while the peroxisomal enzymes are rather less abundant in the heart and kidney compared to the liver. Peroxisomal enzymes are

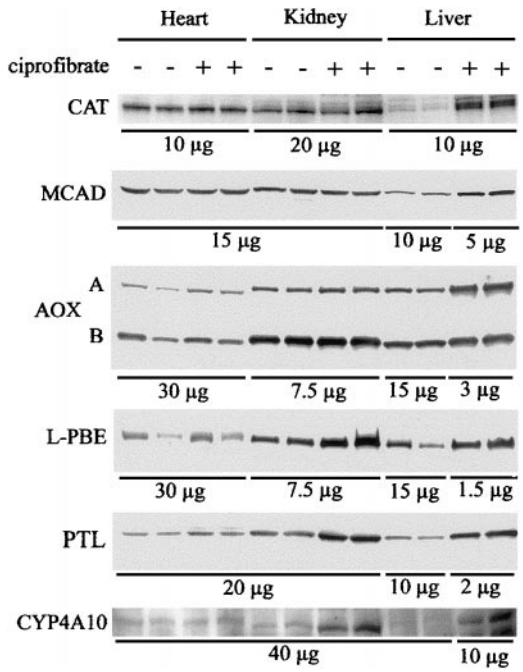

FIG. 1. Mitochondrial and peroxisomal β-oxidation enzymes and a microsomal ω-oxidation enzyme respond similarly to peroxisome proliferators. Immunoblot analysis of some mitochondrial β-oxidation enzymes (CAT and MCAD), peroxisomal β-oxidation enzymes (AOX, L-PBE, and PTL), and a microsomal ω-oxidation enzyme (CYP4A10) showed a consistent response to peroxisome proliferators. Ciprofibrate caused a strong induction in the liver, a moderate response in the kidney, and no response in the heart. The various amounts of total protein loaded in each lane is indicated. Loading was adjusted so that all signals would be in the linear range of the assay for quantitation (data shown in Tables 1 and 2).

especially rare in the heart. These enzymes have been described (25).

Both mitochondrial and peroxisomal enzymes responded to PPARα activation by ciprofibrate (Fig. 1). Carnitine acetyltransferase (CAT) is a mitochondrial enzyme that responded to peroxisome proliferators in a typical manner. Basal levels of CAT were highest in the heart, but the response to ciprofibrate was strongest in the liver. Medium-chain acyl-CoA dehydrogenase (MCAD) is a mitochondrial β-oxidation enzyme expressed approximately equally in the three tissues except after ciprofibrate treatment, when it was increased threefold in the liver. MCAD did not respond to ciprofibrate in the heart or kidney. Peroxisomal fatty acid oxidation enzymes AOX and L-PBE, and CYP4A10, a microsomal enzyme catalyzing ω-hydroxylation (22), responded in a typical fashion to PPARα activation in which a marked increase was seen in the liver, a moderate increase was seen in the kidney, and no response was seen in the heart.

Table 2 summarizes the results of immunoblot analyses of fatty acid oxidation enzymes in the mouse heart, kidney, and liver in response to PPARα activation by ciprofibrate treatment and/or AOX deficiency. Very long chain acyl-CoA synthetase (VLACS) is localized in microsomes and peroxisomes, and plays a role in the activation of very long chain fatty acids to CoA esters. This enzyme has been previously confirmed to be present in liver and kidney, but its content in kidney was lower in the rat. The rat liver enzyme has been shown to be inducible by peroxisome proliferators, but in the current study the contents of the enzyme in liver and kidney were not changed by treatment with ciprofibrate. This enzyme was not detectable in the heart (Table 1).

Palmitoyl-CoA synthetase (PCS) in kidney was induced in the ciprofibrate-treated group and in AOX$^{-/-}$ mice. The content of PCS in heart was not varied either by the ciprofibrate treatment or disruption of AOX gene. PCS is localized in microsomes, mitochondria, and peroxisomes. Previous studies suggested that the PCS activity in both mitochondria and peroxisomes are far higher than the overall β-oxidation activity, suggesting that this activation reaction is not rate limiting (7, 8, 26). Therefore, the physiological role of an approximately twofold increase in the PCS activity in the kidney is obscure.

Octanoyl-CoA synthetase (OCS), a mitochondrial matrix enzyme, activates medium chain fatty acids. This enzyme is rich in liver but lower in extrahepatic tissues. OCS content in kidney was increased both by ciprofibrate treatment and disruption of the AOX gene. The enzyme content in heart was not changed.

Much attention has been focused on regulation at the step of carnitine-dependent uptake of the fatty acyl moiety by mitochondria. Acyl groups are transferred as carnitine esters into the mitochondrial matrix by the concerted action of both carnitine palmitoyltransferase I (CPT I) and acylcarnitine translocase. After transport, CPT II converts fatty acylcarnitines to CoA esters. CPT I exists as two isoforms encoded by separate genes: the liver-type, which is ubiquitously expressed in many tissues including in the kidney (27), and the muscle-type, which is expressed abundantly in heart, skeletal muscle, and brown adipose tissue (28). Kidney CPT I is the liver-type (29), and heart CTP I is the muscle-type (30). Transcription of CPT I (31, 32) is considered to be regulated by PPARα.

In this experiment, only CPT II was quantified. The renal content of CPT II was not increased in wild type mice administered ciprofibrate, and the heart enzyme was not changed either by the administration of ciprofibrate or disruption of AOX gene. However, the renal CPT II content in AOX$^{-/-}$ mice was about twofold higher.

CAT activity in rat and mouse livers was markedly increased by the administration of peroxisome proliferators. CAT in the liver has been previously shown to be a typical peroxisome proliferator-responding enzyme (16). In that study, the CAT content in liver was shown to be markedly increased by the administration

TABLE 2

Quantification of Kidney and Heart Mitochondrial and Peroxisomal Fatty Acid Oxidation Enzymes

	Kidney				Heart				Liver
	Wild		AOX$^{-/-}$		Wild		AOX$^{-/-}$		Wild
Ciprofibrate	−	+	−	+	−	+	−	+	+
VLACS[a,c]	1.0	1.2	1.1	0.95	—	—	—	—	1.0
PCS[c]	1.0	2.2	2.5	2.8	1.0	0.97	1.2	1.2	2.7
Mitochondrial enzymes									
OCS	1.0	13	4.5	5.8	1.0	1.6	0.80	1.4	1.0
CPT II	1.0	1.3	2.3	2.5	1.0	1.0	1.5	1.7	3.2
CAT	1.0	1.8	1.2	1.3	1.0	1.1	1.1	1.0	10
VLCAD	1.0	1.3	1.0	0.80	1.0	1.1	1.7	2.1	1.3
LCAD	1.0	2.6	1.7	1.9	1.0	1.1	1.4	1.8	3.6
MCAD	1.0	1.4	1.3	1.2	1.0	1.1	1.5	2.0	3.8
SCAD	1.0	1.4	1.2	1.2	1.0	1.3	1.2	0.96	5.8
ETF	1.0	1.1	1.0	1.0	1.0	1.1	1.0	1.0	1.0
MH	1.0	1.0	1.0	1.0	1.0	1.1	1.2	1.2	1.0
HADH	1.0	1.1	1.0	1.0	1.0	0.95	0.97	0.95	3.0
MTL1	1.0	1.2	1.1	1.2	1.0	1.1	1.0	1.0	1.2
TFP	1.0	1.0	0.95	1.0	1.0	1.1	1.0	1.0	16
SCOT[b]	1.0	1.5	1.1	0.90	1.0	1.0	1.1	1.1	—
MTL2	1.0	1.7	1.1	1.2	1.0	1.4	0.90	1.2	5.4
Peroxisomal enzymes									
COT[a]	1.0	2.0	1.9	2.0	—	—	—	—	22
AOX[a]	1.0	2.0	—	—	1.0	1.0	—	—	10
L-PBE	1.0	4.7	3.8	3.4	1.0	1.1	1.9	3.3	120
D-PBE[a]	1.0	2.4	2.2	1.8	—	—	—	—	3.0
PTL	1.0	2.2	5.4	5.6	1.0	2.0	9.2	9.0	13

Note. Pooled samples of three mice were subjected to immunoblot analysis, and the signals were quantified. The values are relative to those of the wild type mice fed with the control diet. The ratios of liver are increments in wild-type mice by the ciprofibrate treatment, and these values are adopted from Ref. 16.

[a] VLACS, COT and D-PBE in heart were hardly detected.

[b] SCOT is not present in liver.

[c] VLACS is a bipartite (microsomal and peroxisomal) enzyme and PCS is a tripartite (microsomal, mitochondrial, and peroxisomal) enzyme (see ref. 25).

of ciprofibrate or disruption of AOX gene, and the increase was suggested to be due to PPARα hyperfunction since no increase was observed in the mouse in the absence of PPARα. The CAT contents in the wild-type kidney seemed to be slightly increased by the administration of ciprofibrate. In the AOX$^{-/-}$ mouse kidney CAT content was similar to that of the wild-type mice, and this level was not changed by treatment with ciprofibrate. In heart, the contents were nearly the same among the four groups.

The enzymes involved in the first reaction of the mitochondrial β-oxidation cycle are very long chain acyl-CoA dehydrogenase (VLCAD), long chain acyl-CoA dehydrogenase (LCAD), medium chain acyl-CoA dehydrogenase (MCAD), and short chain acyl-CoA dehydrogenase (SCAD). In liver, the enzyme quantities clearly increased for three classical dehydrogenases, LCAD, MCAD, and SCAD. These increases seem to have physiological significance. However, changes of enzyme levels in kidney and heart among the four groups were not as clear as those in liver. The amount of electron transfer flavoprotein (ETF) remained unchanged.

Enoyl-CoA hydratase (MH), 3-hydroxyacyl-CoA dehydrogenase (HADH), and 3-ketoacyl-CoA thiolase (MTL1) are mitochondrial monofunctional enzymes. The trifunctional protein (TFP) is an enzyme complex catalyzing three reactions: enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase reactions. None of these enzymes were affected by the administration of ciprofibrate in the extrahepatic tissues. The AOX$^{-/-}$ mice also showed no change in these enzyme levels.

Succinyl-CoA: 3-oxoacid transferase (SCOT) and mitochondrial acetoacetyl-CoA-specific thiolase (MTL2) are involved in ketone body utilization. The levels of these two enzymes in kidney and heart were unchanged either by ciprofibrate administration or disruption of AOX gene.

Several peroxisomal proteins in liver are markedly increased both by ciprofibrate treatment and by disruption of AOX gene (16). Carnitine octanoyltransferase (COT) is a matrix enzyme and has a broad specificity of carbon chain length. This enzyme was markedly increased by the administration of peroxi-

some proliferators. However, its physiological role is not clear because peroxisomal β-oxidation is independent of carnitine. COT was detected in kidney, and confirmed to increase both by ciprofibrate treatment and by disruption of the AOX gene. This enzyme was not detected in the heart.

Induction of the peroxisomal straight chain β-oxidation enzymes in liver is a traditional hallmark of the peroxisome proliferator response. The component enzymes AOX, L-PBE, and PTL are transcriptionally regulated by PPARα. The amount of AOX in liver increased 10-fold or more by the administration of peroxisome proliferators. A twofold change in AOX expression was observed in the kidney, but no change was seen in the heart. Treatment with ciprofibrate results in L-PBE becoming the most abundantly expressed protein in the liver, increasing about 120-fold. Both ciprofibrate treatment and AOX disruption resulted in increased levels of L-PBE in the kidney, but only AOX disruption increased the amount of L-PBE in the heart. In either case, the increases were small compared to those seen in the liver.

The D-3-hydroxyacyl-CoA dehydratase/D-3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (D-PBE) content in liver was rather less responsive to peroxisome proliferators when compared to L-PBE. Ciprofibrate treatment increased the amount of D-PBE observed in the kidney. D-PBE was not detected in the heart. The contents of PTL in both kidney and heart were also changed. Sterol carrier protein x (SCPx), a second thiolase, was hardly detected in kidney and heart.

The data suggest that heart, kidney and liver all have a comparable basal capacity for mitochondrial fatty acid oxidation, but the heart and kidney capacities are independent of PPARα activation while capacity in the liver increases with PPARα activation. Kidney and especially heart have a markedly lower capacity for peroxisomal fatty acid oxidation than does liver. The liver enzyme levels are most responsive to PPARα activation. Therefore, the liver plays a major role in regulation of mitochondrial and peroxisomal fatty acid oxidation by regulating enzyme quantities in response to changes in PPARα function.

Transcriptional Regulation

PPARα-regulated transcripts encoding AOX, L-PBE, and CYP4A10 were detected in total RNA samples from heart, kidney and liver using the RNase protection assay. These messages were strongly expressed in liver and kidney, but only weakly expressed in the heart (Figs. 2A and 2B), in agreement with the immunoblot analyses. Administration of ciprofibrate, a potent peroxisome proliferator, to mice resulted in strong induction in the liver, a weaker response in kidney, and no induction in the heart. CYP4A10 and L-PBE were more strongly induced than AOX.

PPARγ and PPARδ are nuclear receptor family members closely related to PPARα, but only PPARα regulates fatty acid β-oxidation enzymes in the liver as demonstrated using PPARα$^{-/-}$ mice (12, 16). PPARγ and PPARδ serve as negative controls in the mRNA analysis, since their expression patterns are not expected to be related to PPARα activity. Basal expression of PPARα was found to be higher in the liver than in kidney or heart, and PPARα mRNA was induced more than twofold in liver by ciprofibrate treatment, but not induced in kidney or heart (Figs. 2A and 2B), providing a possible mechanism explaining the reduced extrahepatic response of fatty acid β-oxidation enzymes to ciprofibrate. As expected, PPARγ and PPARδ mRNA levels were not higher in liver and were not induced by ciprofibrate in any of the tissues.

Significance of PPARα in the Regulation of Metabolism

PPARα functions to regulate energy metabolism in the body, modulating the metabolism of carbohydrates and fatty acids. Indeed, the administration of peroxisome proliferators causes changes in the concentrations of energy fuels in blood such as glucose, free fatty acids and triacylglycerols (5, 6). Changes in energy metabolism are also seen when the PPARα gene is disrupted (17–20). The change in energy metabolite concentrations resulting from disruption of the AOX gene is supposed to be due to the accumulation of endogenous ligand(s) for PPARα (16).

The increased levels of some enzymes seen after administration of ciprofibrate to wild type mice were similar to those seen in AOX$^{-/-}$ mice, which were not significantly changed by ciprofibrate treatment. The general agreement of the data from ciprofibrate-treated and AOX$^{-/-}$ mice suggest that variations in enzyme content between the heart, kidney and liver are not due to variation in ciprofibrate concentrations among these organs.

The current study demonstrates that activation of PPARα results in increased expression of PPARα-responsive genes (both at the mRNA and protein levels) predominantly in the liver and to a lesser degree in the kidney. No response to peroxisome proliferators was observed in the heart. This pattern of induction is consistent with measurements of PPARα mRNA, which is most abundant in the liver. However, the PPARα expression profile does not explain the increased response in kidney over heart, suggesting a requirement for another regulatory molecule differentially expressed in the heart and kidney, such as a PPARα co-activator (33, 34). A recent study of fat metabolism enzymes in the heart of PPARα$^{-/-}$ mice documented a decrease in a subset of mitochondrial enzymes in the heart of null mice (35). However, no change was seen in the levels of peroxisomal enzymes

regulated by PPARα, suggesting that the observed differences may be indirectly related to the absence of PPARα in these mice.

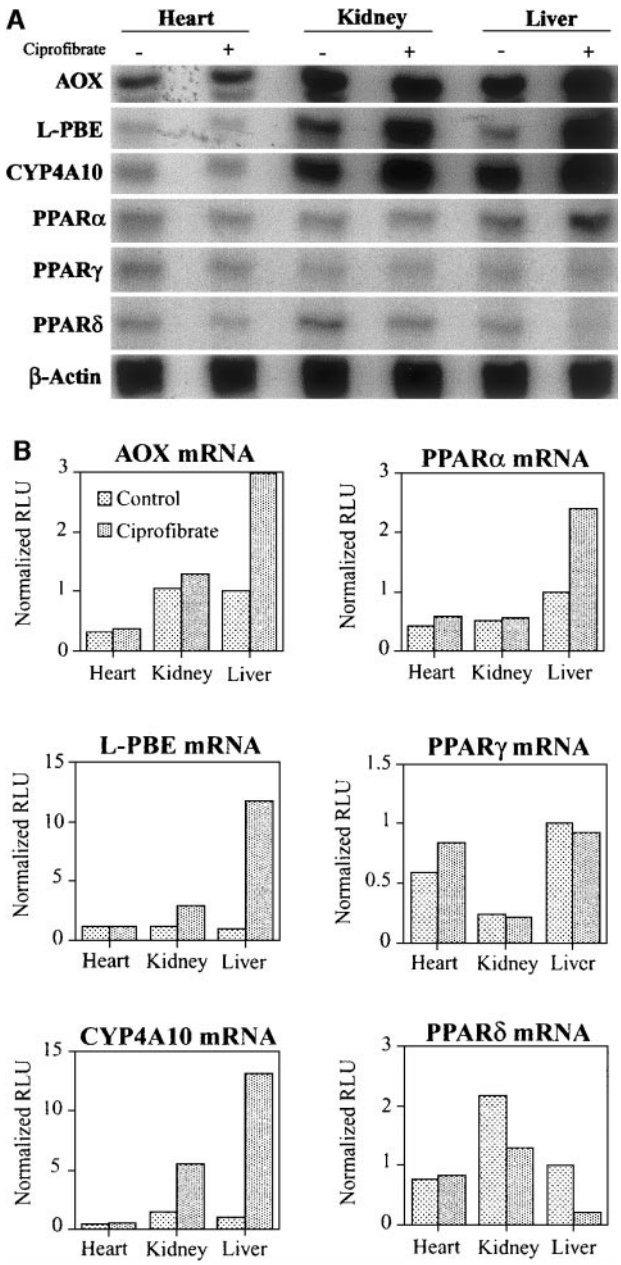

FIG. 2. Variation in peroxisome proliferator response in different tissues correlates with PPARα expression, but not PPARγ or PPARδ. (A) Ribonuclease protection assay of PPARα-responsive transcripts encoding AOX, L-PBE, and CYP4A10 showed a strong response to the peroxisome proliferator ciprofibrate in the liver, a moderate response in the kidney, and no response in the heart. PPARα was most strongly expressed in the liver, where it was induced by ciprofibrate, and was expressed at a lower level in the heart and kidney. PPARγ and PPARδ mRNAs were also detected in the heart, kidney and liver, but their expression patterns appeared to be unrelated to the peroxisome proliferator response. (B) Ribonuclease protection assay signals were quantified using a phosphorimager. Data were normalized to β-actin levels in each sample and displayed relative to untreated liver samples.

TABLE 3

Total Carnitine Measured in Kidney, Heart, and Liver

	Kidney	Heart	Liver
Wild	262 ± 19	659 ± 136	223 ± 17
Wild + ciprofibrate	613 ± 97	986 ± 231	767 ± 116

Note. The values represent mean ± S.D. (n = 3) expressed as nmol/g tissue.

Mitochondrial fatty acid β-oxidation is one of the major ATP-generating processes. The activity of this oxidation system depends upon carnitine. Ciprofibrate administration resulted in a threefold increase in carnitine in the liver, a twofold increase in the kidney, and a smaller increase in the heart (Table 3). The carnitine level in liver of wild type mice was increased after starvation, and this increase was not seen in PPARα$^{-/-}$ mice, and the carnitine level in the liver of AOX$^{-/-}$ mice was also higher (21). It is interesting to note that the mitochondrial fatty acid oxidation system is upregulated by PPARα not only *via* transcriptional induction of β-oxidation enzymes, but also increased carnitine content. The major target organ of PPARα activity is the liver, and extrahepatic tissues are less influenced by PPARα.

Peroxisomal β-oxidation contains two sets of enzymes. One system is composed of AOX, L-PBE, and PTL and oxidizes toxic very long straight-chain fatty acids. The other system consists of branched chain acyl-CoA oxidase, D-PBE, and SCPx and is responsible for branched-chain fatty acid oxidation and bile acid formation. The former system is inducible and the latter is constitutive (25). These processes play major roles in liver metabolism where they are strongly responsive to PPARα activity, while in extrahepatic tissues such as heart and kidney these processes have minor roles and are less responsive to PPARα.

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REFERENCES

1. Reddy, J. K., and Krishnakantha, T. P. (1975) Hepatic peroxisome proliferation: Induction by two novel compounds structurally unrelated to clofibrate. *Science* **190,** 787–789.
2. Lazarow, P. B., and De Duve, C. (1976) A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA* **73,** 2043–2046.
3. Hashimoto, T. (1982) Individual peroxisomal β-oxidation enzymes. *Ann. NY Acad. Sci.* **386,** 5–12.

4. Laker, M. E., and Mayes, P. A. (1979) The immediate and long term effects of clofibrate on the metabolism of the perfused rat liver. *Biochem. Pharmacol.* **28,** 2813–2827.
5. Miyazawa, S., Sakurai, T., Imura, M., and Hashimoto, T. (1975) Effects of ethyl p-chlorophenoxyisobutyrate on carbohydrate and fatty acid metabolism in rat liver. *J. Biochem.* **78,** 1171–1176.
6. Sakurai, T., Miyazawa, S., and Hashimoto, T. (1978) Effects of di-(2-ethylhexyl)phthalate administration on carbohydrate and fatty acid metabolism in rat liver. *J. Biochem.* **83,** 313–320.
7. Shindo, Y., and Hashimoto, T. (1978) Acyl-Coenzyme A synthetase and fatty acid oxidation in rat liver peroxisomes. *J. Biochem.* **84,** 1177–1181.
8. Shindo, Y., Osumi, T., and Hashimoto, T. (1978) Effects of administration of di-(2-ethylhexyl)pthalate on rat liver mitochondria. *Biochem. Pharmacol.* **27,** 2683–2688.
9. Mannaerts, G. P., Debeer, L. J., Thomas, J., and De Schepper, P. J. (1979) Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J. Biol. Chem.* **254,** 4585–4595.
10. Issemann, I., and Green, S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347,** 645–650.
11. Osumi, T., Wen, J.-K., and Hashimoto, T. (1991) Two cis-acting regulatory sequences in the peroxisome proliferator-responsive enhancer region of rat acyl-CoA oxidase gene. *Biochem. Biophys. Res. Commun.* **175,** 866–871.
12. Lee, S.-S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H., and Gonzalez, F. J. (1995) Targeted disruption of the alpha 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.
13. Aoyama, T., Peters, J. M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., and Gonzalez, F. J. (1998) Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα). *J. Biol. Chem.* **273,** 5678–5684.
14. Fan, C.-Y., Pan, J., Chu, R., Lee, D., Kluckman, K. D., Usuda, N., Singh, I., Yeldandi, A. V., Rao, M. S., Maeda, N., and Reddy, J. K. (1996) Hepatocellular and hepatic peroxisomal alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene. *J. Biol. Chem.* **271,** 24698–24710.
15. Fan, C.-Y., Pan, J., Usuda, N., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (1998) Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor α natural ligand metabolism. *J. Biol. Chem.* **273,** 15639–15645.
16. Hashimoto, T., Fujita, T., Usuda, N., Cook, W., Qi, C., Peters, J. M., Gonzalez, F. J., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (1999) Peroxisomal and mitochondrial fatty acid β-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor α and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. *J. Biol. Chem.* **274,** 19228–19236.
17. Djouadi, F., Weinheimer, C. J., Saffitz, J. E., Pitchford, C., Bastin, J., Gonzalez, F. J., and Kelly, D. P. (1998) A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor α-deficient mice. *J. Clin. Invest.* **102,** 1083–1091.
18. Costet, P., Legendre, C., More, J., Edgar, A., Galtier, P., and Pineau, T. (1998) Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273,** 29577–29585.
19. Leone, T. C., Weinheimer, C. J., and Kelly, D. P. (1999) A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: The PPARα-null mouse as a model of fatty acid oxidation disorders. *Proc. Natl. Acad. Sci. USA* **96,** 7473–7478.
20. Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., and Wahli, W. (1999) Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J. Clin. Invest.* **103,** 1489–1498.
21. Hashimoto, T., Cook, W. S., Qi, C., Yeldandi, A. V., Reddy, J. K., and Rao, M. S. (2000) Defect in peroxisome proliferator-activated receptor α-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. *J. Biol. Chem.* **276,** 28918–28928.
22. Hardwick, J. P., Song, B. J., Huberman, E., and Gonzalez, F. J. (1987) Isolation, complementary DNA sequence, and regulation of rat hepatic lauric acid ω-hydroxylase (cytochrome P-450LA ω). Identification of a new cytochrome P-450 gene family. *J. Biol. Chem.* **262,** 801–810.
23. Zhu, Y., Alvares, K., Huang, Q., Rao, M. S., and Reddy, J. K. (1993) Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. *J. Biol. Chem.* **268,** 26817–26820.
24. Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* **91,** 7355–7359.
25. Hashimoto, T. (1999) Peroxisomal β-oxidation enzymes. *Neurochem. Res.* **24,** 551–563.
26. Krisans, S. K., Mortensen, R. M., and Lazarow, P. B. (1980) Acyl-CoA synthetase in rat liver peroxisomes. Computer-assisted analysis of cell fractionation experiments. *J. Biol. Chem.* **255,** 9599–9607.
27. Esser, V., Britton, C. H., Weis, B. C., Foster, D. W., and McGarry, J. D. (1993) Cloning, sequencing, and expression of a cDNA encoding rat liver carnitine palmitoyltransferase I. Direct evidence that a single polypeptide is involved in inhibitor interaction and catalytic function. *J. Biol. Chem.* **268,** 5817–5822.
28. Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W., and McGarry, J. D. (1996) Expression of a cDNA isolated from rat brown adipose tissue and heart identifies the product as the muscle isoform of carnitine palmitoyltransferase I (M-CPT I). M-CPT I is the predominant CPT I isoform expressed in both white (epididymal) and brown adipocytes. *J. Biol. Chem.* **271,** 6972–6977.
29. Kolodziej, M. P., Crilly, P. J., Corstorphine, C. G., and Zammit, V. A. (1992) Development and characterization of a polyclonal antibody against rat liver mitochondrial overt carnitine palmitoyltransferase (CPT I). Distinction of CPT I from CPT II and of isoforms of CPT I in different tissues. *Biochem. J.* **282,** 415–421.
30. Park, E. A., and Cook, G. A. (1998) Differential regulation in the heart of mitochondrial carnitine palmitoyltransferase-I muscle and liver isoforms. *Mol. Cell. Biochem.* **180,** 27–32.
31. Brandt, J. M., Djouadi, F., and Kelly, D. P. (1998) Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor α. *J. Biol. Chem.* **273,** 23786–23792.
32. Mascaro, C., Acosta, E., Ortiz, J. A., Marrero, P. F., Hegardt, F. G., and Haro, D. (1998) Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J. Biol. Chem.* **273,** 8560–8563.
33. Zhu, Y., Kan, L., Qi, C., Kanwar, Y. S., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (2000) Isolation and characterization of peroxisome proliferator-activated receptor (PPAR) interacting

protein (PRIP) as a coactivator for PPAR. *J. Biol. Chem*. **275,** 13510–13516.

34. Zhu, Y., Qi, C., Jia, Y., Nye, J. S., Rao, M. S., and Reddy, J. K. (2000) Deletion of PBP/PPARBP, the gene for nuclear receptor coactivator peroxisome proliferator-activated receptor-binding protein, results in embryonic lethality. *J. Biol. Chem*. **275,** 14779–14782.
35. Watanabe, K., Fuji, H., Takahashi, T., Kodama, M., Aizawa, Y., Ohta, Y., Ono, T., Hasegawa, G., Naito, M., Nakajima, T., Kamijo, Y., Gonzalez, F. J., and Aoyama, T. (2000) Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor α associated with age-dependent cardiac toxicity. *J. Biol. Chem*. **275,** 22293–22299.