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PEROXISOMAL β-OXIDATION AND PEROXISOME PROLIFERATOR–ACTIVATED RECEPTOR α: An Adaptive Metabolic System

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■ **Abstract** β-Oxidation occurs in both mitochondria and peroxisomes. Mitochondria catalyze the β-oxidation of the bulk of short-, medium-, and long-chain fatty acids derived from diet, and this pathway constitutes the major process by which fatty acids are oxidized to generate energy. Peroxisomes are involved in the β-oxidation chain shortening of long-chain and very-long-chain fatty acyl-coenzyme (CoAs), long-chain dicarboxylyl-CoAs, the CoA esters of eicosanoids, 2-methyl-branched fatty acyl-CoAs, and the CoA esters of the bile acid intermediates di- and trihydroxycoprostanoic acids, and in the process they generate H_2O_2. Long-chain and very-long-chain fatty acids (VLCFAs) are also metabolized by the cytochrome P450 CYP4A ω-oxidation system to dicarboxylic acids that serve as substrates for peroxisomal β-oxidation. The peroxisomal β-oxidation system consists of (*a*) a classical peroxisome proliferator–inducible pathway capable of catalyzing straight-chain acyl-CoAs by fatty acyl-CoA oxidase, L-bifunctional protein, and thiolase, and (*b*) a second noninducible pathway catalyzing the oxidation of 2-methyl-branched fatty acyl-CoAs by branched-chain acyl-CoA oxidase (pristanoyl-CoA oxidase/trihydroxycoprostanoyl-CoA oxidase), D-bifunctional protein, and sterol carrier protein (SCP)x. The genes encoding the classical β-oxidation pathway in liver are transcriptionally regulated by peroxisome proliferator–activated receptor α (PPARα). Evidence derived from mice deficient in PPARα, peroxisomal fatty acyl-CoA oxidase, and some of the other enzymes of the two peroxisomal β-oxidation pathways points to the critical importance of PPARα and of the classical peroxisomal fatty acyl-CoA oxidase in energy metabolism, and in the development of hepatic steatosis, steatohepatitis, and liver cancer.

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INTRODUCTION

Peroxisomes are cell organelles present in virtually all eukaryotic cells. Currently, more than 60 proteins have been found to be associated with mammalian peroxisomes, with more than half of these participating in lipid metabolism (16, 92). The regulation of lipid and carbohydrate metabolism is central to energy homeostasis and other vital biological functions of cells in higher organisms (21, 24, 25, 71). Peroxisomes, by virtue of their richness in lipid metabolizing enzymes (44, 48, 92), play a critical role in metabolic systems and physiological processes influencing alternate use of carbohydrate and fatty acids to generate ATP. This regulatory energy consumption, referred to as the glucose fatty acid cycle (71), requires the maintenance of efficient hepatic fatty acid oxidation. It is estimated that in adults, free fatty acids and their ketone body derivatives provide ~80% of caloric requirements after 24 h of fasting, as fasting leads to a dramatic depletion of carbohydrate energy source, especially in infants and children (11). As a consequence, both children and adults exhibit greater dependence on efficient free fatty acid oxidation–dependent ketogenesis during starvation, underscoring the importance of fatty acid oxidation in energy metabolism. Fatty acid oxidation occurs in mitochondria, peroxisomes, and smooth endoplasmic reticulum, and some of the critical enzymes of these oxidation systems are transcriptionally controlled by peroxisome proliferator–activated receptor α (PPARα), a member of the nuclear hormone receptor superfamily (17, 21, 32, 39, 40, 43, 61, 74, 76). PPARs, which derive their designation from structurally diverse compounds called peroxisome proliferators, and their ability to induce predictable pleiotropic responses in

rodent livers, including the development of liver tumors (72–73, 75, 76), consist of three isotypes, PPARα, PPARδ (also called β), and PPARγ, which are products of different genes (4, 17, 39, 81, 98, 102). β-Oxidation is a major process by which fatty acids are oxidized, and this oxidation occurs both in mitochondria and in peroxisomes (21, 24, 25, 47). Fatty acids are also oxidized by ω-oxidation by a cytochrome P450 CYP4A subfamily; this oxidation occurs almost exclusively in smooth endoplasmic reticulum (14, 22, 31, 61). This review focuses on recent developments in the enzymology and functional organization of peroxisomal β-oxidation pathways, and on our perspectives on the critical importance of PPARα in the inducibility of fatty acid oxidation systems that metabolize long-chain and VLCFAs in the pathogenesis of hepatic steatosis, steatohepatitis, and carcinogenesis. Since the previous review on peroxisomal lipid metabolism published in this series (77), several genetically altered mouse models representing some of the enzymatic deficiencies in peroxisomal β-oxidation have become available. New information on genetically determined metabolic defects at the individual enzyme level in mitochondrial and peroxisomal fatty acid β-oxidation pathways in humans has also emerged, and these developments have added new dimensions to our understanding of the interdependencies of the intricate metabolic systems.

FATTY ACID OXIDATION

Fatty acids are energy-rich molecules that are pivotal (*a*) for a variety of cellular processes, such as synthesis of membrane lipids and generation of lipid-containing messengers in signal transduction, and (*b*) for energy storage in the form of triacylglycerol in adipose tissue (21, 24, 28). Adipocytes provide the virtually limitless capacity to store energy in the form of triacylglycerol, which constitutes the most concentrated form of energy storage in higher animals (28). It is rationalized that by its ability to store energy, the adipocyte radically changed the evolutionary hierarchy of life on earth (4, 82, 91). Although most nonadipocytes contain traces of triacylglycerol for homeostatic functions, they are protected from massive accumulation of triacylglycerol during conditions of excess energy consumption. It is hypothesized that leptin functions to confine the storage of energy to the adipocyte, while limiting triacylglycerol storage in nonadipocytes (91). Of all the nonadipocyte cells, the mammalian liver cell is normally capable of storing considerable quantities of triacylglycerol, especially to accommodate plasma nonesterified fatty acids present in excess of the requirement for immediate oxidation and/or secretion as very-low-density lipoproteins (25, 28, 41). The ability of liver to store lipids is viewed as a protective mechanism, neutralizing the potential toxicity of (*a*) long-chain fatty acids and VLCFAs synthesized de novo in individuals consuming excess energy and fat-rich diets and (*b*) those released into the plasma from adipose tissues stores (28, 91). Abnormal quantities of lipids stored in liver manifest as fatty liver (steatosis), a condition most often attributed to the effects of excess alcohol consumption, obesity, diabetes, drugs, and/or metabolic

perturbations resulting from alterations in fatty acid oxidation, fatty acid synthesis, and transport from liver (25, 41).

Fatty acid metabolism is one of the major sources of energy for skeletal muscle and heart, but liver plays a critical role in the energy metabolism of these and other extrahepatic tissues in most animals. Fatty acids are oxidized in three organelles, with β-oxidation confined to mitochondria and peroxisomes, and the CYP4A-catalyzed ω-oxidation occurring in the endoplasmic reticulum. Fatty acid synthesis is enhanced and β-oxidation is lowered when glucose supply is plenty so that excess energy can be stored in the form of triacylglycerol (21, 24, 28). When glucose availability is diminished, however, the synthesis of fatty acids is depressed and β-oxidation is enhanced in liver so as to generate ketone bodies for export to serve as fuels for other tissues, such as the skeletal and cardiac muscle (28, 71). This alternate use of energy sources occurs during short-term fluctuations in energy supply. The fatty acid oxidation in an intact organism is roughly proportional to the plasma concentration of free fatty acids released from adipose tissue, and this mobilization of free fatty acids is stimulated by glucagon and other hormones and inhibited by insulin (28, 71). A brief overview of the three fatty acid oxidation systems is given below as a prelude to a comprehensive review of the peroxisomal β-oxidation system.

Mitochondrial β-Oxidation

Mitochondrial β-oxidation is responsible for the oxidation of the major portion of the of short- ($<C_8$), medium- (C_8–C_{12}), and long- (C_{14}–C_{20}) chain fatty acids and, in the process, contributes to energy production via ATP generating oxidative phosphorylation. Because long-chain fatty acids constitute the bulk of dietary fat, their abundance makes them the predominant source of energy under normal conditions, and during fasting-induced lipolysis they become crucial metabolic substrates (21, 32, 33, 55, 77). The dominant role of mitochondria in the oxidation of long-chain fatty acids appears a logical consequence of the fact that mitochondrial β-oxidation, by means of oxidative phosphorylation, conserves almost double the energy compared with peroxisomes because the energy produced by the first step of the peroxisomal β-oxidation dissipates as heat (33, 48, 49, 77). One important short-term regulatory mechanism of the mitochondrial β-oxidation system involves the carnitine palmitoyltransferase I (CPT I), which, for transport of fatty acids across the inner mitochondrial membrane, catalyzes the formation of fatty acylcarnitine at the outer mitochondrial membrane. Because carnitine concentration is near the K_m value of CPT I, changes in carnitine concentration generally affect the mitochondrial β-oxidation (21, 33). CPT I is inhibited by malonyl-CoA, and therefore, alterations in malonyl-CoA levels affect fatty acid transport (33, 47). Furthermore, two isoforms of CPT I, the liver isoform and the skeletal muscle isoform, exhibit markedly different kinetic characteristics with respect to carnitine and to malonyl-CoA inhibition (33, 47). CPT I is markedly induced by peroxisome proliferators, and by fatty

acids/fatty acyl-CoAs, which activate PPARα, and in this regard this transcription factor plays a critical role in the regulation of mitochondrial β-oxidation (17, 33).

Fatty acids are completely oxidized to acetyl-CoA by mitochondrial β-oxidation, and the acetyl-CoA then either enters the Krebs cycle for further oxidation or condenses to ketone bodies (acetoacetate, acetone, and β-hydroxybutyrate) in liver to serve as oxidizable fuels for extrahepatic tissues (21, 28, 55, 71). The mitochondrial β-oxidation has two distinct components. The first, which is mitochondrial inner-membrane bound, is active with long-chain fatty acyl-CoAs (C_{12}–C_{20}) and generates chain-shortened fatty acyl-CoAs (Figure 1). This inner-membrane–bound mitochondrial β-oxidation system consists of four reactions and generally oxidizes long-chain fatty acyl-CoAs by two membrane-associated proteins (33, 87). The first protein, a very-long-chain acyl-CoA dehydrogenase (VLCAD), performs the first step of β-oxidation, and the second protein, a long-chain enoyl-CoA hydratase/long-chain 3-hydroxyacyl-CoA dehydrogenase/long-chain 3-ketoacyl-CoA thiolase trifunctional β-oxidation protein complex

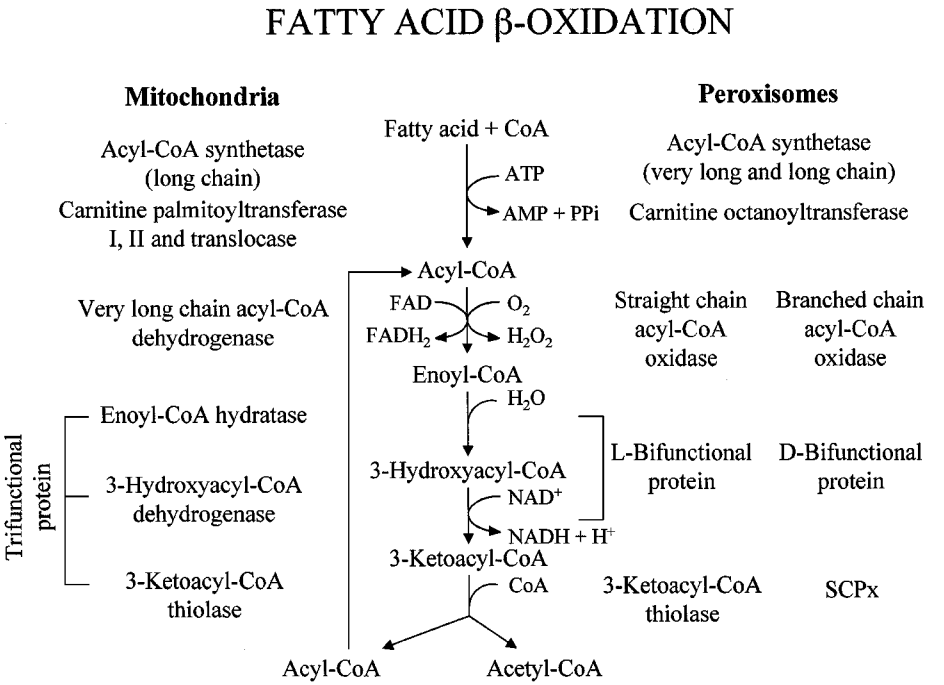

Figure 1 Enzymology of mitochondrial inner-membrane–bound long-chain fatty acid β-oxidation system involving trifunctional protein, and the peroxisomal-inducible classical striaght-chain and the noninducible branched-chain fatty acid β oxidation systems in humans. SCPx, sterol carrier protein x.

(mitochondrial trifunctional protein), carries out the second, third, and fourth β-oxidation steps (Figure 1). The resulting optimally chain-shortened fatty acyl-CoAs are then completely β-oxidized by the second mitochondrial β-oxidation system located in the matrix. This system is active with long-chain (C_{14}–C_{20}), medium-chain (C_8–C_{12}), and short-chain (C_4–C_6) acyl-CoAs (21, 33, 55, 77). In this matrix-bound β-oxidation system, the well-known classical chain-length–specific long-chain (LCAD), medium-chain (MCAD), and short-chain acyl-CoA dehydrogenases (SCAD) catalyze the first step (21, 33, 87). The second, third, and fourth steps, respectively, of this β-oxidation spiral are carried out by the 2-enoyl-CoA hydratase (or crotonse), 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase, all of which are present in the mitochondrial matrix as individual enzymes encoded by separate genes.

The mitochondrial inner-membrane–bound trifunctional protein complex, responsible for the oxidation of very-long-chain acyl-CoAs, is a hetero-octomer made up of four α-subunits with long-chain enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities and four β-subunits with long-chain 3-oxo-/or keto-thiolase activity (60). The two subunits (α and β) are encoded by two adjacent genes linked in a head-to-head fashion, and they are distinctly different from the three genes that encode mitochondrial matrix-bound classical enzymes (60). Comparison of the amino acid sequences of SCAD, MCAD, and LCAD reveal a distinct homology, indicating that these enzymes belong to a gene family, the acyl-CoA dehydrogenase family (21). Primary structures of rat mitochondrial SCAD, MCAD, and LCAD have revealed that their precursors have 414–430 amino acid residues containing a 25- to 30-amino acid signal sequence at the N termini. The subunit of VLCAD is synthesized as a polypeptide with 655 amino acid residues, with a typical cleavable extrapeptide at the N terminus, and the N-terminal region of 98–433 has a high homology with other acyl-CoA dehydrogenases (33). As noted above, long-chain fatty acyl-CoA are actively transported to the mitochondrial matrix, whereas the no-active-transport process involves the transport of short- and medium-chain fatty acids to mitochondrial matrix (21). Enzymes involved in the processing of conjugated double bonds during oxidation of polyunsaturated fatty acids are 2,4-dienoyl-CoA reductase, $\Delta^{3,5},\Delta^{2,4}$-enoyl-CoA isomerase, $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase, and $\Delta^{3,5,7},\Delta^{2,4,6}$-trienoyl-CoA isomerase (21).

Peroxisomal β-Oxidation

The presence of a fatty acid β-oxidation system outside the mitochondrial compartment was first described in 1969 in glyoxysomes (peroxisomes) present in germinating castor bean seedlings (15), and a similar system was found in 1976 in rat liver peroxisomes (48). It is now clear (*a*) that in animals both the mitochondrial and the peroxisomal fatty acid β-oxidation systems are present in the same cell, (*b*) that they are catalyzed by different enzymes encoded by different genes, and (*c*) that they play functionally complementary but different

TABLE 1 Enzymes in the peroxisomal fatty acid β-oxidation system

Very-long-chain acyl-CoA synthetase
Long-chain acyl-CoA synthetase
Carnitine octanoyltransferase
Carnitine acetyltransferase
Straight-chain acyl-CoA oxidase
Branched-chain acyl-CoA oxidase
L-Bifunctional protein
D-Bifunctional protein
3-Ketoacyl-CoA thiolase
Sterol carrier protein 2/3-ketoacyl-CoA thiolase
2,4-Dienoyl-CoA reductase
Δ^3,Δ^2-Enoyl-CoA isomerase
$\Delta^{3,5}$,$\Delta^{2,4}$-Dienoyl-CoA isomerase

roles (32, 33, 55, 77). The enzymes involved in peroxisomal fatty acid oxidation are listed in Table 1. Although long-chain fatty acids are predominantly β-oxidized in mitochondria, the peroxisomal β-oxidation system does indeed participate in the oxidation of these substrates. On the other hand, VLCFAs ($>C_{20}$), are almost exclusively β-oxidized in peroxisomes because mitochondria are devoid of very-long-chain acyl-CoA synthetase (77, 90, 92). Peroxisomal membranes contain at least two acyl-CoA synthetases: a long-chain acyl-CoA synthetase (89), which activates long-chain fatty acids, and a very-long-chain fatty acyl-CoA synthetase, which activates VLCFAs (89). The peroxisomal β-oxidation system also metabolizes long-chain dicarboxylic acids, eicosanoids, bile acid precursors, and side chains of some xenobiotics (55, 77). The three enzymes of the peroxisomal β-oxidation cycle, namely fatty acyl-CoA oxidase (AOX), enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase bifunctional protein (L-PBE), and 3-ketoacyl-CoA thiolase, were purified nearly 20 years ago, but in recent years it has become evident that other enzymes are also involved in the peroxisomal fatty acid oxidation (32, 33, 55, 98). As is discussed later, based on substrate specificity and stereoselectivity of the newly discovered enzymes, two different peroxisomal β-oxidation pathways have been formulated (Figure 1). The classical pathway catalyzed by AOX, L-PBE, and thiolase is generally accepted to be responsible for the oxidation of straight-chain fatty acids (33, 55), and all three enzymes of this classical system are transcriptionally activated by PPARα ligands (23, 39, 74). The oxidation of 2-methyl-branched fatty acids and of the bile acid intermediates, di- and trihydroxycoprostanoic acids, occurs via a second pathway (Figure 1) (see below).

The peroxisomal acyl-CoA oxidases, which are the first and rate-limiting enzyme of each of the two β-oxidation pathways in peroxisomes, reflect distinctive differences between peroxisomal and mitochondrial oxidation pathways (33, 55, 77, 79). The first step in peroxisomal fatty acid β-oxidation is directly coupled to the molecular oxygen, resulting in the cyanide insensitivity of the system, in contrast to the cyanide sensitivity of the mitochondrial system in which the first step is directly coupled to an electron transfer chain (48). Unlike the mitochondrial system, peroxisomal β-oxidation system is carnitine independent, and it does not go to completion, as the chain-shortened acyl-CoAs are exported to the mitochondria for the completion of β-oxidation (21, 32, 33, 77). The peroxisomal acyl-CoA oxidase acts on very-long-chain and long-chain acyl-CoAs but not on medium-chain acyl-CoAs, which are β-oxidized exclusively in mitochondria (33, 55). Therefore, the carbon-chain of palmitate, for example, is stopped at C_8 or so in peroxisomes, and then exported to the mitochondria.

Microsomal ω-Oxidation

The initial step in ω-oxidation is ω-hydroxylation of fatty acid in the smooth endoplasmic reticulum catalyzed by lauric acid ω-hydroxylase, a member of the CYP4A subfamily (14, 31, 43, 61). The CYP4A family of cytochrome P450 enzymes, which are constitutively expressed in liver and kidney, catalyze the ω- and (ω-1)- hydroxylation of long-chain fatty acids (14). All known CYP4A enzymes in rats, namely CYP4A1, CYP4A2, CYP4A3, and CYP4A8, are constitutively expressed in kidney, but only the first three are expressed in liver (14, 43). The major ω-hydroxylase expressed in human liver and kidney is CYP4A11, which is functionally similar to that of rat CYP4A1 (14, 22, 43). The ω-hydroxy fatty acid produced by the initial hydroxylation reaction is then dehydrogenated to a dicarboxylic acid in the cytosol and converted to its CoA derivative by an acyl-CoA synthetase present in the endoplasmic reticulum (14, 77). In humans, long-chain dicarboxylyl-CoAs derived from ω-oxidation of fatty acids are oxidized almost exclusively by peroxisomal AOX of the classical β-oxidation pathway (44, 55, 77). In rats, a significant portion of dicarboxylyl-CoAs also appears to be metabolized via pristanoyl-CoA oxidase (55).

The substrate and regiospecificity studies with rat and human CYP4A enzymes showed highest activity of these enzymes for lauric acid ($C_{12:0}$), and no particular preference for longer-chain fatty acids, such as oleic ($C_{18:1}$) and arachidonic acid ($C_{20:4}$) (18). Human and rat CYP4A enzymes, in general, poorly oxidize long-chain fatty acids (37), and in contrast, rabbit CYP4A4, CYP4A6, and CYP4A7 enzymes appear to be highly active toward arachidonic acid (43). Little information exists as to how effectively the VLCFAs are metabolized by the CYP4A subfamily of enzymes in various species. Aminobenzotriazole, a suicide inhibitor of the CYP4A ω-hydroxylases, significantly inhibited the ω-hydroxylation of VLCFAs in cultured human keratinocytes (10). This compound also altered the permeability barrier in the skin of hairless mice as the inhibitor decreased the

generation of very-long-chain ω-hydroxyceramide ($C_{\geq 30}$), which is required for corneocyte lipid envelope formation, presumably by inhibiting the ω-hydroxylation of the free fatty acid prior to N-acylation of the sphingolipid base (10). These observations, albeit indirect, strongly implicate a role for the CYP4A subfamily in the ω-oxidation of VLCFAs. However, until the ω-hydroxylation of VLCFAs can be demonstrated in vitro by using highly purified CYP4A isoforms, the possibility that an enzyme other than a CYP4A isoform that is also inactivated by aminobenzotriazole inhibits VLCFA oxidation cannot be excluded. For all four known rat isoforms (CYP4A1, -4A2, -4A3, and -4A8), as well as for the single established human isoform (CYP4A11), the rate of NADPH consumption is always higher than the rate of fatty acid hydroxylation, which implies that the enzymes undergo some degree of uncoupled turnover (U Hoch & P Oritz de Montellano, personal communication). Uncoupled turnover implies that NADPH is being used to reduce oxygen to superoxide and/or H_2O_2, and possibly also to H_2O (18, 37). Thus, it is important to note that reactive oxygen species are generated not only during the ω-oxidation of fatty acids to dicarboxylic acids, but also when these dicarboxylic acids undergo ω-oxidation within peroxisomes.

ENZYMES OF THE PEROXISOMAL β-OXIDATION SYSTEM

Fatty Acid Activation and Entry into the Peroxisome

Before a fatty acid can be β-oxidized by peroxisomes or mitochondria, it must be activated to its CoA derivative. As mentioned above, the peroxisomal membrane contains long-chain and very-long-chain acyl-CoA synthetases (84). Long-chain acyl-CoA synthetase, which activates long-chain fatty acids, is also localized in the mitochondrial outer membrane and in the endoplasmic reticulum (84). The enzymes in these three organelles are indistinguishable from each other at the protein level, which has led to the speculation that they are encoded by the same gene (83). The catalytic site of long-chain acyl-CoA synthetase is exposed to the cytosol (83). Medium straight-chain fatty acids can also be activated by the long-chain acyl-CoA synthetases present in the mitochondrial outer membranes and in the peroxisomal and endoplasmic reticulum membranes (21). Very-long-chain acyl-CoA synthetase, which activates VLCFAs, is present in peroxisomes and in the endoplasmic reticulum but is absent from mitochondria (55, 77, 90). The absence of very-long-chain fatty acyl-CoA synthetase in mitochondria may explain why VLCFAs are β-oxidized exclusively in peroxisomes (see below). Very-long-chain acyl-CoA synthetase differs from long-chain acyl-CoA synthetase with respect to molecular, catalytic, and immunochemical properties (89, 90). The deduced primary structures of very-long-chain and long-chain acyl-CoA synthetases are quite different, but it is intriguing that the

amino acid sequence of very-long-chain fatty acyl-CoA synthetase has 40% amino acid identity with that of fatty acid transport protein isolated from adipocytes (78).

Peroxisomes play a prominent role in the β-oxidation of pristanic acid and other branched-chain fatty acids (33, 47, 55, 77, 79). Isoprenoid-derived branched-chain fatty acids can be activated by peroxisomes, mitochondria, and the endoplasmic reticulum, possibly via the long-chain acyl-CoA synthetases present in these organelles (83). Whether peroxisomes contain a branched-chain acyl-CoA synthetase remains unclear. Dicarboxylic acids, prostaglandins, and the C_{27} bile acid intermediates, di- and trihydroxycholestanoic acids, are activated solely in the endoplasmic reticulum (55, 98). The bile acid intermediates, synthesized from cholesterol, are activated by a separate enzyme, trihydroxycoprostanoyl-CoA synthetase, which is present only in liver. The observation that dicarboxylic acids and prostaglandins are not activated by mitochondria or peroxisomes implies that these molecules are substrates not for long-chain acyl-CoA synthetase but for a specific enzyme(s). As indicated before, the dicarboxylyl-CoAs are oxidized almost exclusively in peroxisomes by AOX of the classical β-oxidation pathway (55).

In the mitochondria, the long-chain acyl-CoA esters are transported by a carnitine-dependent mechanism through the inner membrane into the matrix, the place where the β-oxidation reactions occur (21). Short- and medium-chain fatty acid esters do not require specific transport mechanisms to reach the mitochondrial matrix. The peroxisomal membrane does not contain CPT I and carnitine translocase, and as a consequence, the long-chain acyl-CoAs do not require carnitine for their entry into the peroxisome matrix (21, 33). In essence, the nonspecific permeability of the peroxisomal membrane may facilitate the diffusion of amphiphilic fatty acyl-CoAs. In peroxisomes, both carnitine octanoyltransferase and carnitine acetyltransferase are present in the matrix, but their exact functional role in peroxisomal β-oxidation remains unclear (33). Peroxisomal membranes also contain adrenoleukodystrophy protein (ALDP), a member of the ATP-binding cassette (ABC) transporter family, and peroxisomal membrane protein (PMP) 70, but their role, if any, in fatty acid or fatty acyl-CoA transport remains unclear (53, 59, 78).

Presence of Two Sets of Peroxisomal β-Oxidation Enzymes: Inducible and Noninducible Systems

Although mitochondrial β-oxidation is primarily involved in the catabolism of short-, medium-, and long-chain fatty acids, the peroxisomal β-oxidation is largely responsible for the degradation of a number of less-abundant carboxylates of different molecular structure (21, 33, 77). The substrates for peroxisomal β-oxidation include VLCFAs ($>C_{20}$), 2-methyl-branched fatty acids, dicarboxylic acids, prostanoids, and the C_{27} bile acid intermediates, which are converted to the mature C_{24} bile acid intermediates via β-oxidation. Similar to mitochondrial β-oxidation, peroxisomal fatty acid β-oxidation has four steps: (*a*) an oxidation reaction, in

which the acyl-CoA is desaturated to a 2-*trans*-enoyl-CoA; (*b*) a hydration reaction, which converts the enoyl-CoA to a 3-hydroxyacyl-CoA; (*c*) a second oxidation step, which dehydrogenates the hydroxy intermediate to a 3-ketocyl-CoA; and (*d*) thiolytic cleavage, which releases acetyl-CoA and an acyl-CoA that is two carbon atoms shorter than the original molecule and that can reenter the spiral for the next round of β-oxidation (32, 33, 48). The first step is catalyzed by a H_2O_2-generating acyl-CoA oxidase, the second and third steps by a bifunctional protein (hydration plus dehydrogenation), and the fourth (last) step by a thiolase (thiolytic cleavage). During the past 5 years, it has become evident that two or more acyl-CoA oxidases, two different bifunctional proteins, and two different thiolases exist in most mammals (6, 8, 9, 13, 19, 20, 33, 42). Based mostly on substrate specificity of the oxidase that initiates the first and rate-limiting step, two different β-oxidation pathways have been proposed to operate within the peroxisome (Figure 1). The classical pathway generally utilizes straight-chain saturated fatty acyl-CoAs as substrates, whereas the recently discovered second β-oxidation pathway acts on 2-methyl-branched fatty acids and on the bile acid intermediates (33, 55, 98). In the L-hydroxy–specific classical β-oxidation spiral, dehydrogenation of acyl-CoA esters to their corresponding *trans*-2-*enoyl*-CoAs is catalyzed by AOX, whereas the second and third reactions, hydration and dehydrogenation, of enoyl-CoA esters, to 3-ketoacyl-CoA, are carried out by a single enzyme, enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase [L-bifunctional enzyme (L-PBE); also known as multifunctional protein-1] (33, 55, 77). The third enzyme of this classical system, 3-ketoacyl-CoA thiolase, cleaves 3-ketoacyl-CoAs to acetyl-CoA and an acyl-CoA that is two carbon atoms shorter than the original molecule and that can reenter the β-oxidation spiral (49, 77). All enzymes of this classical pathway are found in various species and can be strongly induced by peroxisome proliferators and other biological ligands of PPARα in the liver of rats and mice (23, 74). In the second, D-3-hydroxy–specific β-oxidation pathway, dehydrogenation of acyl-CoA esters to their corresponding *trans*-2-enoyl-CoAs is catalyzed in humans by the branched-chain acyl-CoA oxidase (8, 9, 33, 79, 96). The recently identified D-3-hydroxyacyl-CoA dehydratase/D-3-hydroxyacyl-CoA dehydrogenase [D-bifunctional enzyme (D-PBE); also known as multifunctional protein-2], then converts enoyl-CoAs to 3-ketoacyl-CoAs via D-3-hydroxyacyl-CoAs (19, 20, 33, 42, 56, 70, 98). The third enzyme of this second system is designated sterol carrier protein (SCP)x, the N-terminal part of which exerts thiolytic activity (33, 44, 55, 57, 99). The first desaturation step in this D-3-hydroxy–specific β-oxidation spiral is executed by either trihydroxycoprostanoyl-CoA oxidase (which acts on bile acid intermediates) or pristanoyl-CoA oxidase (which facilitates pristanic acid breakdown) in rats, but in humans this first and rate-limiting step of this second β-oxidation pathway is executed by one enzyme only, the branched-chain acyl-CoA oxidase, which is the counterpart of rat trihydroxycoprostanoyl-CoA oxidase (8). Emerging evidence strongly indicates that separation between two peroxisomal β-oxidation pathways may not be that rigid after the initial first desaturation step catalyzed by specific oxidase (6, 67). It appears that the L- and D-hydroxy intermediates generated in the

two β-oxidations systems can be metabolized to a variable extent by either L-PBE or D-PBE (see below).

Individual Peroxisomal β-Oxidation System Enzymes

First Step of the β-Oxidation Cycle Catalyzed by Acyl-CoA Oxidases The first reaction of peroxisomal β-oxidation is catalyzed by FAD-containing oxidases that donate electrons directly to molecular oxygen, thereby generating H_2O_2 (33, 48, 77). In the classical β-oxidation spiral, which deals with straight-chain acyl-CoAs, this step is catalyzed by acyl-CoA oxidase, a single enzyme, in all species examined. In the branched-chain β-oxidation spiral, the first reaction is also catalyzed by only one oxidase (branched-chain acyl-CoA oxidase) in humans, which appears to be active for both 2-methyl-branched fatty acids and bile acid intermediates (8). However, in rats, two different oxidases are present, namely pristanoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase, which catalyze, respectively, the branched-chain fatty acids and the bile acid intermediates (9, 55, 79).

The acyl-CoA oxidase of the classical β-oxidation system is 140 kDa and consists of two subunits of 72 kDa (component A), which can be proteolytically cleaved into 52-kDa (component B) and 21-kDa (component C) products within the peroxisome matrix (32, 62, 97). This enzyme oxidizes the CoA-esters of medium-chain, long-chain, and VLCFAs, medium-chain and long-chain dicarboxylic acids, and prostaglandins (33, 55). The enzyme is highly active toward substrates with longer carbon chain length but inactive toward substrates having acyl moieties of eight or fewer carbon atoms (77). The human acyl-CoA oxidase gene, localized to chromosome band 17q25, is present as a single copy per haploid genome, with 13 introns in the protein coding region, and it is structurally similar to the rat gene (97). There are two different sequences for exon 3 (3-I and 3-II), resulting from alternate use of exon 3 in splicing. Human acyl-CoA oxidase gene encodes a 660–amino acid residue, whereas the rat gene encodes a 661–amino acid protein. Both rat and human acyl-CoA oxidase cDNAs contain a Ser-Lys-Leu peroxisomal targeting signal (PTS1) in the COOH-terminal end. The enzyme probably exists in three forms: A_2, ABC, and B_2C_2 (32). By immuofluorescence and immunocytochemical methods, this enzyme is localizable to peroxisome matrix and is present in liver cells as well as in extrahepatic tissues. This enzyme is inducible by peroxisome proliferators by activating PPARα (see below).

In humans, the branched-chain acyl-CoA oxidase functions as the first and rate-limiting step (*a*) of the second β-oxidation pathway acting on 2-methyl-branched fatty acids, such as pristanic acid, which is derived from phytol contained in food, and (*b*) of the bile acid intermediates, di- and trihydroxycoprostanoic acids, which also contain a 2-methyl substitution in their side chain (8, 96). Unlike in humans, the branched-chain β-oxidation pathway in rats can be initiated by two different noninducible oxidases, namely pristanoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase (33, 55, 79). Pristanoyl-CoA oxidase acts on the CoA esters of

2-methyl-branched fatty acids, whereas the trihydroxycoprostanoyl-CoA oxidase uses bile acid intermediates as substrates. The human branched-chain AOX cDNA encodes a protein of 681 amino acids, calculated to be 76,739 Da (8). The enzyme is encoded by a single-copy gene, present on chromosome 3p14.3, and is expressed in liver and several other extrahepatic tissues (8). Sequence comparison with the other acyl-CoA oxidases shows that despite its broader substrate specificity, the human branched-chain acyl-CoA oxidase is the homologue of rat trihydroxycoprostanoyl-CoA oxidase and separate gene-duplication events led to the occurrence in mammals of peroxisomal acyl-CoA oxidases with different substrate specificities (8, 55). Rat trihydroxycoprostanoyl-CoA oxidase is 139 kDa and consists of two identical subunits of 69 kDa (9). Rat pristanoyl-CoA oxidase, which mostly uses CoA esters of 2-methyl-branched fatty acids as substrates, also exhibits some activity with the CoA esters of straight-chain fatty acids, but this enzyme does not appear to be sufficient to carry out the straight-chain fatty acid β-oxidation in mice deficient in classical inducible AOX (95). It is 420 kDa and consists of identical subunits of 70 kDa (95). All three AOXs possess a C-terminal PTS 1 signal (9, 70, 83, 95).

Second and Third Steps Catalyzed by Two Different Bifunctional Proteins The second step of the peroxisomal β-oxidation is the hydration of the enoyl-CoAs to 3-hydroxyacyl-CoAs, which are then dehydrogenated to generate 3-ketoacyl-CoAs in the third step (32, 33, 48, 55, 77). A single protein, with both enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase activities, hence called peroxisomal bifunctional enzyme (PBE), catalyzes these two steps. Because this protein also exhibits enoyl-CoA isomerase activity, it is also referred to as trifunctional or multifunctional protein (33, 47, 64). Peroxisomes contain two bifunctional proteins: The first is the L-hydroxy–specific enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase [called L-bifunctional protein (L-PBE) because the hydrated species it generates has the L-configuration] of the classical peroxisome proliferator-inducible β-oxidation spiral, and the second is the D-3-hydroxyacyl-CoA dehydratase/D-3-hydroxyacyl-CoA dehydrogenase [called D-bifunctional protein (D-PBE)] of the branched-chain noninducible β-oxidation system.

The L-PBE, which has been purified from rat and human liver, is a monomeric protein of 79 kDa (32, 33). The enoyl-CoA hydratase activity lies in the N-terminal portion and the L-3-hydroxyacyl-CoA dehydrogenase activity lies in the C-terminal part of the protein. The structure of the N-terminal and C-terminal sides of L-PBE are similar to that of enoyl-CoA hydratase (crotonase) and 3-hydroxyacyl-CoA dehydrogenase, respectively, of the mitochondrial matrix-bound β-oxidation enzymes (33). D-PBE, on the other hand, is a homodimer of two 77-kDa subunits, and it has been shown that D-PBE cleaves in vivo within the peroxisome matrix into the enoyl-CoA hydratase component (45 kDa) and the D-3-hydroxyacyl-CoA dehydrogenase component (35 kDa) (19, 20). The D-PBE also has a C-terminal PTS 1 signal (50). Unlike that of L-PBE, the N-terminal portion of D-PBE contains the D-3-hydroxyacyl-CoA dehydrogenase activity (note that in L-PBE,

the N-terminal part contains enoyl-CoA hydratase activity), and the central part contains the enoyl-CoA hydratase activity (33). In this regard the structure of D-PBE is similar to that of yeast multifunctional proteins catalyzing the two reactions with the D-isomer of 3-hydroxyacyl-CoA (33). The C-terminal part of D-PBE exhibits homology to SCP2 (56, 57). Comparison of the L-PBE and D-PBE cDNAs reveals that they have very little sequence homology and differ markedly in their structure. First, in rats, the L-PBE gene spans about 31 kb and consists of seven exons and six introns. The human L-PBE gene is localized to chromosome 3q26.3-3q28 (38), and its structure is similar to that of the rat L-PBE (M Malki & JK Reddy, unpublished data). On the other hand, the D-PBE gene spans >100 kb and consists of 24 exons and 23 introns (50). The substrate specificities of both enzymes differ in that both enzymes can metabolize straight-chain enoyl-CoAs as substrates, but they differ markedly with respect to branched-chain fatty enoyl-CoAs, with respect to 3α-, 7α-, and 12α-hydroxy-5β-cholest-24-enoyl-CoA, and with respect to 3α- and 7α-dihyroxy-5β-cholest-24-enoyl-CoA, which seem to be more desirable as substrates for D-PBE (33, 55). Finally, the L-PBE, but not the D-PBE, is markedly induced by peroxisome proliferators (33, 77).

Last Reaction is Also Catalyzed by Two Different Enzymes Peroxisomes contain two distinct enzymes capable of thiolytically cleaving 3-ketoacyl-CoA into a chain-shortened acyl-CoA and acetyl-CoA or propionyl-CoA (in case of two methyl branched fatty acids) (13, 33, 55, 57, 62, 77). These two enzymes are the well-known 3-ketoacyl-CoA thiolase of the inducible classic straight-chain β-oxidation system, and the recently discovered SCPx of the noninducible branched-chain β-oxidation system (13, 17, 33, 77). 3-Ketoacyl-CoA thiolase is a homodimer of 89 kDa. It is synthesized as a precursor protein of 44 kDa and is proteolytically cleaved to its mature size of 41 kDa (32). In rats, there are two genes (A and B) for peroxisomal 3-ketoacyl-CoA thiolase (32, 62). Gene A is constitutively expressed at a low level, whereas gene B transcript is barely detectable in normal liver but is dramatically inducible by peroxisome proliferators (33, 62). Thiolase A and B proteins show similar biochemical properties and the same substrate specificities except for some difference in N-terminal amino acid sequences (13). Human liver peroxisomes contain only one 3-ketoacyl-CoA thiolase, and the gene encoding this protein, mapped to chromosome 3p23-p22, shows high homology to both rat genes (77). Rat and human 3-ketoacyl-CoA thiolases contain an N-terminal cleavable PTS2 sequence (77, 83).

The thiolytic function of the second β-oxidation system in peroxisomes is performed by SCPx, a 58-kDa protein with the 3-ketoacyl-CoA thiolase activity in the N-terminal domain and with SCP2 (a lipid carrier or transfer protein) function in the C-terminal domain (33, 57). SCP2 and SCPx are expressed by the same gene, which is mapped to human chromosome 1p32.

The 3-ketoacyl-CoA thiolase and SCPx exhibit distinct substrate specificities: The former enzyme plays a principal but restricted role in the β-oxidation of

straight-chain 3-ketoacyl-CoAs, and the latter plays a broader role in the cleavage not only of branched-chain fatty acids, and the bile acid intermediates, but also of 3-ketoacyl-CoAs of straight-chain fatty acids (33, 55).

β-OXIDATION OF POLYUNSATURATED FATTY ACIDS

Polyunsaturated fatty acids (n-3 and n-6) are β-oxidized in both peroxisomes and mitochondria; however, for this to occur, the double bonds of unsaturated fatty acids must be processed to generate *trans*-2-enoyl-CoA intermediate (36). The removal of preexisting double bonds requires accessory proteins. Two enzymes—a 2,4-dienoyl-CoA reductase and a Δ^3-*cis*,Δ^2-*trans*-enoyl-CoA isomerase—process a double bond in a fatty acid at an even-numbered position (52). Three enzymes—a Δ^3-*cis*,Δ^2-*trans*-enoyl-CoA isomerase, a $\Delta^{3,5}$-$\Delta^{2,4}$-di-enoyl-CoA isomerase, and a 2,4-dienoyl-CoA reductase—process fatty acids with double bonds at odd-numbered positions. The peroxisomal 2,4-dienoyl-CoA reductase has a PTS1 signal, and Δ^3-*cis*,Δ^2-*trans*-enoyl-CoA isomerase is a function of the L-PBE, as indicated before (52). Human and rat peroxisomal $\Delta^{3,5}$-$\Delta^{2,4}$-di-enoyl-CoA isomerase proteins are 36 kDa and contain PTS1 signal (52). Dietary γ-linolenic acid– and fish oil–containing diets rich in omega-3 polyunsaturated fatty acids, such as docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA), are known to increase peroxisomal β-oxidation in liver and lower serum triglycerides (3, 47). DHA is essential for normal growth and functional development of brain, and it preferentially binds brain fatty acid binding protein (B-FABP), a member of the fatty acid binding protein family.

The role of peroxisomal β-oxidation in the synthesis and metabolism of DHA (3, 5, 9, 12, 14, 17–20:6) and retroconversion of DHA to EPA is depicted in Figure 2. Synthesis of DHA requires (*a*) the enzymes in both peroxisomes and smooth endoplasmic reticulum and (*b*) regulated movement of fatty acids between these two compartments (3, 55, 77). Chain elongation of 7, 10, 13, 16, 19–22:5 fatty acid to 9, 12, 15, 18, 21–24:5 and its desaturation to 6, 9, 12, 15, 18, 21–24:6 occurs in microsomes. This fatty acid is then moved to peroxisomes without esterification. In peroxisomes, it is either partially β-oxidized (3, 5, 9, 12, 14, 17–20:6) or transported to microsomes to form 1-acyl-sn-glycerol-3-phosphocholine, or fully β-oxidized to yield EPA (4, 7, 10, 15–18:5).

LIGAND BINDING PROTEINS

Very-long-chain and long-chain fatty acids and their CoA esters are not only the molecules involved in lipid metabolism, as substrates, they also function as PPAR ligands (12, 22, 86). The intracellular concentrations of fatty acids and their derivatives are strictly regulated, which implies the existence of integrated circuits to sense changes in serum and cellular fatty acid levels. Because of extremely low aqueous solubility, the intracellular distribution and trafficking of these PPAR

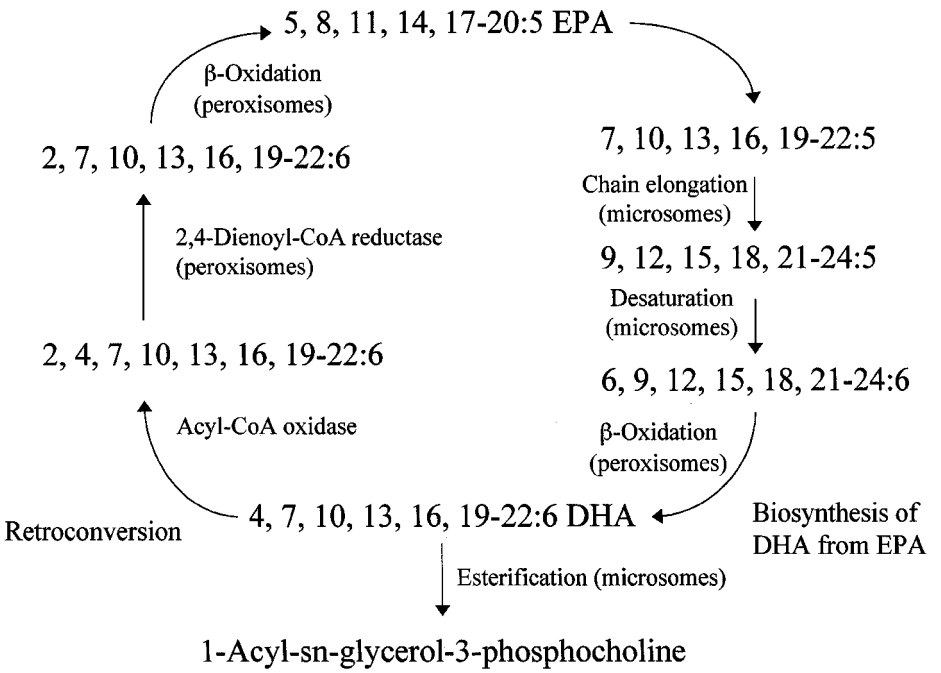

Figure 2 Peroxisomal β-oxidation of polyunsaturated fatty acids and retroconversion of docosahexaenoic acid (DHA) to eicosapentaenoic acid (EPA). DHA [22:6(n-3)] and EPA [20:5(n-3)] are interconverted and the process involves the participation of smooth endoplasmic reticulum (microsomes) and peroxisomes.

ligands is modulated by lipid binding proteins (FABP), fatty acyl-CoA binding protein (ACBP), and SCP2 (also called nonspecific lipid carrier protein). These proteins modulate free ligand concentrations in several ways: (*a*) concentrations of binding proteins, (*b*) association constants, (*c*) specificities, (*d*) intracellular localization, and (*e*) cell-tissue–specific expression. FABP and ACBP are cytosolic in distribution, and SCP2 is present exclusively within the peroxisome matrix. Despite the identification of proteins that bind these lipid ligands of PPAR, little is known about the mechanisms by which ligand-binding proteins control receptor-mediated gene expression.

Fatty Acid Binding Proteins

FABPs form a conserved family of intracellular monomeric lipid binding proteins with low molecular mass (~15 kDa). The following FABPs are of particular relevance: liver (L-FABP), heart (H-FABP), intestine (I-FABP), and brain (B-FABP). They form 1:1 complexes with fatty acids or other hydrophobic ligands, except that L-FABP is capable of binding two molecules of fatty acids (12). Many of

the cell types express more than one type of FABP: L-FABP in liver, L-FABP and H-FABP in kidney, H-FABP and B-FABP in brain, L-FABP and I-FABP in intestine, and H-FABP and I-FABP in stomach. FABPs are thought to bind and target fatty acids to various sites within the cell. L-FABP is induced in response to fatty acids and peroxisome proliferators (12).

Acyl-CoA Binding Protein

Various cells contain a cytosolic high-affinity acyl-CoA binding protein (ACBP). It is a low-molecular-mass protein (10 kDa) and is identical to a protein known as diazepam-binding inhibitor (86), a modulator of the GABA receptor in brain membranes. The main function of ACBP in lipid metabolism appears to be the modulation of acyl-CoA concentrations within the cell. Acyl-CoA affects the activities of various enzymes. For example, by its ability to bind acyl-CoAs, ACBP effectively opposes the product feedback inhibition of the long-chain acyl-CoA synthetase and shows a string-attenuating effect on the long-chain acyl-CoA inhibition of acetyl-CoA carboxylase. Acyl-CoAs function as ligands for transcription factors and regulate intracellular signaling. ACBP promoter has a sterol regulatory element-like sequence motif (86), which has led to the speculation that ACBP may play a role in steroidogenesis.

Sterol Carrier Protein-2

SCP2 binds sterols, phospholipids, long-chain isoprenoids, long-chain fatty acids, and long-chain fatty acyl-CoAs. SCP2 also binds branched-chain fatty acids and may be specifically involved in their peroxisomal β-oxidation (44). SCP2, a 15-kDa protein, contains a PTS1 and is one of the most abundant proteins present in peroxisomes. SCP2 and SCPx are derived from the same gene by alternate use of promoters (57). SCP2 and SCPx share the C-terminal 123–amino acid sequence and a PTS1 (57). SCP2 interacts with long-chain acyl-CoAs and forms specific complexes with acyl-CoA oxidase and other β-oxidation enzymes. In this capacity, SCP2 appears to facilitate peroxisomal β-oxidation by supplying fatty acyl-CoA esters and by removing the oxidized or chain-shortened products of β-oxidation (33). It is conceivable that SCP2 stabilizes β-oxidation enzymes by protein-protein interaction, a function that may be altered in diabetes because of a change in the SCP2 content in liver (33).

REGULATORY ROLE OF PPARα

Peroxisome Proliferators (Ligands for PPARα)

Several structurally diverse chemicals with hypolipidemic properties have been shown to induce peroxisome proliferation in the livers of rats and mice, and a receptor-mediated mechanism for the induction of these pleiotropic responses

was proposed (75, 76). These agents, designated as peroxisome proliferators (75), currently encompass a broad spectrum of synthetic and naturally/biologically occurring compounds that function as PPARα ligands (39). Synthetic peroxisome proliferators include certain hypolipidemic drugs, phthalate ester plasticizers, herbicides, food flavors, and leukotriene D_4 receptor antagonists (30, 76). In general, peroxisome proliferators exhibit little obvious structural similarity; the only shared chemical feature is that each of these either is or has the potential to be transformed into a carboxylic acid derivative, and these acid properties may be of critical importance to their ability to induce the peroxisome proliferative response. Natural/biological factors that are capable of increasing fatty acid β-oxidation activity and inducing variable degrees of peroxisome proliferation in hepatocytes include high-fat diets [especially diets rich in VLCFAs ($>C_{20}$) and polyunsaturated fatty acids, such as DHA and EPA], phytanic acid, the adrenal steroid dehydroepiandrosterone, and eicosanoids derived from arachidonic acid either via the lipoxygenase pathway, leading to the formation of leukotrienes and dehydroxyeicosatetraenoic acids, or via a cyclooxygenase pathway generating prostaglandins (30). Despite their structural diversity, the synthetic peroxisome proliferators, as a group, induce in rats and mice qualitatively predictable pleiotropic responses consisting of hepatomegaly, proliferation of peroxisomes in liver parenchymal cells, and the induction of several hepatic enzymes, particularly those responsible for lipid metabolism (30, 48, 75, 76). Long-term exposure to synthetic peroxisome proliferators and the sustained induction of peroxisome proliferation in livers leads to the development of hepatocellular carcinoma in rats and mice (30, 72, 72a). Peroxisome proliferators, in general, have been consistently found to be nonmutagenic (nongenotoxic), in that they do not interact with or damage DNA either directly or after metabolic activation, thereby leading to the proposal that the development of liver tumors is attributable to sustained induction of peroxisome proliferation (30, 72a, 76). In this regard, it is pertinent to note that in mice with disrupted AOX gene, sustained peroxisome proliferation occurring spontaneously also causes liver tumors, indicating that substrates for the classical peroxisomal β-oxidation system function as ligands for PPARα and that unmetabolized ligands cause sustained hyperstimulation of PPARα transcriptional activity (23) (see also below). Proliferation of peroxisomes in liver is associated with >15-fold increases in the activities of the enzymes required for peroxisomal β-oxidation of fatty acids, and these now fall under the category of the inducible classical L-hydroxy–specific peroxisomal β-oxidation pathway (72a, 74). Peroxisome proliferation is also associated with profound induction of microsomal CYP4A family of fatty acid ω-oxidation system enzymes (31, 43). The increases in the activities of these enzymes also parallel the increases in the peroxisomal β-oxidation enzyme system and reflect transcriptional activation of the respective genes (31, 74). Several other genes are also induced in livers with peroxisome proliferation, and the recent application of cDNA microarray technology is providing new insights into the hepatic gene expression profiles during peroxisome proliferation.

PPAR Isotypes

Significant progress has been made during the past decade in understanding the mechanisms responsible for the induction of genes involved in peroxisomal, microsomal, and mitochondrial fatty acid oxidation systems in liver by peroxisome proliferators. The existence of a specific receptor(s) responsible for the action of peroxisome proliferators was first postulated in 1983, based the cell/tissue specificity of pleiotropic responses, rapid transcriptional activation of fatty acid oxidation system genes, response of extrahepatic hepatocytes to the inductive effects of peroxisome proliferators, and the presence of specific binding protein(s) in liver cytosol for amphipathic carboxylates (73, 74, 76). These tenets, in essence, formed the impetus for the identification and molecular cloning of a receptor, now known as PPARα, from mouse liver that is activated by structurally diverse peroxisome proliferators (39). The induction of some of the critical enzymes of the peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems by peroxisome proliferators is transcriptionally controlled by PPARα, as these effects are abrogated in PPARα null mice (49). Two other PPAR isotypes, PPARγ and PPARδ, also appear to be important in lipid homeostasis, but they do not participate in the mediation of peroxisome proliferator–induced pleiotropic responses (4, 17, 35, 81). These isotypes are encoded by distinct genes, which are located on different chromosomes in humans and mice (for a review, see 17). The PPARγ gene generates two transcripts, designated PPARγ1 and PPARγ2, resulting from differential mRNA splicing and promoter usage (103). It plays an important role in adipocyte differentiation and, therefore, storage of fatty acids (4, 82). In conditions associated with excess energy consumption, acetyl-CoA generated from glucose is utilized for fatty acid synthesis, and their subsequent conversion to triglycerides leads to PPARγ promotion of adipogenesis and lipid storage (28, 82, 91). PPARγ null mutation is lethal around embryonic day (E)10, and it appears that this receptor is required for epithelial differentiation of trophoblast tissue, and for proper placental vascularization (7). Lipodystrophy, abnormalities of cardiac development, and multiple hemorrhages occur in tetraploid-rescued mutants surviving to term, indicating that PPARγ orchestrates a spectrum of physiological processes (7). The specific functional role of PPAR$\beta(\delta)$ remains elusive, as little is known about the target genes of this receptor. Disruption of PPARβ gene results in myelination and epidermal cell proliferation defects (65).

PPARs, like other members of the nuclear receptor superfamily, display six regions or domains (4, 17, 39, 69, 100). The N-terminal portion of the receptor contains a variable A/B region, which exhibits a ligand-independent transactivation function (AF-1 domain). The central part of the receptor depicts a highly conserved C region composed of two type II zinc fingers that are responsible for sequence-specific DNA recognition and dimerization (DNA-binding domain). The C-terminal half of the PPAR is subdivided into D, E, and F regions, with the E region functioning as the ligand-binding domain. The crystal structures of

ligand-binding domain of the human PPARγ and human PPARβ/δ reveal that the ligand-binding pocket is unusually large ($\sim$1300 Å^3), which may account for the accommodation of a diverse spectrum of synthetic and natural ligands and their variable ability to bind and to transactivate all three PPAR isoforms (100). This E region is relatively large, and highly conserved, and because of its ligand-binding property, it also functions as a ligand-dependent transactivation domain (AF-2 domain). The C-terminal F region is relatively small compared with other regions and is considered important in the interaction of nuclear receptors with nuclear receptor coactivators and corepressors (29, 69, 105).

The tissue distribution of PPARs to some extent reflects their function. PPARα is highly expressed in hepatocytes, enterocytes, proximal tubular epithelium of kidney, and cardiac muscle, and the expression pattern parallels, to a certain extent, the sensitivity of various tissues to the β-oxidation induction by synthetic peroxisome proliferators (4, 17). PPARγ is expressed predominantly in adipose tissue, liver, mammary gland, urinary bladder, and colonic mucosa (4, 17). PPARγ is involved in adipogenesis, and maintenance of differentiation may be an important function of this receptor in breast, colon, and urinary bladder. PPARβ is expressed widely in most tissues, but its function remains largely elusive (17).

PPAR Response Elements

The PPARs form heterodimers with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR), and the PPAR/RXR heterodimers bind to DNA sequences, termed PPAR response elements (PPREs), containing direct repeats (DR) of the hexanucleotide sequence AGGTCA separated by one nucleotide, known as a DR-1 response element, present in the 5′-flanking region of target genes. PPREs transcriptionally activated by peroxisome proliferators and that are present in the promoter sequences of some of the genes, such as AOX, involved in fatty acid oxidation have been characterized (17, 73). Contrary to some of the claims, the PPRE of the human AOX gene is functionally active (97).

PPAR Cofactors

The biochemical and molecular mechanisms by which nuclear receptors achieve transcriptional activation, in a gene-, tissue-, and species-specific fashion, are the subjects of intense research activity (29). The current models call for participation of a series of cofactors (accessory proteins) that bind to nuclear receptors in a ligand-dependent fashion (29). During the past 5 years, several "coactivator" proteins for nuclear receptors have been identified (29). Of these, steroid receptor coactivator (SRC)-1 (103), PPAR-binding protein (PBP) (106), PPARγ-coactivator-1 (PGC-1) (66), PPAR interaction protein (PRIP) (104), and others have been identified as PPAR coactivators (for a review, see 29, 69). A model depicting the interaction of PPARα-coactivator complexes with the basal transcription machinery is depicted in Figure 3. Mice lacking SRC-1 are viable and

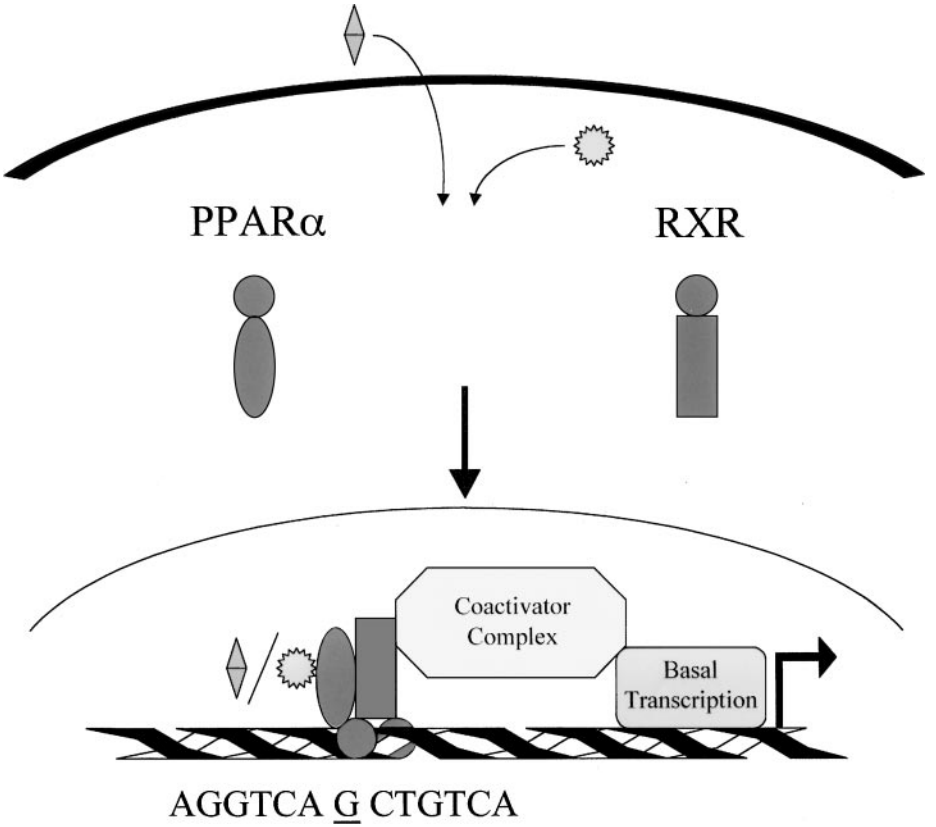

Figure 3 A model depicting the interaction of the peroxisome proliferator–activated receptor α (PPARα)-coactivator complexes with basal transcription machinery, leading to the enhanced transcription of target genes. Liganded PPARα interacts with 9-*cis*-retinoic acid receptor (RXR) to form PPARα/RXR heterodimers and recruit nuclear receptor coactivator protein complexes for linking with the basal transcription apparatus. The sequence AGGTCA G CTGTCA represents the PPAR response (DR1) element of the human AOX gene of the classical peroxisomal β-oxidation spiral. The synthetic ligands (diamonds) and the natural/biological ligands (asterisks) interact with PPARα.

fertile (68, 101) and responded to peroxisome proliferators in a fashion analogous to wild-type mice exposed to peroxisome proliferators, indicating functional redundancy of SRC-1 with regard to PPARα function (68). In contrast, mice homozygous for PBP null mutation exhibited lethality around E11.5, indicating the critical importance of this coactivator in development (40, 107). Elucidation of the tissue- and cell-specific mechanisms of transcriptional activation of genes involved in peroxisomal β-oxidation is essential for a greater appreciation of the importance of lipid homeostasis in health and disease.

DISORDERS OF PEROXISOMAL LIPID METABOLISM

Peroxisomal Diseases

Since the first description of peroxisomal absence in liver and kidney in patients with Zellweger syndrome, several peroxisomal disorders, a majority manifesting as disturbances in lipid metabolism, have been documented (92, 98). Peroxisomal disorders, classified on the basis of biochemical defects, generally fall into three categories, depending on whether there is a generalized (group A), multiple (group B), or a single (group C) loss of peroxisomal functions (98). Groups A and B are mostly related to defects in posttranslational import of peroxisomal matrix proteins due to mutations in peroxins (83). Peroxins PEX5 and PEX7 function as receptors for PTS1 and PTS2, respectively, and mutations in these receptors lead to defects in the import of PTS1, or PTS1 and PTS2, proteins (83). Because peroxisomal β-oxidation is not a functional duplication of the mitochondrial system, abnormalities in the peroxisomal β-oxidation pathway involve specifically the metabolism of VLCFAs, long-chain dicarboxylic acids (DCAs), pristanic acid, certain prostaglandins and leukotrienes, and 15-hydroxyeicosatetraenoic acid, among others (55, 77, 98).

The Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum's disease are examples of group A with generalized defects in peroxisome assembly, manifesting in the functional loss of many peroxisomal enzymes (98). In these group A diseases, the molecular basis appears to be a dysfunction of *PEX5*, which encodes the PTS1 receptor, necessary for peroxisomal import of such proteins as AOX, L-PBE, D-PBE, and many others with the C-terminal PTS1 (83). Dysfunction of PEX7, which encodes the PTS2 receptor, is also found in group A diseases. These patients reveal abnormalities in the β-oxidation of VLCFAs and multiple dysmorphogenetic features, including craniofacial, neurological, ocular, hepatological, and skeletal anomalies. Peroxisomes are generally absent, but mitochondrial abnormalities become prominent, in liver cells of Zellweger syndrome patients. Hepatic steatosis is variable, but detailed information on the evolution of hepatic changes in these genetic disorders and the status of PPARα expression in liver is lacking.

Rhizomelic chondrodysplasia punctata is an example of a group B peroxisomal disorder with defects in ether-phospholipid metabolism due to abnormalities in dihydroxyacetonephosphate acyltransferase and alkyldihydroxyacetonephosphate synthase and to an abnormality in phytanic acid α-oxidation (98). The molecular basis is attributed to dysfunction of *PEX7*, which encodes the PTS2 receptor, necessary for peroxisomal import of enzymes such as thiolase, which contain N-terminal PTS2 (83). The hepatic phenotype in this rare genetic disease is not well delineated.

Peroxisomal disorders, characterized by the loss of a single peroxisomal function due to loss/mutation in a single gene, belong to group C (single enzyme deficiency). The several disorders relevant to the discussion of peroxisomal

β-oxidation include X-linked adrenoleukodystrophy (X-ALD), straight-chain AOX deficiency, D-PBE deficiency, and thiolase deficiency (98). X-ALD, a most frequent peroxisomal genetic disorder, presents either as a lethal childhood form or as a mild "Addison's only" form (98). In this disorder, VLCFAs accumulate because of impaired peroxisomal β-oxidation. The defect in this condition was initially thought to be a deficiency of very-long-chain acyl-CoA synthetase, which activates VLCFAs to their corresponding CoA esters at the site of peroxisomal membrane, but it is now attributed to a defect in a 80-kDa peroxisomal membrane protein, called adrenoleukodystrophy protein (ALDP), which belongs to the ATP-binding cassette (ABC) transporter superfamily (53, 54, 98). ALDP functions as a homo- or heterodimer and participates in the transport of $C_{26:0}$ CoA esters across the peroxisome membrane into the peroxisome matrix. In X-ALD patients, a variety of mutations, including deletions, point mutations, and insertions, in the ALDP gene have been observed (98). The other diseases belonging to group C include deficiency of one of the enzymes of the straight-chain or branched-chain β-oxidation systems. Only a few patients with classical AOX deficiency have been reported, and some of these have a large deletion of the oxidase gene (26). The absence of straight-chain AOX leads to deficient oxidation of C26:0 fatty acids only, without affecting the oxidation of branched-chain fatty acids (26). Peroxisomes are present in liver cells in this condition, but detailed descriptions of liver morphology are lacking. Few patients with D-PBE deficiency or 3-ketoacyl-CoA thiolase deficiency have been identified (93, 94, 98). VLCFA and pristanic acid oxidation is affected in D-PBE deficiency, resulting in increased plasma levels. Evidence indicates that D-PBE deficiency is more severe than AOX deficiency but less severe than thiolase deficiency (98). The magnitude of functional anomalies in thiolase deficiency is similar to that encountered in Zellweger syndrome (98). D-PBE deficiency is less severe in part because of the functional redundancy between L-PBE and D-PBE.

The major unresolved issue is how individuals with genetic defects in peroxisomal β-oxidation or PPARα-inducible genes react to nutritional stress. Data from genetically altered animal models points to the intricacies of basal metabolism and to such rapid, egregious amplification of response to stress as short-term energy deprivation. It is important to note that children with an inherited deficiency of mitochondrial medium-chain acyl-CoA dehydrogenase, a PPARα-inducible mitochondrial β-oxidation enzyme, develop normally and are mostly asymptomatic, but during fasting-related stress they decompensate and die suddenly (11, 87).

Fatty Acid Oxidation in Genetically Altered Mice

Of the three PPARs, the PPARα isotype plays a prominent role in the catabolism of fatty acids and is solely responsible for the peroxisome proliferator–induced pleiotropic responses, including the transcriptional activation of genes involved in fatty acid oxidation in livers of rats and mice (49). Sustained activation of PPARα and the induction of PPARα-responsive genes that participate in lipid catabolism in the liver leads to the development of liver tumors (23, 30, 72, 76, 72a).

The purported relative nonresponsiveness of human liver cells to the peroxisome proliferator–induced pleiotropic effects may be attributed, in part, to differences in the levels of PPARα expression in rodents and humans (63). The identity of PPARα target genes involved in hepatic lipid catabolism is well delineated, and the use of gene targeting to disrupt PPARα and some of the genes involved in the peroxisomal β-oxidation system has led to major advances in our knowledge about the role of peroxisomal β-oxidation in regulating PPARα activity and in the pathogenesis of macrovesicular and microvesicular hepatic steatosis (5, 6, 23, 35, 44, 49, 67, 80). The studies with genetically altered mice underscore the importance of PPARα and of the peroxisomal β-oxidation systems, in particular the critical role of classical AOX, in hepatic lipid metabolism.

Classical AOX Deficiency Leads to PPARα Activation The fatty acyl-CoA oxidase (AOX) deficiency disease (pseudoneonatal adrenoleukodystrophy) manifests in the neonatal period with hypotonia, varying extent of dysmorphic features, and pyschomotor retardation, with death occurring by 4–5 years of age (26). Mice with a disrupted classical AOX gene (AOX$-/-$) exhibit, during the first 2–4 months of age, high levels of VLCFAs in the serum, growth retardation, hepatomegaly with severe microvesicular steatohepatitis, and lipogranulomatous reaction (23). Lipogranulomas in liver contain macrophages, lymphocytes, eosinophils, and polymorphonuclear leukocytes, and in many of these lipogranulomas a central fat globule is discernable (23). AOX knockout mice reveal age-progressive hepatocellular regeneration commencing in the periportal region and extending toward the centrizonal region of the liver lobule (23). Between 6 and 8 months of age, almost all steatotic hepatocytes in AOX$-/-$ mice are replaced by regenerated hepatocytes devoid of steatosis, but these cells display an abundance of peroxisomes (23). This spontaneous peroxisome proliferation is associated in liver with increased expression of genes that are transcriptionally regulated by PPARα. Among others that contain PPRE in their 5-flanking regions, these include L-PBE, thiolase, CYP4A1, and CYP4A3 (23). Increased H_2O_2 levels are evident in AOX$-/-$ livers compared with age-matched controls. Hepatocellular adenomas and hepatocellular carcinomas develop in AOX$-/-$ mice between 10 and 15 months of age (23). In essence, the phenotypic alterations in AOX$-/-$ mice are similar to wild-type mice exposed chronically to potent synthetic peroxisome proliferators (23, 72a).

The AOX null mouse model serves as a paradigm for several important pathophysiological processes (23). First, disruption of the first and rate-limiting step of the classical peroxisomal β-oxidation system affects the metabolism of VLCFAs and other substrates of AOX, such as the proinflammatory arachidonic acid metabolites, leukotriene B_4, and 8(S)-hydroxyeicosatetraenoic acid. Second, it is evident that the unmetabolized acyl-CoAs derived from VLCFAs and DCAs—proinflammatory molecules such as leukotriene B_4 and 8(S)-hydroxyeicosatetraenoic acid and possibly other substrates of AOX—function as ligands for PPARα, leading to sustained hyperactivation of this receptor and up-regulation of downstream genes that contain PPRE (23). Sustained induction of CYP4A family genes,

which metabolize LCFAs and VLCFAs, results in the excess generation of dicarboxylic acids (14). Rate of NADPH consumption is higher for human (CYP4A11) and rat (CYP4A1, CYP4A2, CYP4A3, and CYP4A8) isoforms than the rate of fatty acid hydroxylation, which implies that the enzymes undergo some degree of uncoupled turnover, indicating reduction of oxygen to superoxide and/or H_2O_2 (18, 37; U Hoch & P Oritz de Monetllano, personal communication). The toxic dicarboxylic acids cannot be further metabolized in the absence of AOX in these animals. Third, microvesicular hepatic steatosis and steatohepatitis observed in AOX−/− mice appear to be the result of toxic effects of unmetabolized AOX substrates, which include VLCFAs and DCAs. In addition, the excess production of H_2O_2 due to inflammatory changes, to CYP4A-mediated ω-hydroxylation, and to other H_2O_2-generating oxidases, such as urate oxidase in the liver of AOX−/− mice (103), can further contribute to the "second hit" capable of inducing necroinflammatory steatohepatitis, leading to the formation of lipogranulomas (41). Fourth, sustained enhanced transcriptional activity of PPARα on PPARα-regulated genes in liver in this AOX knockout model leads to the development of liver tumors. In AOX−/− mouse liver, increased mRNA levels of genes regulated by PPARα, such as L-PBE, CYP4A1, and CYP4A3, are found, implying sustained activation of this receptor in AOX deficiency. Evidence points to the oncogenic potential of PPARα (23, 30, 49), and to the fact that AOX gene function is indispensable for the physiological regulation of this receptor (23). It is evident that ligands for PPARα function as substrates for the AOX, and because the gene encoding this enzyme is transcriptionally regulated by PPARα, this constitutes a critical cross talk because sustained up-regulation of PPARα-controlled genes leads to liver tumor development (23, 103). The principal function of AOX is to keep the PPARα in check by β-oxidizing VLCFAs and other biological ligands of this receptor, including DCAs, the products of CYP4A-mediated ω-oxidation. Evidence indicates that the mechanism of liver tumorigenesis in AOX knockout mice is most likely the result of sustained activation of PPARα and oxidative stress emanating from proinflammatory responses and the induction of H_2O_2-generating enzymes.

Disruption of Peroxisomal β-Oxidation Pathway Proximal to AOX For the β-oxidation process to begin, very-long-chain fatty acyl-CoAs have to enter peroxisome matrix. In X-ALD, a peroxisomal disorder with impaired VLCFA metabolism, the VLCFAs and/or their acyl-CoAs are not effectively transported into peroxisome because of defective ALDP, which is a transporter (53, 58, 59, 78). In this disease, there is progressive VLCFA accumulation, resulting in mental and motor function deterioration, with demyelination of the central and peripheral nervous system (98). A mouse model for x-linked adrenoleukodystrophy developed by gene targeting (54, 102) reveals no hepatic steatosis and no spontaneous peroxisome proliferation in liver cells, which suggests that disruption of classical peroxisomal β-oxidation proximal to AOX does not affect the generation and metabolism of PPARα ligands. Also, because PPARα is not hyperactivated in this x-linked adrenoleukodystrophy mouse model, the production of toxic dicarboxylic acids

by CYP4A is also curtailed, unlike in AOX null mouse (23). These observations imply that VLCFAs are not the direct ligands of PPARα and that intact functional AOX is highly efficient in inactivating these ligands to keep PPARα in check (23).

Disruption of Peroxisomal β-Oxidation Pathway Distal to AOX Mice homozygous for a disruption of the L-PBE gene, which encodes the second enzyme of the β-oxidation spiral, demonstrate that disruption of this classical β-oxidation pathway distal to AOX does not affect the metabolism of natural ligands of PPARα (67). Mice deficient in L-PBE displayed no hepatic steatosis, a feature that is striking in young AOX-deficient mice (23, 67). Disruption of the L-PBE gene also fails to induce spontaneous peroxisome proliferation such as that encountered in the regenerated liver cells of mice lacking AOX. These observations suggest that the hepatic steatogenic stimuli prominent in AOX null background are not present in L-PBE knockout mice, implying that AOX is necessary for their detoxification. The absence of spontaneous peroxisome proliferation in L-PBE null mice also indicates that classical AOX of the L-hydroxy–specific β-oxidation system is responsible for the metabolism of all putative ligands of PPARα (67), and that L-PBE enzyme, which is immediately downstream of AOX, is not essential for the successful completion of the L-hydroxy–specific β-oxidation (67). It would appear that once long and very long straight-chain acyl-CoAs, long-chain dicarboxylyl-CoAs, and the CoA esters of eicosanoids are converted into their respective enoyl-CoAs by the classical AOX, they can be metabolized by D-PBE of the D-hydroxy–specific branched-chain peroxisomal β-oxidation system (67), or even by the mitochondrial β-oxidation system. The AOX and L-PBE null mouse models convincingly prove that classical AOX is pivotal for the metabolic degradation of a bulk of PPARα ligands, and this class of ligands (substrates of classical AOX) is not metabolized by the branched-chain AOX of the second system (23, 67). Thus, it is reasonable to surmise that the functions of AOX (especially the metabolism of critical substrates of AOX, which serve as PPARα ligands) cannot be handled by the branched-chain AOX. Mice with a disrupted D-PBE (6) or SCPx gene (80) of the second system are generally devoid of hepatic steatosis, implying that L-PBE and thiolase of the classical system are capable of completing the β-oxidation reaction of either straight- or branched-chain enoyl-CoAs generated after the initial oxidase step. Further studies are needed to rule out the possibility that if left unmetabolized, straight-chain and branched-chain enoyl-CoAs serve as PPARα ligands (67). Generating mice deficient in both L-PBE and D-PBE and characterizing their liver phenotype should resolve this issue. The observations from these gene knockout mouse models indicate that the two peroxisomal β-oxidation pathways are not strictly separable after the oxidation (desaturation) step.

Mice Nullizygous for Both PPARα and AOX PPARα knockout mice (PPARα $-/-$) established that this receptor is essential for hepatic peroxisome proliferation and coordinate transcriptional activation of AOX, L-PBE, peroxisomal thiolase, CYP4A1, CYP4A3, and other genes by structurally diverse synthetic peroxisome

proliferators (49). PPAR$\alpha-/-$ mice display a normal complement of peroxisomes in hepatocytes, but these mice remain nonresponsive to the inductive influence of synthetic peroxisome proliferators (49). They also fail to develop liver tumors when exposed to peroxisome proliferators, indicating the potential oncogenic nature of PPARα and the contribution of PPARα-regulated genes to the neoplastic process (30). A mild degree of centrilobular macrovesicular fatty change develops in the liver of these mice. This is attributed to reduction in the constitutive levels of mitochondrial fatty acid β-oxidation, because the constitutive or basal oxidation of VLCFAs by the peroxisomal β-oxidation system appears unaffected by PPARα deficiency, although this system in these mutant mice fails to respond to the inductive effects of peroxisome proliferators (49). The PPARα null mice have normal levels of the classical peroxisomal AOX, L-PBE, and peroxisomal thiolase but lower constitutive expression of D-PBE. This indicates that this basal activity is sufficient to metabolize the VLCFAs and other AOX substrates and therefore shows no adverse microvesicular steatotic response in liver (49). It is of particular interest, therefore, that disruption of this basal classical β-oxidation system at the AOX level causes profound microvesicular steatosis in AOX knockout mice, affirming the criticality of AOX in preventing steatohepatitis (23). Mice nullizygous for both PPARα and AOX (PPAR$\alpha-/-$AOX$-/-$) fail to exhibit extensive microvesicular steatohepatitis, spontaneous peroxisome proliferation, and induction of PPARα-regulated genes by biological ligands that remain largely unmetabolized in the absence of AOX (35). In AOX null mice, the hyperactivity of PPARα enhances the severity of steatohepatitis by inducing CYP4A family proteins that generate DCAs and H_2O_2 (18; U Hoch & P Ortiz, personal communication), and because DCAs are not metabolized in the absence of AOX, they damage mitochondria and affect β-oxidation (Figure 4). Blunting of microvesicular steatosis in PPAR$\alpha-/-$AOX$-/-$ mice suggests a role for PPARα-induced genes, especially members of CYP4A family, in determining the severity of steatohepatitis with defective classical peroxisomal β-oxidation (35). In these PPAR$\alpha-/-$AOX$-/-$ mice, under a fed state, only a few scattered hepatocytes in the periportal region show microvesicular fatty change (35).

Defect in PPARα-Inducible Fatty Acid Oxidation and Severity of Hepatic Steatosis in Response to Fasting PPARα null mice under fed conditions do not manifest hepatic steatosis except for an occasional hepatocyte in the centrizonal area showing large-droplet fatty change as the animals age (35, 49). During starvation, fatty acids entering into the liver constitute the major source of energy, and they require efficient hepatic oxidation to generate ketone bodies to serve as fuels for other tissues (71). It is now well recognized that fasting causes a rapid transcriptional activation of genes encoding peroxisomal, microsomal, and certain mitochondrial fatty acid oxidation enzymes in liver in healthy subjects (45, 46, 51). These observations point to the importance also of regulatory step(s) controlling the levels of inducible fatty acid oxidation enzymes. In response to fasting, any abnormality in the inducibility of such enzymes can, in a manner similar to that encountered

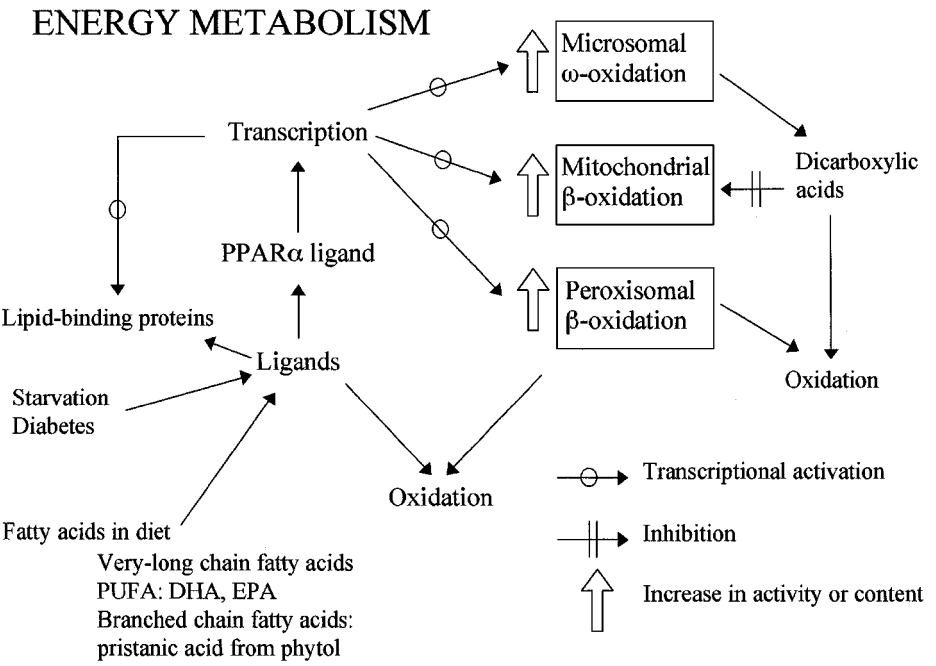

Figure 4 Cross talk among fatty acid oxidation systems and the regulatory role of peroxisome proliferator–activated receptor α (PPARα) in the oxidation of fatty acids and energy metabolism. Microsomal ω-oxidation generates dicarboxylic fatty acids, which are further degraded by peroxisomal β-oxidation by the classical fatty acyl-CoA oxidase (AOX). The chain-shortened fatty acyl-CoAs produced by the peroxisomal β-oxidation are shunted to the mitochondrial β-oxidation pathway for completion of oxidation. Substrates for peroxisomal classical β-oxidation system (for AOX) act as ligands for PPARα and increase the transcription of PPARα target genes of the microsomal, mitochondrial, and peroxisomal fatty acid oxidation systems. In the absence of classical peroxisomal β-oxidation (AOX deficiency), the unmetabolized substrates cause hyperstimulation of PPARα-regulated genes. PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

with metabolic defects at the enzymatic level, impact energy metabolism and degree of hepatic steatosis. The availability of mice (*a*) deficient in peroxisomal AOX, (*b*) deficient in PPARα (49), and (*c*) nullizygous for both PPARα and AOX (35) enabled studies on the effect of genotype on energy utilization during fasting and on hepatic phenotype (34, 45, 46, 51). In wild-type mice, fasting for 24–72 h caused a modest induction of the hepatic expression of PPARα target genes encoding AOX, L-PBE, PTL, CYP4A1, CYP4A3, mitochondrial medium-chain acyl-CoA dehydrogenase, carnitine palmitoyltransferase I, and very-long-chain acyl-CoA synthetase (34, 45, 46, 51). In the wild-type mice, hepatic steatosis was minimal during 24–72 h of starvation and essentially disappeared when animals were starved for 96 h (34). In contrast, hepatic expression of PPARα-regulated

fatty acid oxidation system genes is not induced by fasting in PPAR$\alpha-/-$ mice, and when these mice are stressed by short-term fasting, there is an exaggeration of fatty liver phenotype (45, 46, 51). As these double nulls lack AOX in addition to PPARα, because of added deficiency of peroxisomal β-oxidation, fasting caused a more pronounced steatotic phenotype in the liver of mice nullizygous for both PPARα and AOX compared with PPARα knockout mice (34). Following fasting, the hepatic triglyceride/protein ratio was substantially higher in mice nullizygous for both PPARα and AOX than that noted in PPAR$\alpha-/-$ mice (34). Fasting for 24–72 h did not lead to significant increases in triglyceride levels in kidney and heart of wild-type, PPAR$\alpha-/-$, AOX$-/-$, and PPAR$\alpha-/-$AOX$-/-$ mice (34). Differences in lipid metabolism between liver and these extrahepatic organs under starvation are attributed to rapid activation of PPARα-responsive genes in liver and to the fact that PPARα-inducible fatty acid oxidation systems in liver play a vital role in energy metabolism and in the prevention of hepatic steatosis. When fasted for 48 and 72 h, both PPAR$\alpha-/-$ and PPAR$\alpha-/-$AOX$-/-$ mice exhibit severe hypoglycemia, hypoketonemia, and elevated serum free fatty acids, indicating a dramatic inhibition of fatty acid oxidation in liver, as the defect in PPARα essentially negates the induction of fatty acid oxidation systems in response to fasting (34). These observations point to the critical importance of PPARα in the transcriptional regulatory responses to fasting and in determining the severity of hepatic steatosis. In mice deficient in AOX alone, fasting-related amplification of hepatic steatosis is not prominent (34). Although the basal content of hepatic triglycerides in fed AOX null mice, reflecting the presence of preexisting microvesicular steatosis in some centrizonal hepatocytes, is somewhat higher than that of other groups, fasting only induced a minimal increase in triglyceride/protein ratios. This is attributed to sustained hyperactivation of PPARα and up-regulation of mitochondrial β-oxidation and microsomal ω-oxidation systems in liver. As a consequence these animals are highly effective in generating ketone bodies. Increases in plasma 3-hydroxybutyrate levels occurred in AOX$-/-$ mice similar to those observed in fasted wild-type mice (34). In contrast, the 3-hydroxybutyrate levels decreased in fasted PPAR$\alpha-/-$ and PPAR$\alpha-/-$AOX$-/-$ mice. It is also worth noting that the regenerative nature of a majority of hepatocytes containing numerous spontaneously proliferated peroxisomes in the livers of older AOX$-/-$ mice are resistant to fatty change.

Relevance to Humans In humans, the hepatic PPARα level is reported to be lower than that found in rats and mice (63). This raises issues related to (*a*) species differences in responses to peroxisome proliferators, (*b*) the critical levels of this transcription factor required to maintain lipid homeostasis, and (*c*) the effectiveness of PPARα-inducible fatty acid oxidation systems in different species in dealing with conditions of stress that lead to reduced energy intake and in dealing with dietary energy overload occurring in obesity and other metabolic conditions, such as type 2 diabetes. If the functions of PPARα, vis-à-vis the inducible levels of PPARα-enzymes in liver, are indeed less efficient in humans, the hepatic consequences of

starvation, increased energy consumption, and other metabolic conditions involving lipid catabolism may appear more dire in humans than in rats and mice. A PPARα splice variant that may negatively interfere with wild type of PPARα has been described in human liver, which raises the question of countering the induction of PPARα-regulated genes leading to abnormal energy utilization. The relevance of the purported low levels of PPARα and the presence of a splice variant in humans, leading to reduced lipid metabolism and energy storage in hepatic and extrahepatic tissues in obesity, need to be explored (27, 63). If humans have low levels of PPARα, the unmetabolized energy can stimulate PPARγ-mediated adipogenesis for storage functions, which suggests cross talk between PPARα and PPARγ. In PPAR$\alpha-/-$ mice as well as in PPAR$\alpha-/-$AOX$-/-$ mice, fasting caused a slight increase in hepatic PPARγ level (34). Detailed quantitative studies are needed to ascertain whether humans have more PPARγ and reduced PPARα levels in general to account for the increased incidence of obesity and associated hepatic steatohepatitis.

PERSPECTIVE

The demonstration that the phenomenon of peroxisome proliferation can be induced by many structurally diverse synthetic chemicals, now known as peroxisome proliferators, led to the proposal that peroxisome proliferation is linked to lipid metabolism and subsequently to the identification of the peroxisomal β-oxidation system. The tissue/cell specificity of pleiotropic responses and the coordinated rapid transcriptional activation of β-oxidation system genes led to the hypothesis that peroxisome proliferators exert their pleiotropic effects, including the development of liver tumors, by a receptor-mediated mechanism. Ten years ago, the first PPAR, now known as PPARα, was cloned and characterized and the identification of two other isoforms, PPARγ, and PPARβ/δ, soon followed. During the past 5 years, the functional roles of PPARα in peroxisome proliferation and in lipid catabolism, and that of PPARγ in adipogenesis and in lipid storage, have been fully recognized. Fatty acid β-oxidation occurs in both mitochondria and peroxisomes, with peroxisomes playing a specialized role in the metabolism of VLCFAs, long-chain dicarboxylic acids, branched-chain fatty acids, eicosanoids, and bile acid precursors. The genes encoding some of the enzymes responsible for fatty acid oxidation are transcriptionally regulated by PPARα. Recent evidence has demonstrated that in both mitochondria and peroxisomes, two distinct β-oxidation systems operate, and each system has different enzymes with different but some overlapping substrate specificities (Table 2). In peroxisomes, the inducible classical β-oxidation system oxidizes straight-chain fatty acids, dicarboxylic acids, and eicosanoids, whereas the noninducible system oxidizes branched-chain fatty acids and bile acid precursors. Long-chain fatty acids and VLCFAs are also metabolized by the cytochrome P450 CYP4A ω-oxidation system to dicarboxylic acids, which serve as substrates for

TABLE 2 Functions of the two fatty acid β-oxidation systems

Determinant	Mitochondria	Peroxisomes
Substrates	Long-chain fatty acids Medium-chain fatty acids	Very-long-chain fatty acids Long-chain fatty acids Long-chain dicarboxylic acids Branched-chain fatty acids Bile acid precursors Some xenobiotics
Component	Inner membrane and matrix associated	Enzymes having different fatty acid specificities and enzymes haivng overlapping carbon chain-length specificities
Regulation		
Short term	Carnitine, malonyl-CoA	None known
Long term	Some enzymes inducible	Enzymes of classical set inducible
Clinical manifestation	Hypoketotic hypoglycemia	Progressive degeneration of central nervous system
Main roles	ATP production	Detoxification

the inducible peroxisomal β-oxidation system but which inhibit mitochondrial β-oxidation (Figure 4). The AOX of the classical inducible peroxisomal β-oxidation system appears critical in metabolizing PPARα ligands, and in the absence of this enzyme, the unmetabolized natural/biological ligands of PPARα cause sustained hyperstimulation of PPARα, leading to hepatic steatosis, hepatocellular regeneration, spontaneous peroxisome proliferation, and liver tumors. Thus, the substrates of AOX function as ligands for PPARα, the receptor, which controls the transcription of the enzyme. Because the substrate for the enzyme constitutes the biological/natural ligands of the receptor, this cross talk between the receptor and the enzyme it regulates is an important consideration in extrapolating the species response to peroxisome proliferators and in the blanket dismissal that humans are not susceptible to hepatomegaly, hepatic peroxisome proliferation, induction of peroxisomal β-oxidation, and liver tumors. The interdependencies between PPARα and the fatty acid oxidation that occur in three different subcellular organelles, namely peroxisomes, mitochondria, and microsomes, point to delicate, but vital, metabolic circuits (Figure 4). Evidence derived from genetically altered mice with a deficiency of PPARα, peroxisomal AOX, and some of the other enzymes of the two peroxisomal β-oxidation pathways highlights the critical importance of PPARα and the classical peroxisomal AOX in energy metabolism, and in the development of hepatic steatosis, steatohepatitis, and liver cancer.

Fatty liver is a common histological finding in human liver biopsies and presents as macrovesicular, with a large fat droplet in hepatocyte cytoplasm displacing the nucleus, or as microvesicular, with numerous small fat droplets surrounding a centrally located nucleus. Macrovesicular steatosis, a reflection of long-standing disturbance of hepatic lipid metabolism, is generally encountered in alcoholic liver injury, obesity, and type 2 diabetes mellitus. Microvesicular steatosis is regarded as a severe condition involving impairment of fatty acid β-oxidation, and emerging evidence points to the role of abnormalities in the metabolism of VLCFAs, DCAs, and other substrates of peroxisomal AOX that activate PPARα. This nuclear receptor is a key regulator of energy homeostasis and of peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems, and perturbations in the levels of PPARα, and the inducibility of PPARα-regulated enzyme systems that catabolize fatty acids in liver, can play a significant role in the pathogenesis of steatohepatitis and liver tumorigenesis.

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