Genetic Disorders of Mitochondrial and Peroxisomal Fatty Acid Oxidation and Peroxisome Proliferator-Activated Receptors

Ronald J. A. Wanders

Department of Pediatrics, Academic Medical Center; Department of Clinical Chemistry, University of Amsterdam, Amsterdam, The Netherlands

Fatty acids are an important source of energy in humans, especially during fasting. Most tissues are able to degrade fatty acids to carbon dioxide and water, but in addition, some organs—notably the liver—have the capacity to convert the acetyl-CoA units produced during β oxidation into the ketone bodies acetoacetate and 3-hydroxybutyrate. These are important fuels for certain organs, especially the brain. Fatty acids are the main source of energy in the heart. Indeed, under normal conditions 60% to 70% of the energy requirements of the heart are provided by fatty acid oxidation, and even more under certain conditions such as fasting and diabetes.

In principle, fatty acids can be degraded by several mechanisms, including α, β, and ω oxidation. However, most fatty acids are degraded by β oxidation, which can occur in the mitochondria or in the peroxisomes. It is generally agreed that the mitochondria form the major site of fatty acid β oxidation, catalyzing the oxidation of all major dietary fatty acids. In recent years, tremendous progress has been made in the identification of patients affected by a genetic deficiency in either the mitochondrial or the peroxisomal β oxidation system, and defects in virtually all the steps involved have now been identified. Thanks to these efforts, reliable laboratory methods have now become available to identify patients with these conditions. Much remains to be learned, however, about the pathophysiologic mechanisms behind these disorders. In this respect, the role of peroxisome proliferator-activated receptors (PPARs) is intriguing, especially since the metabolites accumulating as a result of a block in mitochondrial or peroxisomal β oxidation are PPAR activators.

In this chapter, I will describe our current state of knowledge about mitochondrial and peroxisomal fatty acid oxidation, with particular emphasis on the various inherited diseases and the role of PPARs.

FATTY ACID OXIDATION

Fatty acids may undergo α, β, or ω oxidation.
**Alpha-Oxidation**

α-oxidation is required for fatty acids with a methyl group at the 3-position, as 3-methyl branched chain fatty acids cannot undergo direct β oxidation. The mechanism of fatty acid α oxidation has remained mysterious ever since its discovery in the early 1960s, when the accumulation of the 3-methyl branched chain fatty acid phytanic acid (3,7,11,14-tetramethylhexadecanoic acid) was identified in patients suffering from a rare genetic disease known as Refsum disease. Patients with Refsum disease show various abnormalities including retinitis pigmentosa, cerebellar ataxia, peripheral neuropathy, anosmia, and cardiac abnormalities (1). The finding of markedly raised phytanic acid concentrations has led to detailed studies of phytanic acid oxidation. These studies showed that phytanic acid undergoes oxidative decarboxylation, with pristanic acid (2,4,6,10-tetramethylpentadecanoic acid) and CO₂ as the end products, and that patients with Refsum disease cannot convert phytanic acid to pristanic acid and CO₂. Despite intense efforts, the mechanism of phytanic acid α oxidation remained unclear until very recently. However, work from our laboratory and others has now established the sequence of events (Fig. 1A), and most of the enzymes involved have been characterized, purified, and studied at the molecular level (2). This led to the identification of the enzyme defect in Refsum disease—at the level of phytanoyl-CoA hydroxylase (3)—and later to the resolution of the molecular basis of Refsum disease (4). Interestingly, phytanic acid has been found to be a powerful ligand for some nuclear receptors, including PPARα (5–8), as discussed below.

**Omega-Oxidation**

Ω Oxidation is a relatively minor pathway, like α oxidation. The initial step occurs in the smooth endoplasmic reticulum by ω hydroxylases belonging to the CYP4A subfamily, with a ω-hydroxy fatty acid as the product. The CYP4A family of cytochrome P450 enzymes, which are constitutively expressed in liver and kidney, catalyze both the ω and the (ω-1) hydroxylation of long-chain fatty acids (9). The major ω-hydroxy- lase expressed in human liver and kidney is CYP4A11, which is functionally similar to the rat CYP4A1. The ω-hydroxy fatty acids produced by the initial hydroxylation reaction then undergo two dehydrogenations to generate the corresponding ω-keto fatty acids and ω-carboxy fatty acids. These reactions are catalyzed by alcohol and aldehyde dehydrogenases in the cytosol. Subsequently, the dicarboxylic acids are activated to their CoA esters by an acyl-CoA synthetase present in the endoplasmic reticulum (10). The long-chain dicarboxylyl-CoA esters produced then appear to undergo several β oxidation cycles in the peroxisome to produce medium-chain dicarboxylyl-CoA esters that undergo final oxidation to CO₂ and H₂O in the mitochondrion (Fig. 1B).

**Beta-Oxidation**

β Oxidation is the main mechanism whereby fatty acids are broken down, and involves a sequence of four reactions including dehydrogenation, hydration, dehydrogenation again, and thiolytic cleavage (Fig. 2). Both mitochondria and peroxisomes
FIG. 1. Schematic representation of the fatty acid α oxidation system (A) and the ω oxidation system (B).
FIG. 2. The mitochondrial and peroxisomal β oxidation of fatty acids. The first step in the β oxidation of fatty acids in mitochondria is catalyzed by acyl-CoA dehydrogenases but in peroxisomes by acyl-CoA oxidases. Both acyl-CoA dehydrogenases and acyl-CoA oxidases are flavoproteins which use different mechanisms for the reoxidation of enzyme-bound FADH$_2$. In case of mitochondrial acyl-CoA dehydrogenases, enzyme-bound E-FADH$_2$ is reoxidized by electron transfer flavoprotein (ETF) followed by reoxidation of reduced ETF by ETF-dehydrogenase, so that the electrons ultimately enter the respiratory chain at the level of ubiquinone. In case of acyl-CoA oxidases, enzyme-bound FADH$_2$ is directly reoxidized by molecular oxygen to produce H$_2$O. Steps 2, 3, and 4 are catalyzed by enoyl-CoA hydratases, 3-hydroxyacyl-CoA dehydrogenases, and 3-ketoacyl-CoA thiolases, which catalyze identical reactions. RC, respiratory chain.

use the same mechanism for β oxidation of fatty acids, although the two systems differ in many respects as summarized below.

**Oxidation of Fatty Acids to CO$_2$ and H$_2$O in Mitochondria Versus Peroxisomes**

Peroxisomes lack a citric acid cycle and thus cannot degrade the acetyl-CoA units produced during fatty acid oxidation to CO$_2$ and H$_2$O. Available evidence indicates that the acetyl-CoA units produced in peroxisomes are transferred to mitochondria in the form of acetylcarnitine (11,12).
Peroxisomal β Oxidation Versus Mitochondrial β Oxidation

In mitochondria the first step in the β oxidation of fatty acids is catalyzed by various acyl-CoA dehydrogenases, all FAD-linked, which donate their electrons to the respiratory chain, thus generating adenosine triphosphate (ATP). In peroxisomes, however, the acyl-CoA oxidases—which are also FAD linked—donate their electrons directly to molecular oxygen to produce H₂O₂ which is subsequently decomposed to H₂O and O₂. Consequently, one cycle of β oxidation in peroxisomes is at most half as efficient as β oxidation in mitochondria in terms of ATP production.

Role of Carnitine in Peroxisomal and Mitochondrial β Oxidation

Carnitine plays an indispensable role in both mitochondrial and peroxisomal β oxidation, but at different levels. In the mitochondria, carnitine is involved in the transfer of long-chain fatty acids across the mitochondrial inner membrane by the concerted action of carnitine palmitoyltransferase 1 (CPT1), mitochondrial carnitine/acylcarnitine translocase (CACT), and carnitine palmitoyltransferase 2 (CPT2) (13). In peroxisomes, however, carnitine plays no role in fatty acid uptake but does play an indispensable role in the transfer of chain-shortened fatty acids from peroxisomes to mitochondria (12) (Fig. 3).

Regulation of Mitochondrial and Peroxisomal β Oxidation

Mitochondrial but not peroxisomal β oxidation is under rapid short-term control by malonyl-CoA, the key regulator of mitochondrial fatty acid β oxidation at the level of CPT1. On the other hand, despite this difference in short-term control, the long-term control of both systems shares some features, with major effects on both systems exerted by PPARα ligands.

Genetic Basis of Enzymes Involved in Fatty Acid β Oxidation in Peroxisomes and Mitochondria

Although the reactions involved in peroxisomal and mitochondrial β oxidation are identical, these reactions are catalyzed by different enzymes that are in general encoded by distinct genes—with a few exceptions where a single gene codes for a protein directed to both peroxisomes and mitochondria. Examples are carnitine acetyltransferase, Δ3,5,Δ2,4-dienoyl-CoA reductase, and 2-methylacyl-CoA racemase (2).

Substrate Specificities of the Mitochondrial and Peroxisomal β Oxidation Systems

From a physiologic viewpoint, the most important difference between the mitochondrial and the peroxisomal β oxidation systems is that the two systems have different substrate specificities. Realization that the peroxisomal system handles a distinct set of
FIG. 3. Illustration of the different roles of carnitine in mitochondrial (A) and peroxisomal (B) β oxidation. See text for details. CAT, carnitine acetyltransferase; COT, carnitine octanoyltransferase; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane.
substrates has largely come from studies on a rare genetic disease—the so-called cerebrohepatorenal syndrome of Zellweger or Zellweger syndrome. In patients with Zellweger, peroxisome biogenesis is defective owing to a genetic defect in one of the many genes involved in peroxisome biogenesis, leading to the complete absence of peroxisomes. Careful studies in plasma and cells from these patients have shown that peroxisomes play an indispensable role in the β oxidation of the following fatty acids (Fig. 4):

- Very long-chain fatty acids, notably hexacosanoic acid (C26:0);
- Certain 2-methyl branched chain fatty acids including pristanic acid [2,4, 6,10-tetramethylpentadecanoic acid], which is in part derived from dietary sources and in part from α oxidation of phytanic;
- Di- and trihydroxycholestanolic acids, as produced in the liver from cholesterol: After activation to their CoA esters, these undergo β oxidation in the peroxisome to produce chenodeoxycholoyl-CoA and choloyl-CoA, respectively, which are

![Diagram of fatty acid oxidation]

**FIG. 4.** Simplified scheme depicting the different roles of mitochondria and peroxisomes in cellular fatty acid β oxidation.
then converted into the corresponding tauro- and/or glycoconjugates with
tauro/glycochenodeoxycholate, and tauro/glycocholate as end products;

- Peroxisomes play a major role in the β oxidation of a series of mono- and polyunsaturated fatty acids; recent studies have shown that the conversion of C24:6 into C22:6, the last step in the synthesis of docosahexaenoic acid (DHA, C22:6) from linolenic acid, is mediated by the peroxisomal β oxidation system and not by the mitochondrial system (Fig. 4).

In addition to the substrate listed above, peroxisomes also catalyze the β oxidation of a range of additional fatty acids and fatty acid derivatives (14).

FATTY ACID TRANSPORT

Before fatty acids can undergo β oxidation in the mitochondrion or peroxisome, transport across the plasma membrane must occur. Although it was long believed that this was by simple diffusion, increasing evidence favors the involvement of a carrier-mediated process, although the precise mechanism is still to be worked out. Three different proteins seem to be involved in transmembrane translocation of long-chain fatty acids (LCFA): FARPpm, FAT/CD36, and FATP. All three proteins probably play a role in LCFA transport, with FAT/CD36 as the best candidate for the actual transmembrane translocation (15). Following transport, fatty acids and fatty acyl-CoA esters are further carried through the cytosol to their actual site of action or metabolism by the proteins FABP and ACBP, respectively.

Although the mechanism of transport of fatty acids across the mitochondrial membrane has been resolved in detail (Fig. 3A), much remains to be learned about the transport across the peroxisomal membrane, although there is increasing evidence to suggest the involvement of members of the family of ATP binding cassette (ABC) proteins. Four of these proteins have been identified in peroxisomal membranes. These are ALDP, ALDRP, PMP70, and PMP69 (2).

ENZYMEOLOGY OF THE MITOCHONDRIAL AND PEROXISOMAL β OXIDATION SYSTEMS

Mitochondrial β Oxidation Enzymes

Studies over the years have clearly shown that the four reactions of the β oxidation spiral are not catalyzed by single enzymes covering the whole spectrum of different substrates. Instead, each reaction is catalyzed by multiple enzymes, each having a certain chain length specificity (16). Full oxidation of a LCFA such as palmitate to its 8 acetyl-CoA units requires the active participation of the following:

- Three acyl-CoA dehydrogenases [very long-chain (VLCAD), medium-chain (MCAD), and short-chain (SCAD) acyl-CoA dehydrogenase];
- Two enoyl-CoA hydratases [short-chain (SCEH) and long-chain (LCEH) enoyl-CoA hydratase]; it should be noted that LCEH is not a monofunctional enzyme
but part of the mitochondrial trifunctional protein (MTP), an octamer of $4\alpha$ and $4\beta$ subunits with long-chain enoyl-CoA hydratase (LCEH), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), and long-chain 3-ketothiolase (LCKT) activity;

- Two 3-hydroxyacyl-CoA dehydrogenases, including short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) and long-chain 3-hydroxyacyl-CoA dehydrogenase, again part of MTP;
- Two 3-kethothiolases, including medium-chain 3-ketothiolase (MCKT) and long-chain thiolase, again part of MTP.

The fact that the mitochondrial enzymes involved in long-chain fatty acid β oxidation (VLCAD and MTP) are membrane-bound, whereas the other enzymes are soluble enzymes localized in the mitochondrial matrix, suggests a different spatial organization, as shown schematically in Fig. 5.

**FIG. 5.** Intramitochondrial organization of the mitochondrial fatty acid β oxidation machinery. The fact that carnitine palmitoyltransferase 2, VLCAD, and MTP are all membrane-bound enzymes, in contrast to the other enzymes involved in the complete oxidation of long-chain fatty acids, suggests that the β oxidation of long-chain and medium-/short-chain acyl-CoAs occurs in different submitochondrial compartments.
Peroxisomal β Oxidation Enzymes

Like the mitochondria, the peroxisomes contain multiple enzyme proteins to catalyze the oxidative chain shortening of the various acyl-CoA esters, including two acyl-CoA oxidases, two so-called bifunctional proteins with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, and two 3-ketothiolases (14).

Acyl-CoA Oxidases

Human peroxisomes contain two acyl-CoA oxidases with different substrate specificities. The first oxidase (ACOX1) is the human equivalent of the clofibrate inducible rat enzyme identified by Hashimoto and coworkers, and handles straight chain acyl-CoAs. The enzyme is not reactive with 2-methyl branch chain acyl-CoAs, like pristanoyl-CoA and trihydroxycholestanoyl-CoA, which are handled by the second oxidase (ACOX2), also known as branched chain acyl-CoA oxidase.

Bifunctional Proteins

Human peroxisomes contain two bifunctional proteins. Both enzymes catalyze the conversion of enoyl-CoA esters into the respective 3-keto esters, but they do this in different ways through an L-3-hydroxy and a D-3-hydroxy intermediate, respectively. Different names have been given to these proteins, including L-BP/D-BP, L-PBE/D-PBE, MFEI/MFEII, and MFP1/MFP2. Studies in humans (17–19) and in mutant

---

FIG. 6. Enzymology of the peroxisomal fatty acid β oxidation system. See text.
mice (20) have shown that D-BP is the enzyme involved in the oxidation of C26:0, pristanic acid, and di- and trihydroxycholestanolic acids. The function of L-BP remains to be established. Clofibrate feeding in rats leads to a large induction of L-BP but not of D-BP.

3-Ketothiolases

Human peroxisomes contain two thiolases, one of which is the human counterpart of the clofibrate inducible thiolase identified by Hashimoto (21). The second thiolase is the 58-kDa sterol carrier protein/3-ketothiolase identified by Seedorf et al. (22), which goes by the name SCPx or alternatively pTH2 (2). SCPx/pTH2 plays an indispensable role in the β oxidation of 2-methyl branched chain fatty acids such as pristanic acid and di- and trihydroxycholestanolic acids, as revealed by several studies, including ones carried out in SCPx (−/−) mice (23) (Fig. 6).

INHERITED DISORDERS OF MITOCHONDRIAL AND PEROXISOMAL OXIDATION

As described above, much has been learned about inherited disorders of mitochondrial and peroxisomal β oxidation, and defects in almost all the enzymes and transporters involved have been identified recently. The introduction of tandem mass spectrometry for the analysis of plasma acylcarnitine species has greatly stimulated progress in the field of mitochondrial fatty acid oxidation disorders, while detailed studies on patients with Zellweger syndrome have paved the way for the identification of peroxisomal fatty acid oxidation disorders.

Mitochondrial Fatty Acid Oxidation Disorders

Table 1 lists the various mitochondrial fatty acid oxidation defects. The clinical manifestations of these defects are diverse and depend on the nature of the enzyme block. The main organs directly affected by a defect in mitochondrial fatty acid oxidation are the liver (fatty change, microvesicular steatosis, liver failure), the heart (acute heart block, progressive cardiomyopathy, arrhythmia, tachycardia), skeletal muscle (rhabdomyolysis), brain (energy deficit), and the kidneys (renal failure, renal tubular acidosis). Depending on the primary organ involved, the clinical manifestations of the fatty acid oxidation defects may range from a pure hepatic or cardiac presentation to one dominated by skeletal or kidney involvement. In many cases, these clinical manifestations occur in different combinations and these may vary with the age of the affected individual. In general, patients affected by a defect in the oxidation of long-chain fatty acids—such as in mitochondrial carnitine/acylcarnitine translocase (CACT) deficiency, carnitine palmitoyltransferase 2 deficiency, VLCAD deficiency, and LCHAD/MTP deficiency—have a bad prognosis, usually with early death from cardiac abnormalities, especially in cases of severe enzyme deficiency.
TABLE 1. Disorders of mitochondrial and peroxisomal fatty acid oxidation

<table>
<thead>
<tr>
<th>Mitochondrial FAO disorder</th>
<th>Gene involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Plasma membrane Na⁺/carnitine transport defect</td>
<td>OCTN2</td>
</tr>
<tr>
<td>2. Carnitine palmitoyl transferase 1 deficiency (CPT1)</td>
<td>M-CPT1</td>
</tr>
<tr>
<td>3. Mitochondrial carnitine/acylcarnitine translocase (CACT)</td>
<td>CACT</td>
</tr>
<tr>
<td>4. Carnitine palmitoyl transferase 2 deficiency (CPT2)</td>
<td>CPT2</td>
</tr>
<tr>
<td>5. Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency</td>
<td>VLCAD</td>
</tr>
<tr>
<td>6. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency</td>
<td>MCAD</td>
</tr>
<tr>
<td>7. Short-chain acyl-CoA dehydrogenase (SCAD) deficiency</td>
<td>SCAD</td>
</tr>
<tr>
<td>8. Medium-chain 3-ketothyroxyacyl-CoA dehydrogenase (LCHAD)</td>
<td>MTP</td>
</tr>
<tr>
<td>9. Mitochondrial trifunctional protein (MTP) deficiency</td>
<td>MTP</td>
</tr>
<tr>
<td>10. X-linked adrenoleukodystrophy</td>
<td>ABCD1</td>
</tr>
<tr>
<td>2. Acyl-CoA oxidase 1 (ACOX1) deficiency</td>
<td>ACOX1</td>
</tr>
<tr>
<td>3. D-Bifunctional protein (D-BP) deficiency</td>
<td>D-BP</td>
</tr>
<tr>
<td>4. Peroxisomal 3-ketothyroxyase (pTH1) deficiency</td>
<td>pTH1?</td>
</tr>
<tr>
<td>5. 2-Methylacyl-CoA racemase (AMACR) deficiency</td>
<td>AMACR</td>
</tr>
</tbody>
</table>

* Molecular defect as yet unresolved.

**Hepatic Presentation**

This presentation, consisting of acute attacks of life-threatening coma associated with impaired hepatic ketone body production, is most commonly observed in infants and young children. The first attacks may occur in the newborn period as a result of prolonged fasting associated with unsuccessful attempts to breastfeed. Usually, however, attacks occur later in life at around 3 to 6 months of age, when the frequency of feeding declines. In the older infant or child, intercurrent infections or missed morning meals are events that typically provoke hypoketotic hypoglycemic attacks. An example of a fatty acid oxidation disorder often associated with the hepatic presentation is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. A large retrospective analysis of 120 MCAD patients by Lafolla et al. (24) showed a median age of presentation of 12 months, ranging from the newborn period (2 days) to 6.5 years of age. Importantly, 20% of the patients died during their first attack.

**Cardiac Presentation**

Cardiac involvement in fatty acid oxidation disorders may be associated with either dilated or hypertrophic cardiomyopathy. The cardiomyopathy may resemble endocardial fibroelastosis on echocardiography. In some fatty acid oxidation disorders such as primary carnitine deficiency, cardiac involvement may be acute, with overt heart block early in life. This is especially true for those fatty acid oxidation disorders in which the oxidation of LCFAs is impaired.

**Skeletal Muscle Presentation**

In early infancy and childhood, muscle weakness may be an isolated feature of some fatty acid oxidation defects, but it may also accompany acute attacks of the hepatic
presentation. Muscle weakness is rarely seen in MCAD deficiency, but is common in defects that interfere with the oxidation of LCFAs. An example of pure skeletal muscle involvement is the mild form of CPT2 deficiency first described by DiMauro and DiMauro (25), which presents later in life with exercise-induced myalgia, rhabdomyolysis, myoglobinuria (sometimes leading to renal shut down), and greatly increased creatine kinase concentrations in plasma.

Treatment of Mitochondrial Fatty Acid Oxidation Disorders

Avoidance of Fasting

The most important therapeutic measure in fatty acid oxidation disorders is avoidance of prolonged fasting, especially during an intercurrent illness. Although the need for a late evening meal has not been established in all these disorders, it is recommended for the long-chain defects by most clinicians. This guarantees suppression of lipolysis by ensuring sufficient carbohydrate absorption with high insulin levels. In the case of an infectious disease, which in children is generally accompanied by anorexia and vomiting, the utmost care must be taken to supply the desired amount of carbohydrates by gavage feeding or intravenous glucose. During a severe metabolic derangement with hypoketotic hypoglycemia, intravenous glucose should be given at a rate of at least 7 mg/kg body weight per minute, with careful monitoring of plasma glucose concentrations. A high rate of glucose intake not only normalizes glycemia but also efficiently suppresses lipolysis, thus diminishing the production of toxic long-chain acylcarnitines in the case of a long-chain fatty acid oxidation defect and, probably, the production of other toxic metabolites such as octanoate in the case of medium- or short-chain defects.

Dietary Treatment

The role of a low-fat/high-carbohydrate diet in the management of fatty acid oxidation disorders is not well established. In long-chain disorders, treatment with medium-chain triglycerides (MCT) and a diet containing very small amounts of long-chain triglycerides (LCT) is generally considered beneficial. MCT supplementation should be avoided in patients with medium- or short-chain disorders. For a more prolonged glycemic effect, the use of uncooked cornstarch in the late evening meal is recommended by some investigators. In patients treated with a diet low in LCT, one has to be aware that this may easily result in a deficiency of essential fatty acids, making supplementation necessary.

L-Carnitine Supplementation

Primary carnitine deficiency will benefit greatly from oral carnitine supplementation. In most fatty acid oxidation disorders, low plasma levels of free carnitine are observed, owing to the accumulation of acylcarnitines. In medium- and short-chain disorders, total carnitine levels can be very low as a result of urinary losses. L-Carnitine supplementation (50–100 mg/kg/d) is considered beneficial in patients with a short- or
medium-chain defect to replenish these urinary losses. In addition, L-carnitine may work as a detoxifying agent by binding toxic intermediates such as octanoate, facilitating urinary excretion. During an acute metabolic derangement, intravenous L-carnitine can be given in doses exceeding 100 mg/kg/d.

It is not clear whether L-carnitine supplementation is justified in patients with a long-chain disorder. In these disorders, it may even be harmful by increasing the levels of toxic long-chain acylcarnitines. However, in some patients with a long-chain defect (VLCAD/LCHAD), L-carnitine supplementation in combination with dietary treatment is thought to ameliorate the cardiac abnormalities.

**Riboflavin**

While a trial of riboflavin should be started in all patients with a multiple acyl-CoA dehydrogenase defect, its effects in the treatment of true fatty acid β oxidation disorders are disputable. In only a few case reports on MCAD deficiency has a beneficial effect of riboflavin been suggested.

**Peroxisomal Fatty Acid Oxidation Disorders**

To date, five defined disorders of peroxisomal fatty acid β oxidation have been identified: X linked adrenoleukodystrophy (XALD); acyl-CoA oxidase 1 (ACOX1) deficiency; D-bifunctional protein (D-BP) deficiency; peroxisomal thiolase (pTH1) deficiency; and 2-methylacyl-CoA racemase (AMACR) deficiency.

**X-linked Adrenoleukodystrophy**

X-linked adrenoleukodystrophy (XALD) is a devastating disease showing marked clinical variability even within families. At least six phenotypic variants can be distinguished (26). The classification of the different phenotypes is somewhat arbitrary and is based on the age of onset and the organs principally involved. The two most frequent phenotypes, accounting for approximately 80% of all cases, are childhood cerebral adrenoleukodystrophy (CCALD) and adrenomyeloneuropathy (AMN). CCALD is characterized by rapidly progressive cerebral demyelination. The age of onset ranges from 3 to 10 years. Frequent early neurologic symptoms are behavioral disturbances, a decline in school performance, deterioration of vision, and impaired auditory discrimination. The course is relentlessly progressive, and seizures, spastic paraplegia, and dementia develop within months. Most patients die within 2 to 3 years after the onset of neurologic symptoms.

Adrenomyeloneuropathy (AMN) presents later in life, with neurologic symptoms usually starting in the third or fourth decade. Neurologic deficits are primarily caused by myelopathy, and to a lesser extent by neuropathy. Patients gradually develop a spastic paraparesis, often in combination with a disturbed vibration sense in the legs and sphincter dysfunction.

The biochemical hallmark of all forms of XALD is the accumulation of VLCFAs, notably C26:0, in plasma owing to a defect in the peroxisomal β oxidation of
VLCFAs. The defective gene was discovered in 1993 (27) and codes for a peroxisomal membrane protein which belongs to the ATP binding cassette (ABC) family of transporters. The protein involved, called ALDP, is a half ABC transporter and forms either homo- or heterodimers. Although this has not been resolved definitively, ALDP is supposed to catalyze the transport of VLCFAs across the peroxisomal membrane (2).

**Acyl-CoA Oxidase 1 Deficiency**

Acyl-CoA oxidase 1 (ACOX1) deficiency has been reported in a few patients only. All patients reported showed neurologic abnormalities including early onset seizures, hypotonia, hearing impairment, and visual failure resulting from retinopathy. Most patients die early in life (28).

**D-Bifunctional Protein Deficiency**

D-Bifunctional protein (D-BP) deficiency is the second most common disorder of peroxisomal β oxidation after XALD. The clinical presentation of D-BP patients is usually severe and resembles Zellweger syndrome in many respects, with hypotonia, craniofacial dysmorphism, neonatal seizures, hepatomegaly, developmental delay, and usually early death. A particular feature is that patients with D-BP deficiency often show disordered neuronal migration. The central role of D-BP in the peroxisomal β oxidation of both straight chain and 2-methyl branched chain fatty acids explains why there is accumulation of VLCFAs, pristanic acid, and di- and trihydroxysterolanoic acids in most but not all patients (17–19,28).

**Peroxisomal Thiolase 1 Deficiency**

Peroxisomal thiolase 1 (pTH1) deficiency has so far been described only in a single patient, with a Zellweger-like phenotype (29).

**2-Methylacyl-CoA Racemase Deficiency**

2-Methylacyl-CoA racemase (AMACR) deficiency is a newly identified disorder of peroxisomal β oxidation in which only the peroxisomal oxidation of the 2-methyl branched chain fatty acids pristanic acid and di- and trihydroxysterolanoic acids is impaired. Following the description of three patients in our first report (30), we have identified a few additional patients, all of whom show a late onset neuropathy.

**Treatment of Peroxisomal Oxidation Disorders**

In the past few years, little progress has been made in the treatment of peroxisomal fatty acid oxidation disorders. Initially it was hoped that dietary treatment based on administration of Lorenzo’s oil—made from trioleate and trierucate—would be
beneficial for XALD patients. Unfortunately, long-term studies have shown no beneficial effect despite the fact that plasma VLCFA levels are reduced on this treatment. Bone marrow transplantation remains the only option for XALD patients. There have been no therapeutic trials in the other peroxisomal fatty acid oxidation disorders.

PPARs and Fatty Acid Oxidation Disorders

So far, studies in inherited diseases of mitochondrial and peroxisomal fatty acid oxidation have largely concentrated on the identification of patients and the development of methods for detecting the different syndromes. A great deal of work has been done to determine the molecular basis of the defects after cloning the genes that code for the various enzymes and transporters. Much remains to be learned, however, about the consequences of these enzyme defects and the pathophysiologic mechanisms involved. This is especially true for the peroxisomal fatty acid oxidation disorders, including D-bifunctional protein deficiency. Patients suffering from this enzyme defect show severe abnormalities including neonatal hypotonia, dysmorphic features, neonatal seizures, hepatomegaly, developmental delay, and early death.

A block in mitochondrial or peroxisomal fatty acid oxidation will lead to the accumulation of certain fatty acids, determined by the site of the block in the pathway. Although not studied in patients directly, there are clear indications of the involvement of PPARs under these conditions, as shown by in vitro studies and by studies in experimental animals.

PPARs and Mitochondrial β Oxidation Deficiencies

Patients suffering from a defect in the mitochondrial oxidation of long-chain fatty acids have increased plasma concentrations of a range of free fatty acids and acylcarnitines, including C18:0, C18:1, C18:2, C16:0, C16:1, C16:2, C14:0, C14:1, and C14:2 in cases of VLCAD deficiency for instance. In case of CPT1 deficiency, there is no accumulation of acylcarnitines in plasma but only of long-chain free fatty acids. Early studies by Keller et al. (31) have shown that free fatty acids, especially polyunsaturated fatty acids, are powerful activators of PPARα, with C22:6ω3, C18:3ω3, and C18:2ω6 being about equally effective. To study the role of PPARα under conditions of an impaired mitochondrial oxidation of long-chain fatty acids, Djouadi et al. (32) used etomoxir as a pharmacologic inhibitor of CPT1. Administration of etomoxir for 5 days led to a marked increase in the expression of PPARα target genes like acyl-CoA oxidase, CYP4A1, CYP4A3, and medium-chain acyl-CoA dehydrogenase in the liver. Studies in heart from the etomoxir-treated animals revealed that acyl-CoA oxidase and MCAD were also markedly induced in this tissue, which is in line with the fact that PPARα is not only expressed in liver but also in brown adipose tissue (BAT), and to a lesser extent in kidney, skeletal muscle, and heart. All the mice tolerated the perturbation induced by etomoxir. These findings indicate that the metabolic changes induced by etomoxir lead to an activation of PPARα, which subsequently causes an increase in LCFA β oxidation.
When the same experiments were repeated in PPAR(−/−) mice, there were some surprising findings. First, in mice lacking PPARα, inhibition of cellular fatty acid flux by etomoxir caused massive hepatic and cardiac lipid accumulation, hypoglycemia, and death in 100% of male but only 25% of female PPAR(−/−) mice. When male PPAR(−/−) mice were treated for 2 weeks with β-estradiol, virtually all the mice survived. The cause of death of the PPAR(−/−) mice treated with etomoxir turned out to be extreme hypoglycemia, which was not observed in PPAR(−/−) female mice or in male PPAR(−/−) mice pretreated with β-estradiol. These results clearly show the important role of PPARα under conditions where mitochondrial fatty acid oxidation is impaired.

PPARs and Peroxisomal β Oxidation Deficiencies

In recent years, several mouse models have been generated in which one of the peroxisomal β oxidation genes has been disrupted. The acyl-CoA oxidase 1 (ACOX1)-deficient mouse generated by Fan et al. (33) has growth retardation, hepatomegaly with severe microvesicular steatohepatitis, and lipogranulomas. These ACOX1(−/−) mice show age-progressive hepatocellular regeneration beginning in the periportal region and extending towards the centrilobular region of the liver lobule. Between 6 and 8 months of age, almost all steatotic hepatocytes in ACOX1(−/−) mice are replaced by regenerated hepatocytes without steatosis. Interestingly, these cells show an abundance of peroxisomes. This spontaneous peroxisome proliferation is associated with the increased expression of genes that are transcriptionally regulated by PPARα. The investigators conclude that the principal function of ACOX1 is to keep PPARα in check by β oxidizing very long-chain fatty acids and a range of other biologic ligands of PPARα. Interestingly, in mice nullizygous for both PPARα and ACOX1 (PPARα(−/−)/ACOX1(−/−)) the extensive microvesicular steatohepatitis, spontaneous peroxisome proliferation, and induction of PPARα-regulated genes, as observed in ACOX1(−/−) mice, was not present (34).

In addition to the ACOX1(−/−) mice, several other knock-out mouse models have been made including the L-bifunctional protein (L-BP)(−/−) (35), D-bifunctional protein (D-BP) (−/−) (20), and the sterol carrier protein X (SCPx/pTH2) (−/−) mouse (23). The latter mouse model was initially constructed to try to shed new light on the role of the sterol carrier protein in sterol metabolism. The SCPx/pTH2(−/−) mice, however, were completely normal and no abnormalities in sterol metabolism were found. This led to a shift in interest to the potential role of the 58-kDa SCPx in fatty acid oxidation, as SCPx contains both a thiolase and a sterol carrier protein (SCP) domain. This was especially relevant because our own work (36) and that of Antonenkov et al. (37) had shown that the thiolase domain of SCPx reacts with 3-ketoacyl-CoA esters of 2-methyl branched chain fatty acids like pristanic acid, in contrast to the other peroxisomal thiolase, pTH1. The studies done in SCPx(−/−) mice have clearly shown the importance of SCPx for peroxisomal fatty acid oxidation. When phytol was given to the (−/−) mice to put pressure on the system, the mice developed a severe phenotype characterized by
lethargy, reduced muscle tone, weight loss, ataxia, peripheral neuropathy (incoordinated movements, unsteady gait, and trembling), and death. Remarkably, inspection of the livers from control and phytol-fed SCPx(−/−) mice revealed an increased number of peroxisomes, and subsequent studies showed that various PPARα target genes were up-regulated. Because SCPx deficiency leads to a block in pristanic acid β oxidation, pristanic and phytanic acid were subsequently tested for their potential to activate PPARα. Ellinghaus et al. (7) reported that phytanic acid is indeed a ligand for PPARα. Earlier studies by Lemotte et al. (5) had already identified phytanic acid as a ligand for the retinoid X receptor (RXR). Subsequent studies by Zomer et al. (8) confirmed that phytanic acid is a ligand for both PPARα and all RXRs. Furthermore, pristanic acid was found to be a more powerful ligand of PPARα than phytanic acid (8). These findings must have important consequences for patients suffering from peroxisomal fatty acid oxidation defects, especially as pristanic and phytanic acid can reach extremely high levels in these patients.

Preliminary studies in liver biopsy specimens from D-BP-deficient patients have shown that PPARα target genes are indeed up-regulated in such patients (Ferdinandusse S, Wanders RIA, August, 2001 unpublished data).

ACKNOWLEDGMENTS

I gratefully acknowledge the help of Mrs. Maddy Festen for her expert preparation of the manuscript and Mr. Jos Ruiter for the artwork.

REFERENCES


DISCUSSION

Dr. Superti-Furga: In the mouse model of acyl-CoA oxidase deficiency, has it been shown that the hepatocytes that repopulate the liver are genetically corrected, as in tyrosinemia?

Dr. Wanders: It is believed that there is de novo synthesis of hepatocytes, and that we are not dealing with old hepatocytes going through some phase and being restored to normality.

Dr. Superti-Furga: But the hepatocytes do not have that defect, because they can make peroxisomes. They must somehow be corrected.

Dr. Wanders: No, they are genetically identical, but for some reason they are repopulating the liver. The situation is not like tyrosinemia.

Dr. Bachmann: Could you clarify the term “PPAR”? It seems misleading, as not all PPARs are important for peroxisomes.

Dr. Wanders: The nomenclature is awful, I agree. This nuclear hormone receptor was discovered through work on clofibrate and other peroxisome proliferators and it was given the name “peroxisome proliferator-activated receptor.” Later on, the β form was discovered and then the γ form. These two forms have very little to do with peroxisome proliferation, but as always, once the name has stuck you can’t get rid of it.

Dr. Kon: What is the frequency of peroxisomal disorders?

Dr. Wanders: The current estimate in our institute and Moser’s, which are the largest centers in this field, is between 1/5000 and 1/6000, of which X-linked adrenoleukodystrophy makes up about half.

Dr. Kon: Are there any reliable clinical tests to diagnose this disorder?

Dr. Wanders: I didn’t go into any detail about this, but the diagnosis of peroxisomal and mitochondrial fatty acid oxidation disorders has improved tremendously over the last few years. For peroxisomal disorders, the initial investigation is to measure the very long-chain fatty acids in the plasma. This does not cover all the disorders, but it will pick up most of them. It is an extremely reliable test if you use a suitable assay. For mitochondrial β oxidation disorders, which used to be very difficult to identify, acylcarnitine analysis has now been available for a couple of years. When we receive a sample, we first run acylcarnitines, which you can do overnight because it’s a very simple straightforward assay, and then we measure the enzyme in the lymphocytes, which we retain. Using these procedures, you can usually reach a diagnosis in 1 or 2 days.

Dr. Yamashiro: Does peroxisomal β oxidation decline with age? The reason I ask is that there has been a report that hexaenoic acid in adults with obesity or diabetes is increased in comparison with normal healthy adults.

Dr. Wanders: That’s true. From animal experiments, we know that peroxisomal β oxidation declines with age. I don’t have any data in humans.

Dr. Scott: As it appears that you have very good biochemical markers, can you tell us whether in the noninborn errors there is a wide range of what you would consider to be normal people? I suspect that in many of these conditions it will turn out that there are polymorphisms of these systems. Obviously, it would be a place to start looking, if some people have interme-
Dr. Wanders: We are not doing that, and I don’t know of any other group that is doing so at this time. In principle, you are right, and it should be studied.

Dr. Steinmann: You mentioned the great intrafamilial variability in adrenoleukodystrophy—for instance, a boy who was severely affected and had a maternal uncle with only benign adrenal insufficiency. Can you speculate on the factors that might be involved in this variability? It’s an important question because bone marrow transplantation could be planned better.

Dr. Wanders: The problem is that this variability is not only seen between families, but also within a single family, so you can have a child with ALD (adrenoleukodystrophy) presentation in the same family as another with AMN (adrenomyeloneuropathy). I know that the Baltimore group is looking for modifier genes, and there have been several reports on monozygotic twins showing widely discrepant clinical presentations. The idea now is that if you compare AMN with ALD, the basic difference is that in AMN the inflammatory reaction is missing. The crucial question is why this should be so. We don’t know at the moment, but people are looking into it.

Dr. Sies: As well as peroxisomes, there are also microperoxisomes, especially in organs where there is steroid biosynthesis. Are there any diseases of the microperoxisomes?

Dr. Wanders: Microperoxisomes are identical to peroxisomes, but smaller. Both the enzyme content and everything that takes place in peroxisomes are the same as in microperoxisomes.

Dr. Baerlocher: Can you comment on the dietary treatment of adrenoleukodystrophy?

Dr. Wanders: “Lorenzo’s oil” was supposed to be helpful, and all ALD patients were put on it. Unfortunately, the treatment was not double-blind or placebo-controlled. Now, after many years, it has been shown in Baltimore that there is no benefit from this treatment, but even so, it is still being used.

Dr. Baerlocher: I understand that the European Working Group on treatment with Lorenzo’s oil has been dissolved.

Dr. Bohles: Dr. Martinez from Barcelona showed that C22:6 was beneficial. She published a paper on this (1).

Dr. Wanders: That’s another story obviously. In fact, patients with ALD don’t have a deficit of C22:6. Dr. Martinez claims that C22:6 helps in Zellweger patients also, but I know many other people who disagree completely with this. Again, there have been no double-blind studies.

Dr. Batshaw: In Zellweger syndrome, have you any idea about the origin of the migrational defect, and how this primary defect could affect migration in early embryogenesis?

Dr. Wanders: We don’t have any good clues about that. It appears to be related to one of the β oxidation intermediates which in some way or other inhibits neuronal migration.

Dr. Superti-Furga: This is speculative, but we could be dealing with the same mechanism as in the Smith–Lemli–Opitz syndrome—that is, interference mediated through a cholesterol deficit or membrane fluidity changes. Signal transduction in the so-called “sonic hedgehog” pathway is disturbed, and that is like a master switch for morphogenesis.

Dr. Wanders: That’s a good possibility.

Dr. Batshaw: The problem with that hypothesis, however, is that sonic hedgehog is primarily involved in prosencephalic development, which occurs earlier than migration. There does not seem to be a problem with prosencephalic development in Zellweger syndrome.

REFERENCE