Overexpression of Peroxisome Proliferator-activated Receptor-α (PPARα)-regulated Genes in Liver in the Absence of Peroxisome Proliferation in Mice Deficient in both \( L \)- and \( D \)-Forms of Enoyl-CoA Hydratase/Dehydrogenase Enzymes of Peroxisomal \( \beta \)-Oxidation System*

Received for publication, June 16, 2003, and in revised form, August 7, 2003

Published, JBC Papers in Press, September 18, 2003, DOI 10.1074/jbc.M306363200

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Peroxisomal \( \beta \)-oxidation system consists of peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \))-inducible pathway capable of catalyzing straight-chain acyl-CoA\( s \) and a second noninducible pathway catalyzing the oxidation of 2-methyl-branched fatty acyl-CoA\( s \). Disruption of the inducible \( \beta \)-oxidation pathway in mice at the level of fatty acyl-CoA oxidase (AOX), the first and rate-limiting enzyme, results in spontaneous peroxisome proliferation and sustained activation of PPAR\( \alpha \), leading to the development of liver tumors, whereas disruptions at the level of the second enzyme of this classical pathway or of the noninducible system had no such discernible effects. We now show that mice with complete inactivation of peroxisomal \( \beta \)-oxidation at the level of the second enzyme, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydratase (L-PBE), of the inducible pathway and 3-hydroxyacyl-CoA dehydratase/3-hydroxyacyl-CoA dehydrogenase (D-PBE) of the noninducible pathway (L-PBE\( ^{−/−} \) D-PBE\( ^{−/−} \)), exhibit severe growth retardation and postnatal mortality with none surviving beyond weaning. L-PBE\( ^{−/−} \) D-PBE\( ^{−/−} \) mice that survived exceptionally beyond the age of 3 weeks exhibited overexpression of PPAR\( \alpha \)-regulated genes in liver, despite the absence of morphological evidence of hepatic peroxisome proliferation. These studies establish that peroxisome proliferation in rodent liver is highly correlatable with the induction mostly of the \( L \)- and \( D \)-PBE genes. We conclude that disruption of peroxisomal fatty acid \( \beta \)-oxidation at the level of second enzyme in mice leads to the induction of many of the PPAR\( \alpha \) target genes independently of peroxisome proliferation in hepatocytes, raising the possibility that intermediate metabolites of very long-chain fatty acids and peroxisomal \( \beta \)-oxidation act as ligands for PPAR\( \alpha \).

In mammals, fatty acid oxidation occurs in mitochondria, peroxisomes, and smooth endoplasmic reticulum (1). Whereas mitochondria and peroxisomes oxidize fatty acids via \( \beta \)-oxidation, the cytochrome P450 CYP4A subfamily of enzymes located in the smooth endoplasmic reticulum metabolizes fatty acids by \( \omega \)-oxidation (1–3). Mitochondrial \( \beta \)-oxidation is responsible for the oxidation of the major portion of the short- (\( < C_{20} \)), medium-(C\( _{20} \)–C\( _{24} \)), and long-(C\( _{24} \)–C\( _{28} \)) chain fatty acids and, in the process, constitutes the primary source of energy derived from fatty acids. On the other hand, peroxisomal \( \beta \)-oxidation is responsible for the metabolism, almost exclusively, of very long straight-chain fatty acids (\( > C_{20} \)), 2-methyl-branched fatty acids (e.g. pristanic acid that is generated after \( \omega \)-oxidation of the 3-methyl-branched fatty acid phytanic acid), prostanooids, dicarboxylic acids, and the C\( _{27} \) bile acid intermediates di- and trihydroxycoprostanolic acids (1, 2, 4). The importance of peroxisomes and peroxisomal fatty acid \( \beta \)-oxidation for human health is underscored by the existence of peroxisomal biogenesis disorders such as Zellweger syndrome and other genetic diseases affecting peroxisomal \( \beta \)-oxidation (1, 2) and by the ability of many structurally diverse chemicals designated as peroxisome proliferators to induce peroxisome proliferation and increase fatty acid oxidation in liver cells, leading to the development of liver tumors in rodents (5–7).

Peroxisomal \( \beta \)-oxidation consists of four steps. Each metabolic conversion can be carried out by at least two different enzymes. The classical peroxisome proliferator-inducible pathway utilizes straight-chain acyl-CoA as substrates, whereas the second noninducible pathway catalyzes the oxidation of 2-methyl-branched fatty acyl-CoA\( s \) (1, 2, 4). In the classical inducible \( \beta \)-oxidation pathway, dehydrogenation of acyl-CoA esters to their corresponding trans-2-enoyl-CoA\( s \) is catalyzed by fatty acyl-CoA oxidase (AOX), whereas the second and third

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* This work was supported by National Institutes of Health Grants GM23750 (to J. K. R.) and CA84472 (to M. S. R.) and by Fonds Weten schappelijk Onderzoek Vlaanderen G 0235 01, Geconcerteerde Onderzoeksacties GOA/99/09 and European Community Grant QLG1-CT2001-01299 (to M. B. and P. P. V.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: AOX, peroxisomal fatty acyl-CoA oxidase; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid-x-receptor for 9-cis-retinoic acid; L-PBE, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydratase bi- (multi) functional enzyme; D-PBE, 3-hydroxyacyl-CoA dehydratase/3-hydroxyacyl-CoA dehydrogenase bi- (multi) functional enzyme; DKO, L-PBE\( ^{−/−} \) D-PBE\( ^{−/−} \) double null mice; PTL, peroxisomal thiolase; SCP2/SCPx, sterol carrier protein 2, sterol carrier protein 20, sterol carrier protein X; CYP4A1 and CYP4A3, encode microsomal cytochrome P450 fatty acid \( \omega \)-hydroxylase; ACTE, acyl-CoA thioesterase PTE-2; UOX, urate oxidase; CTL, catalase; PEX11a, peroxisome membrane protein; PMP70, 70-kDa peroxisomal membrane protein; CD36, cluster of differentiation 36; SHLR, endogenous virus-
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Like sequence; PDK-4, pyruvate dehydrogenase kinase protein; CSf, gene-rich cluster CSf; Ly-6D, lymphocyte antigen 6 complex locus D; mLipase, monoglyceride lipase.

reactions, hydration and dehydrogenation of enoyl-CoA esters to 3-ketoacyl-CoA, are catalyzed by a single enzyme, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (l-bi/multifunctional enzyme (l-PBE/MFP2)) (1, 2, 4). The third enzyme of this inducible system, 3-ketoacyl-CoA thiolaize (PFTL), converts 3-ketoacyl-CoA to acetyl-CoA and an acyl-CoA that is two carbon atoms shorter than the original molecule, and the shortened acyl-CoA re-enters the β-oxidation cycle (1, 2, 4). In the noninducible β-oxidation pathway, dehydrogenation of branched fatty acyl-CoA esters to their corresponding trans-2-enoyl-CoAs is catalyzed by the branched chain acyl-CoA oxidase or by pristanoyl-CoA oxidase (4). These enzymes are also capable of desaturating straight-chain acyl-CoAs. The second and third reactions of this pathway are performed by 3-hydroxyacyl-CoA dehydratase/3-hydroxyacyl-CoA dehydrogenase (l-bi/multifunctional enzyme (l-PBE/MFP2)), and the resulting 3-ketoacyl-CoAs are cleaved by the third enzyme of this system, designated sterol carrier protein x (SCPx), which possesses thiolaize activity (4, 8). The gene encoding SCPx, a 58-kDa protein with 3-ketoacyl-CoA thiolaize activity, also codes for a smaller 5.3-kDa protein designated sterol carrier protein 2 (SCP2 or nonspecific lipid transfer protein) (4, 8). The three enzymes of the classical β-oxidation pathway are markedly induced in conjunction with peroxisome proliferation by peroxisomal proliferators (9). These agents exert their pleiotrophic effects in liver by activating a nuclear receptor designated as peroxisome proliferator-activated receptor α (PPARα) (10). The PPARα subfamily consists of three members, α, β/δ, and γ (11), that heterodimerize with retinoid X receptor (12), and the PPAR/retinoid X receptor complex binds to peroxisome proliferator-response element, a region consisting of a degenerate direct repeat of the canonical AGGTCA sequence separated by 1 base pair (DR1), present in the 5′-flanking region of target genes (12, 13). Sustained activation of PPARα in liver by these synthetic ligands in rats and mice leads to the development of liver tumors (7, 14). Activation of PPARα by natural ligands in the liver of AOX−/− mice also leads to the development of liver cancer (15). In this sense, the functional integrity of the classical β-oxidation pathway at the level of the first enzyme AOX appears to be essential to keep PPARα in check and prevent spontaneous peroxisome proliferation by effectively metabolizing AOX substrates, which appear to function as natural PPARα ligands (15). Disruption of the gene encoding l-PBE, the second enzyme of this classical system, did not manifest either in spontaneous peroxisome proliferation or in the transcriptional activation of PPARα target genes in liver, indicating that inactivation of classical β-oxidation system distal to AOX step does not interfere with the inactivation of endogenous ligands of PPARα (15, 16). The absence of appreciable defects in lipid metabolism in l-PBE−/− mice suggested that enoyl-CoAs, generated in the classical β-oxidation system, are diverted to the noninducible branched chain β-oxidation pathway for degradation by d-PBE (16). In this study, to investigate the functional implications of the disruption of the metabolism of enoyl-CoAs on lipid metabolism, peroxisome proliferation, and PPARα activation in liver, we generated mice deficient in both l-PBE and d-PBE enzymes so that both peroxisomal β-oxidation pathways are effectively disrupted at the second enzyme level. Mice deficient in both l-PBE and d-PBE enzymes (l-PBE−/−/d-PBE−/−) exhibited severe growth retardation and postnatal mortality. They revealed microvesicular hepatic steatosis, abnormalities in mitochondrial structure, and overexpression of PPARα-regulated genes in liver, despite the lack of morphological evidence of peroxisome proliferation. These findings establish that the induction of many of the PPARα target genes can occur independently of peroxisome proliferation, further extending previous results (16), and that peroxisome proliferation in rodent liver is highly correlatable with the induction mostly of the l-PBE gene.

MATERIALS AND METHODS

Generation of l-PBE−/−/d-PBE−/− Mice—The generation of AOX−/−, l-PBE, and d-PBE (MFP-2) null mice has been described elsewhere (16–18). Since d-PBE deficiency resulted in reduced fertility of l-PBE−/− mice (18), l-PBE−/− mice were mated with heterozygous d-PBE+/− mice to obtain l-PBE−/−/d-PBE+/− mice. These were intercrossed to generate l-PBE−/−/d-PBE−/− and l-PBE+/−/d-PBE−/− mice. The l-PBE−/−/d-PBE−/− mouse lineages were used to generate l-PBE−/−/d-PBE−/− and l-PBE+/−/d-PBE−/− double knockout (DKO) mice. DNA extracted from the tail tips of mice and from the yolk sac of embryos was used for genotyping as described elsewhere (16, 18). All animal procedures used in this study were approved by the Institutional Review Board for Animal Research of the Northwestern University.

Morphological Analysis, Catalase Cytochemistry, and AOX and l-PBE Immunohistochemistry—Wild-type (C57BL/6J), l-PBE−/−, and d-PBE−/− mice, 6–8 weeks of age, were fed powdered diet with or without Wy-14,643 (0.0125% w/v), a peroxisomal proliferator (5), for 2 weeks. l-PBE−/−/d-PBE−/− mice were with the dams that were maintained on a normal diet without Wy-14,643. AOX−/− mice were also on a normal diet without the drug. For light microscopy, tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Histological analysis was performed using 4-μm-thick sections stained with hematoxylin and eosin. For cytochemical localization of catalase (CTL), the peroxisomal marker enzyme, tissues were incubated in alkaline 3,3-diaminobenzidine reaction mixture and processed for light and electron microscopy as described (15, 17). Immunohistochemical localization of AOX and l-PBE proteins in liver fixed in 10% formalin or 70% ethanol was performed using monospecific polyclonal antibodies as described (16, 17).

Northern Analysis—Total RNA extracted from liver with TRizol reagent (Invitrogen) was glyoxylated, separated on 0.8% agarose gel, transferred to nylon membrane, and then hybridized at 42 °C in 50% formamide hybridization solution using 32P-labeled cDNA probes as described (17, 19). Changes in mRNA levels were estimated by densitometric scanning of autoradiograms.

SDS-PAGE Analysis and Immunoblotting—Liver homogenates were separated by SDS-PAGE and stained with Coomassie Brilliant R Blue for assessing the induction of 78-kDa protein, which represents L-PBE, and D-PBE, and D-PBE (MFP-2) null mice has been described elsewhere (16, 17).

RESULTS

Growth Retardation and Postnatal Mortality in l-PBE−/−/d-PBE−/− Mice—l-PBE−/− (16) and d-PBE−/− (18) mice were crossed to generate mice nullizygous for both l-PBE and d-PBE (l-PBE−/−/d-PBE−/−) (Fig. 1A). Genotype analysis performed on litters during the late gestational age (embryonic day 17.5) or on postnatal day 1 revealed the expected Mendelian ratios from l-PBE−/−/d-PBE−/− animals, suggesting no intrauterine mortality (data not shown). l-PBE−/−/d-PBE−/−/DKO litter revealed variable but striking growth retardation at birth as compared with l-PBE−/−/d-PBE−/− and l-PBE−/−/d-PBE−/− littersmates. Mice deficient in both l-PBE and d-PBE that died during the first 3 postnatal days were grossly underized, underweight, and hypotonic with difficulties in suckling as evidenced by the reduced quantity of milk in their stomach. Some of the DKO mice that were stronger appeared to suckle adequately but showed marked retardation in weight gain and growth rate (Fig. 1B). Almost all of the l-PBE and d-PBE double null mice died before weaning, and a rare animal (<10% of double nulls) that survived up to 5 weeks was still with the dam suckling and did not consume solid diet. As described previously, mating of l-PBE−/− mice yielded l-PBE−/− mice with the expected Mendelian inheritance ratio of 25%; likewise,
the failure of L-PBE
most of the remaining mice survived to adulthood (18). Thus,
the enoyl-CoAs generated by the first step of the peroxisomal

some density and numerical distribution in mice deficient in
3,3-diaminobenzidine cytochemical method to visualize peroxi-

cal architecture of liver and the morphologic appearance of
PPAR
3,3-diaminobenzidine cytochemical method to visualize peroxi-

Characterization of Liver Phenotype in L-PBE

mating of d-PBE<sup>−/−</sup> mice also generated d-PBE<sup>−/−</sup> mice at the
expected ratio of ~25%, and these single nulls were indistin-
guishable at birth from their heterozygous and wild-type li-
ter-mates (16, 18). No detectable phenotype was noted in
L-PBE<sup>−/−</sup> mice, which were viable and fertile (16), whereas,
30% of the d-PBE<sup>−/−</sup> mice died before postnatal day 12, and
most of the remaining mice survived to adulthood (18). Thus,
the failure of L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice to survive beyond 5
weeks of age is considered a reflection of the consequences of
the total disruption of two β-oxidation pathways to metabolize
the enoyl-CoAAs generated by the first step of the peroxisomal
β-oxidation cycle catalyzed by acyl-CoA oxidases (1). Increases
in C<sub>26:0</sub> (3.5-fold) and phytanic acid (4-fold) levels were noted in
the serum of L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice. A 7-fold increase in
C<sub>26:0</sub>/C<sub>22:0</sub> was noted in L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice, as
compared with wild-type mice. There was also a significant
decrease in docosahexaenoic acid level (3.5-fold) in the serum of
these double knockout mice. These observations indicate that
in DKO mice, peroxisomal β-oxidation is abnormal.

Characterization of Liver Phenotype in L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice

Mice That Survived beyond the Age of 3 Weeks—The histologi-
cal architecture of liver and the morphologic appearance of
hepatocytes in mice deficient in either L-PBE or d-PBE did not
differ significantly from each other or from that of wild-type
animals during the first 5 weeks of their postnatal development
(Fig. 2, A–C). In contrast, the liver of mice deficient in both
L-PBE and d-PBE revealed a mild degree of microvesicular
fatty change between 3 and 5 weeks of age (Fig. 2D). The hepatic steatosis observed in L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice was
considerably less pronounced as compared with that noted in
AOX<sup>−/−</sup> mice (15, 17). Survey for alterations in peroxisome
population in liver cells of L-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice using 0.5-
μm-thick liver sections that were processed using the alkaline
3,3-diaminobenzidine cytochemical method to visualize peroxi-
somal marker enzyme catalase (15) revealed similar peroxi-
somal density and numerical distribution in mice deficient in
either L-PBE (Fig. 2F) or d-PBE (Fig. 2G) as compared with
wild type (Fig. 2E). In contrast, L-PBE and d-PBE double
knockout mouse livers did not reveal the presence of discrete
brown dots in the hepatocyte cytoplasm that represent peroxi-
somes (Fig. 2H). The hepatocyte cytoplasm and nuclei exhib-
it a diffuse brown reaction, suggesting the presence of solu-
ble catalase in these locations instead of in the expected
peroxisomal compartment.

At the ultrastructural level, peroxisomes in hepatocytes of
wild-type mouse livers appeared as discrete single membrane
limited particles with dense osmophilic material in sections
that were processed for the localization of catalase (Fig. 3A).
Similar distribution of peroxisomes was also noted in L-PBE<sup>−/−</sup>
or d-PBE<sup>−/−</sup> mouse livers (not illustrated). In contrast, the
liver cells of mice deficient in both L-PBE and d-PBE revealed
a marked reduction in peroxisome population, and when pres-
ent, they were seen as microperoxisomes (Fig. 3B). Liver cells
in DKO mice also revealed severe abnormalities in the appear-
cence of mitochondrial structure (Fig. 3B). The mitochondrial
abnormalities include condensations with curvilinear alter-
ations of cristae, myelin-like rings, evaginations and invagina-
tions of the outer membrane, and an occasional mitochondrial
ghost. Microvesicular steatosis is also seen in the cytoplasm.
Defects in mitochondrial structure have also been described in
Zellweger syndrome patients and in mouse models of defective
peroxisomal biogenesis (24).

Up-regulation of PPARα Target Gene Expression in the Absence of Peroxisome Proliferation—Previously, we demon-
strated that mice deficient in AOX, the first and rate-limiting
enzyme of the inducible classical peroxisomal β-oxidation sys-

tem, exhibit spontaneous peroxisome proliferation and tran-
scriptional activation of PPARα target genes in liver (15).
These observations indicated that AOX substrates that remain
unmetabolized by the branched-chain oxidase function as nat-
ural ligands of PPARα, leading to sustained activation of this
receptor (15, 25). Because no such changes occurred in the
livers of mice deficient in L-PBE, the second enzyme of this
inducible β-oxidation system, it appeared that d-PBE, the sec-
ond enzyme of the noninducible peroxisomal β-oxidation path-
way, would metabolize the substrates of L-PBE (16). The dis-
ruption of both L-PBE and d-PBE genes in the present study
provided an opportunity to examine whether the induction of
PPARα target genes occurs in liver when the two β-oxidation
pathways are interrupted at the level of the second enzyme,
although there was no morphological evidence of spontaneous
peroxisome proliferation (Fig. 2H). Northern analysis of liver
RNA obtained from 4-week-old animals for changes in the
levels of PPARα-regulated fatty acid oxidation system genes,
namely AOX, L-PBE, PTL, CYP4A1, and CYP4A3, revealed

Fig. 1. l-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice. A, Southern blot analysis of
genomic DNA for l-PBE and d-PBE gene deletion. B, retarded growth
and hypotonia in 3.5-week-old l-PBE and d-PBE double null mouse
(lower) as compared with wild-type (upper) littermate.

Fig. 2. Liver morphology in l-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> double null
mice. A–D, liver sections stained with hematoxylin and eosin. E–H,
sections of liver that were processed for the cytochemical localization
peroxisomal catalase. A and E, wild type; B and F, l-PBE<sup>−/−</sup>; C and G,
d-PBE<sup>−/−</sup>; and D and H, l-PBE<sup>−/−</sup> d-PBE<sup>−/−</sup> mice.
marked increases in the mRNA levels of AOX, PTL, CYP4A1, and CYP4A3 in L-PBE and D-PBE mice with the expected absence of L-PBE and D-PBE mRNAs due to the gene disruption (Fig. 4A). We noted marked increase in the mRNA content of Riken clone W09719 in earlier cDNA microarrays, which was identified as a PPARα-regulated gene in liver (19, 26). Recently, this Riken clone W09719 has been found identical to SCP2/SCPx (27, 28), encoding for the second thiolase enzyme (1–4). Increase in hepatic SCP2/SCPx mRNA level indicates (Fig. 4B) that this gene, like AOX, L-PBE, and PTL, is also regulated by PPARα. Modest increases in liver fatty acyl-CoA synthetase and fatty acid synthetase mRNA levels were noted in L-PBE−/− and D-PBE−/− mice (Fig. 4A). These results strongly suggest spontaneous up-regulation of PPARα target gene expression in liver in double knockout mice, similar to that seen in AOX−/− livers (Fig. 4A). It is of interest to note that modest increases in the mRNA levels of ACTE, PEX11α, and PMP70 also occurred in the L-PBE−/− and D-PBE−/− mouse livers. As expected, no perceptible alterations in UOX and CTL mRNA levels were noted (Fig. 4A). No significant changes in PPARα and PPARγ mRNA levels were detected among various groups except that PPARα levels appeared slightly increased in AOX−/− livers (Fig. 4A).

Northern analysis of the mRNA levels of some of the recently identified PPARα-regulated genes in liver, namely CD36, Ly-6D, PDK-4, SHLR, Riken clone W09719 (now identified as SCP2/SCPx), monoglyceride lipase (mLipase), and C3f (19, 26) showed increases in expression in L-PBE−/− and D-PBE−/−, and AOX−/− mouse livers and in the livers of wild-type mice treated with Wy-14,643, a synthetic peroxisome proliferator (Fig. 4B).

These observations clearly establish that induction of PPARα target genes occurs in liver in the absence of peroxisome proliferation in L-PBE−/− and D-PBE−/− double null mice. The mRNA
that noted in AOX (Fig. 4B). Immunoblot analysis confirmed the absence of L-PBE and D-PBE proteins in L-PBE−/− mouse liver and increases in the content of 72-kDa subunit A (but decrease or absence of 51-kDa subunit) of AOX protein (8, 29, 30) and in the amount of SCPx (Fig. 5). The 78-kDa protein seen in liver samples revealed very little of this protein in DKO livers (Fig. 5) despite the presence of UOX mRNA (Fig. 4). Immunoblotting for UOX in liver proteins for changes in peroxisomal proteins (20–22) and in the amount of PTL (Fig. 5). Also of interest is the marked decrease in the amount of the 40-kDa subunit of SCPx (8, 29, 30) and in the amount of PTL (Fig. 5). Immunoblot analysis confirmed the absence of 51-kDa subunit) of AOX protein (8, 29, 30). Immunoblot analysis of liver proteins for changes in peroxisomal β-oxidation system enzymes, AOX, L-PBE, D-PBE, PTL, and SCPx. For peroxisomal UOX and CTL, 20 and 5 μg of liver protein, respectively, was used. UOX signal was barely detectable in L-PBE−/− (DKO) mouse liver.

**DISCUSSION**

Peroxisomes are single-membrane bound organelles that are present in all eukaryotic cells. These organelles contain many enzyme systems responsible for a wide variety of metabolic functions, including the β-oxidation of very long-chain fatty acids.
acids (1, 2, 4). Abnormalities of peroxisomal fatty acid β-oxidation and absence or reduction in the number of peroxisomes in cells play a significant role in the pathophysiological manifestations of Zellweger syndrome and other peroxisome biogenesis disorders (1, 2, 24, 32, 33). Also important is that the induction of Zellweger syndrome and other peroxisome biogenesis disorders (1, 2, 24, 32, 33). It is further indicated that peroxisomal fatty acid oxidation catalyzed by inducible and noninducible pathways leads to alterations in hepatic mitochondrial structure, reminiscent of changes occurring in Zellweger patients and some mouse models for disturbances in peroxisome biogenesis (24, 36). In aggregate, microvesicular steatosis, paucity of peroxisomes and mitochondrial abnormalities encountered in L-PBE−/−d-PBE−/− mouse liver mimic changes described in Zellweger patients, and this is most likely related to the combined bifunctional enzyme deficiency and total interruption of fatty acid oxidation at the enoyl-CoA level.

Of considerable interest is that this study presents evidence for the up-regulation of PPARα target genes in liver in the absence of the morphological phenomenon of peroxisome proliferation. In L-PBE−/−d-PBE−/− mouse liver, increases in the hepatic AOX, PTL, CYP4A1, CYP4A3, and ACTE mRNA levels (Fig. 4A) were evident, and these are known PPARα target genes (3, 6, 34, 37, 38). Increases in PEX11α and PMP70 mRNA levels were also observed. PEX11α has been shown to be dispensable for PPARα-mediated peroxisome proliferation, although overexpression of this peroxin is known to enhance peroxisome formation (32, 33). Increases in PEX11α mRNA in the absence of peroxisome proliferation in L-PBE−/−d-PBE−/− mouse liver may appear paradoxical, but it provides an indication of PPARα activation. Overexpression of other putative PPARα-regulated genes such as macrophage scavenger receptor CD36 (37, 39), Ly-6D (19, 40), pyruvate dehydrogenase kinase protein (PDK) (41), SCP2/SCPx (19, 27, 28, 42), SHLR (uncharacterized endogenous virus-like sequence) (43), mLipase (44), and others (19) in the liver of these double nulls is further indication of PPARα activation by natural ligands that are unmetabolized substrates of peroxisomal β-oxidation. Although fatty acids are generally considered to be PPARα ligands (45, 46), we entertain the possibility that very long-chain acyl-CoAs (>20 C) or acyl-carnitines in AOX−/− mice and very long-chain enoyl-CoAs in L-PBE−/−d-PBE−/− mice can act as PPARα ligands, and these possibilities should be properly evaluated. Since acyl-CoAs enter peroxisomes, they can also exit peroxisomes by diffusion or transported out as acyl-carnitines. Although the possibility that acyl-CoAs can be converted or hydrolyzed to free fatty acids by peroxisomal thioesterases (ACTE) (38) and that enoyl-CoAs can be used by 2-enoyl-CoA reductase and be converted to acyl-CoAs (47) exists, this is considered futile because it wastes NADPH. It is
possible that in these double null mice, under limiting peri-
osomal NADPH concentration, there will be increases in the concentra-
tion of cellular enoyl-CoAs, which may diffuse out of peroxisomes to act as PPARα ligands. Evidence also suggests that phytanic acid activates PPARα in SCPx−/− mice (48), and consistent with this is the increased levels of phytic acid in the serum of L-PBE−/−/PBE−/− mice. These knockout models of peroxisomal β-oxidation raise the question that different endogenous ligands generated during very long-chain fatty acid oxidation can serve as PPARα ligands. Additional studies are needed to analyze the amounts and composition of acyl-CoAs, acyl-carnitines, and enoyl-CoAs in AOX−/− and L-PBE−/−/PBE−/− mice.

These studies also indicate that PPARα target gene overexpression can occur in the absence of peroxisome proliferation and that these two events can be dissociable. Studies with a variety of structurally diverse synthetic peroxisome proliferators have unequivocally established a strong correlation between hepatic peroxisome proliferation, PPARα target gene overexpression, and the development of liver cancer in rats and mice (5, 6, 7, 14, 49). Sustained activation of PPARα overexpression, and the development of liver cancer in rats and mice. These studies also indicate that PPARα ligands are negligible to nonexistent because peroxisome proliferation is somewhat diffuse in the cytoplasm. Because of the disruption of lipid peroxidation and increased staining in DKO livers, but unlike in wild-type mice cells and marked reduction in the processing of AOX and of certain Zellweger syndrome patients (29, 30, 56). The near absence of UOX protein in DKO livers also suggests that peroxisomal proteins are not appropriately targeted to the peroxisome (17, 31). Immunohistochemical staining for AOX shows increased staining in DKO livers, but unlike in wild-type mice fed Wy-14,643, the staining is not granular (peroxisomal) but somewhat diffuse in the cytoplasm. Because of the disruption of branched chain fatty acid oxidation system in the L-PBE−/−/PBE−/− mice, it is likely that these animals may have abnormalities in bile acid metabolism (57) in addition to defects in the metabolism of very long-chain fatty acids.

Acknowledgments—We thank Drs. Amiya K. Hajra and Stefan Alex-

References—We thank Drs. Amiya K. Hajra and Stefan Alex-

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J. Biol. Chem. 2003, 278:47232-47239.
doi: 10.1074/jbc.M306363200 originally published online September 18, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M306363200

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