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Functional Aspects of Peroxisomal β-oxidation: an Overview

Abstract

This overview describes the different substrates that are β-oxidized in mammals; in which cell compartments and by which acyl-CoA synthetases these substrates are activated; how the activated substrates penetrate the peroxisome; and by which peroxisomal acyl-CoA oxidases the activated substrates are oxidized. Which substrates are oxidized by mitochondria and which ones by peroxisomes is explained. Finally, the article discusses the metabolic fate of the peroxisomal β-oxidation products and the regulation of peroxisomal β-oxidation.

I. Introduction

In lower eukaryotes such as yeasts and fungi, peroxisomes are the only subcellular compartment that can degrade fatty acids via β-oxidation [48]. Also plant peroxisomes are capable of β-oxidizing fatty acids [10, 26], but whether plant mitochondria possess a functional β-oxidation system remains controversial [17]. In animal cells fatty acids and fatty acid derivatives are β-oxidized in peroxisomes as well as mitochondria [51, 52]. This overview will be limited to a description of the functional aspects of peroxisomal (and mitochondrial) β-oxidation in mammals.

1. Substrates for β-oxidation include a wide variety of different molecules

Substrates for β-oxidation in mammals include short ($<C_6$), medium (C_6-C_{12}), long (C_{14}-C_{20}) and very long ($>C_{20}$) chain fatty acids, dicarboxylic fatty acids, isoprenoid-derived methyl-branched fatty acids, eicosanoids, the side chain of cholesterol during bile acid synthesis, the side chains of the fat-soluble vitamins and the side chains of certain xenobiotics (for references, see later). By far the most abundant β-oxidation substrate in the organism are the long chain fatty acids. It should be realized that, because of this abundance, long chain fatty acids are the only β-oxidizable substrate that constitutes an important source of metabolic fuel.

2. The β-oxidation substrates are activated to their CoA esters by several acyl-CoA synthetases located in different cell compartments

Before a fatty acid can be β-oxidized, it needs first to be activated to its CoA ester. Several acyl-CoA synthetases located in different cell compartments are involved in the activation of the β-oxidation substrates (Table 1). Short and medium chain fatty acids, which are oxidized mainly in the mitochondria (see later), can penetrate the mitochondria in unaltered form and are activated in the mitochondrial matrix [96]. Long chain fatty acids are activated by long chain acyl-CoA synthetases present in the endoplasmic reticulum, the mitochondrial outer membrane and the peroxisomal membrane [47, 60, 88]. The enzymes have the same molecular, kinetic and immunochemical properties, indicating a high degree of homology [65]. The catalytic site of the peroxisomal enzyme faces the cytosol [50, 54, 60, 90] implying that the acyl-CoA ester and not the fatty acid is transported into the peroxisome. The long chain acyl-CoA synthetases display also some activity towards medium chain fatty acids. Very long chain fatty acids are activated by very long chain acyl-CoA synthetases found in the endoplasmic reticulum and the peroxisomal membrane [54, 89]. Mitochondria lack the enzyme [55, 89]. As a consequence, isolated mitochondria, although capable of oxidizing very long chain acyl-CoAs, do not oxidize very long chain fatty acids, when incubated in the presence of the appropriate cofactors. Therefore, the absence of very long chain acyl-CoA synthetase from mitochondria appears to be the reason why in the intact cell very long chain fatty acids are oxidized predominantly in the peroxisome (see later). Singh and coworkers have provided evidence that the catalytic site of the peroxisomal very long chain acyl-CoA synthetase faces the matrix and that very long chain fatty acids are transported into the peroxisome in unesterified form [54, 90]. In contrast, Wanders and collaborators concluded that the catalytic site of the enzyme, like that of the long chain acyl-CoA synthetase, faces the cytosol [50]. Their conclusions are based on the enzyme's susceptibility to exogenous proteases, added to intact purified peroxisomes. 3-Methyl-branched fatty acids such as phytanic (3,7,11,15-tetramethylhexadecanoic) acid and 2-methyl-branched fatty acids such as pristanic (2,6,10,14-tetramethylpentadecanoic) acid are activated in the endoplasmic reticulum, peroxisomes and mitochondria, possibly by the long chain acyl-CoA synthetases located to these cell compartments [114, 124]. Preliminary evidence that the peroxisomal membrane contains a separate phytanoyl-CoA synthetase [77] requires further confirmation. Dicarboxylyl-CoA synthetase activity is found in the endoplasmic reticulum but not in peroxisomes or mitochondria [108]. Likewise, prostaglandins [84] and leukotriene E_4 [35] appear to be activated solely in the endoplasmic reticulum. Leukotriene B_4 is activated in the endoplasmic reticulum and perhaps also in the peroxisome [35]. The fact that dicarboxylic acids and eicosanoids are activated in the endoplasmic reticulum and not in peroxisomes (with the possible exception of leukotriene B_4) or mitochondria suggests that the long chain acyl-CoA synthetases are not responsible for the CoA ester formation but that separate

Table 1. Activation of β-oxidation substrates[1]

Substrate	Subcellular compartment	Enzyme[2]
Short and medium chain fatty acids	Mitochondria (matrix)	
Long chain fatty acids	Mitochondria (outer membrane)	Long chain acyl-CoA synthetase
	Peroxisomes (membrane)	Long chain acyl-CoA synthetase
	Endoplasmic reticulum (membrane)	Long chain acyl-CoA synthetase
Very long chain fatty acids	Peroxisomes (membrane)	Very long chain acyl-CoA synthetase
	Endoplasmic reticulum (membrane)	Very long chain acyl-CoA synthetase
Long chain dicarboxylic fatty acids	Endoplasmic reticulum	Dicarboxylyl-CoA synthetase
2-Methyl-branched fatty acids (e.g. pristanic acid)	Mitochondria Peroxisomes (membrane) Endoplasmic reticulum	
3-Methyl-branched fatty acids (e.g. phytanic acid)	Mitochondria Peroxisomes (membrane) Endoplasmic reticulum	Phytanoyl-CoA synthetase?
Prostaglandins	Endoplasmic reticulum	
Leukotriene E_4	Endoplasmic reticulum	
Leukotriene B_4	Endoplasmic reticulum Peroxisomes?	
Di- and trihydroxycoprostanic acids	Endoplasmic reticulum (membrane)	Trihydroxycoprostanoyl-CoA synthetase
Chenodeoxycholic and cholic acids	Endoplasmic reticulum (membrane)	Choloyl-CoA synthetase

[1] For explanation and references, see text. [2] No entry in this column means that the enzyme remains to be identified.

enzymes are involved. The bile acid intermediates di- and trihydroxycoprostanic acids (see later) are converted to their CoA esters by trihydroxycoprostanoyl-CoA synthetase, an enzyme that is located in the endoplasmic reticulum [81, 85]. The enzyme, which is present only in liver, is different from choloyl-CoA synthetase [85]. The latter enzyme is most probably involved in the conjugation of bile acids reabsorbed from the gut. The exact subcellular location of the enzymes responsible for xenobiotic acyl-CoA ester formation has not been studied in detail. Depending on the chemical structure of the molecules, several acyl-CoA synthetases may be involved.

3. The penetration of acyl-CoA esters into the peroxisome is not dependent on carnitine

Acyl-CoA esters cannot cross the mitochondrial inner membrane. They are first converted to acylcarnitines, a reaction catalyzed by carnitine palmitoyltransferase I, the catalytic site of which is localized at the inner side of the outer membrane [71]. After diffusion through the inner membrane via an acylcarnitine: carnitine exchange carrier, the acylcarnitines are re-converted to acyl-CoAs by carnitine palmitoyltransferase II present at the matrical aspect of the inner membrane [64]. The peroxisomal membrane does not contain carnitine palmitoyltransferase and acyl-CoA esters formed at the outer aspect of the peroxisomal membrane (e.g. long chain acyl-CoAs) or in the endoplasmic reticulum (e.g. the CoA esters of dicarboxylic acids, eicosanoids, bile acid intermediates) penetrate the peroxisome without need for carnitine [8, 59, 84, 104].

Isolated peroxisomes and peroxisomes in detergent-permeabilized rat hepatocytes are permeable to a whole spectrum of small hydrophilic molecules including substrates and cofactors for peroxisomal enzymes [115, 117, 120]. As a result, peroxisomal enzymes are not latent when measured in broken cell systems. An exception is catalase. The latency of catalase is not due to a restricted permeability of the peroxisomal membrane to exogenously added hydrogen peroxide but to the high concentration and activity of catalase in the peroxisome so that the diffusion of hydrogen peroxide within the peroxisome becomes limiting [5]. The non-specific permeability of isolated peroxisomes is caused by the presence in the peroxisomal membrane of proteinaceous pores [49, 57, 117]. The diameter of the pore has been estimated at 1.7 nm, which is large enough to allow for the free diffusion of substrates, products and cofactors [57]. It remains to be investigated whether the permeability of the pore is regulated in the intact cell and whether amphiphiles such as acyl-CoA esters, which tend to accumulate in the lipid phase of membranes, also diffuse through the pores. Dihydroxyacetone-phosphate acyltransferase, a peroxisomal integral membrane enzyme, the catalytic site of which is exposed to the matrix [12], appears to be latent in detergent-permeabilized skin fibroblasts [127], suggesting that at least one of its substrates, perhaps acyl-CoA, does not freely diffuse through the membrane. Hashimoto and coworkers have speculated that a 70 kDa peroxisomal

integral membrane protein, which is a member of the P-glycoprotein-related ATP-binding protein superfamily, is involved in membrane transport, possibly that of acyl-CoAs [37]. Their speculation is based on the transport function of other members of the ATP-binding protein family.

4. Several acyl-CoA oxidases are involved in the peroxisomal oxidation of the different CoA esters

Peroxisomal β-oxidation proceeds via four consecutive reactions catalyzed by three matrix enzymes. The first reaction consists of the desaturation of the acyl-CoA to a 2-enoyl-CoA. It is catalyzed by an FAD-containing acyl-CoA oxidase that reduces molecular oxygen to hydrogen peroxide, which is subsequently decomposed by catalase. The second (hydration to an L-3-hydroxyacyl-CoA) and third (NAD^+-linked dehydrogenation to a 3-ketoacyl-CoA) reactions are catalyzed by a multifunctional protein that also displays Δ^3, Δ^2 isomerase activity required for the oxidation of unsaturated fatty acids. The last reaction is catalyzed by 3-ketoacyl-CoA thiolase and releases acetyl-CoA and an acyl-CoA shortened by two carbon atoms, that can re-enter the spiral for a next round of b-oxidation.

The broad spectrum of β-oxidation substrates raises the question as to whether each step of peroxisomal β-oxidation is catalyzed by a single enzyme. It has recently become clear that this is not the case, at least as far as the first step is concerned. Rat liver peroxisomes contain three acyl-CoA oxidases (Table II) [86, 118, 119]. A first enzyme, palmitoyl-CoA oxidase, oxidizes the CoA esters of long chain fatty acids, long chain dicarboxylic fatty acids and prostaglandins. It also shows activity towards the CoA esters of very long chain fatty acids (e.g. lignoceric acid), medium chain fatty acids and medium chain dicarboxylic fatty acids (e.g. glutaric acid). Maximal activitities and substrate affinities are markedly lower with the medium chain acyl-CoAs than with the long chain acyl-CoAs [118, 123]. A second enzyme, pristanoyl-CoA oxidase, oxidizes the CoA esters of 2-methyl-branched fatty acids such as the synthetic 2-methylpalmitic acid and the naturally occurring pristanic (2,6,10,14-tetramethylpentadecanoic) acid. It is not active towards the branched short chain isobutyryl-CoA and isovaleryl-CoA, catabolites of valine and leucine, respectively. However, it also oxidizes the CoA esters of long and very long chain fatty acids and long chain dicarboxylic acids but not those of prostaglandins, medium chain fatty acids shorter than octanoic acid, and medium chain dicarboxylic fatty acids such as glutaric acid [118, 123]. The CoA esters of the bile acid intermediates di- and trihydroxycoprostanic acids are oxidized by a third acyl-CoA oxidase, trihydroxycoprostanoyl-CoA oxidase, which also shows some activity towards the CoA esters of 2-methyl-branched fatty acids [118]. Thus, the CoA esters of prostaglandins, medium chain fatty acids shorter than octanoic acid and medium chain dicarboxylic fatty acids (glutaric acid) are oxidized exclusively by palmitoyl-CoA oxidase; those of the bile acid inter-

Table II. Contribution of the different acyl-CoA oxidases to peroxisomal β-oxidation in rat liver[1]

Substrate	Palmitoyl-CoA oxidas	Pristanoyl-CoA oxidase	Trihydroxyco-prostanoyl-CoA oxidase
Glutaryl-CoA	+	−	−
Hexanoyl-CoA	+	−	−
Octanoyl-CoA	+ +	+	−
Palmitoyl-CoA	+ +	+	−
Lignoceroyl-CoA	+	+	−
Hexadecanedioyl-CoA	+ +	+	−
2-Methylpalmitoyl-CoA	−	+ +	(+)
Pristanoyl-CoA	−	+ +	(+)
Prostaglandin E_2-CoA	+	−	-
Di- and trihydroxycoprostanoyl-CoA	−	−	+
Isobutyryl-CoA	−	−	−
Isovaleryl-CoA	−	−	−

[1] For explanation and references, see text.

mediates exclusively by trihydroxycoprostanoyl-CoA oxidase. The CoA esters of the long chain 2-methyl-branched fatty acids are oxidized for approx. 90% by pristanoyl-CoA oxidase and for approx. 10% by trihydroxycoprostanoyl-CoA oxidase. The contribution of pristanoyl-CoA oxidase to the overall hepatic peroxisomal oxidation of long chain mono-and dicarboxylyl-CoAs and to the oxidation of very long chain acyl-CoAs may amount to 30% and 65%, respectively, the remaining part being catalyzed by palmitoyl-CoA oxidase [118].

Palmitoyl-CoA oxidase and pristanoyl-CoA oxidase are present not only in rat liver but also in rat extrahepatic tissues; trihydroxycoprostanoyl-CoA oxidase is found only in liver [86, 119]. Relative to palmitoyl-CoA oxidase, pristanoyl-CoA oxidase is markedly less active in extrahepatic tissues than in liver, so that in these tissues the contribution of pristanoyl-CoA oxidase to the overall peroxisomal oxidation of long and very long chain acyl-CoAs and long chain dicarboxylyl-CoAs becomes almost negligible.

Surprisingly, human liver peroxisomes contain only two acyl-CoA oxidases: palmitoyl-CoA oxidase and branched chain acyl-CoA oxidase (Table III) [113]. The substrate spectrum of the human palmitoyl-CoA oxidase is similar to that of its rat counterpart, except that the human enzyme does not seem to oxidize glutaryl-CoA. The branched chain acyl-CoA oxidase oxidizes the CoA esters of 2-methyl-branched fatty acids (e.g. pristanic and 2-methylpalmitic acids) but also those of the bile acid intermediates di- and trihydroxycoprostanic acids, which also possess a 2-methyl substitution in their side chain. Hence the name branched chain acyl-CoA oxidase. The enzyme does not oxidize isobutyryl-CoA or isovaleryl-CoA. It shows some activity towards the CoA esters of long and very long chain fatty acids and long chain dicarboxylic acids but much less than rat pristanoyl-CoA oxidase, so that its contribution to the oxidation of straight chain mono- and dicarboxylic acids is minor [113]. Human palmitoyl-CoA

Table III. Contribution of the different acyl-CoA oxidases to peroxisomal β-oxidation in human liver[1]

Substrate	Palmitoyl-CoA oxidase	Branched chain acyl-CoA oxidase
Glutaryl-CoA	–	–
Hexanoyl-CoA	+	(+)
Octanoyl-CoA	++	(+)
Palmitoyl-CoA	++	(+)
Lignoceroyl-CoA	++	(+)
Hexadecanedioyl-CoA	++	(+)
2-Methylpalmitoyl-CoA	–	++
Pristanoyl-CoA	–	++
Prostaglandin E_2-CoA	+	(+)
Di- and trihydroxycoprostanoyl-CoA	–	+
Isobutyryl-CoA	–	–
Isovaleryl-CoA	–	–

[1] For explanation and references, see text.

oxidase and branched chain acyl-CoA oxidase are also found in extrahepatic tissues. Because of the presence of the latter enzyme in extrahepatic tissues, these tissues may have the potential of converting bile acid intermediates into the primary bile acids, chenodeoxycholic and cholic acids (see later).

A separate glutaryl-CoA oxidase has been described in rat liver [110] and in human fibroblasts [6]. As long as the enzyme has not been identified and further characterized, the data must be viewed with caution. Rat palmitoyl-CoA oxidase displays weak glutaryl-CoA oxidase activity (see above) and rat liver glutaryl-CoA oxidase copurifies with palmitoyl-CoA oxidase [79, 118]. Human palmitoyl-CoA oxidase does not oxidize glutaryl-CoA [113]. However, glutaryl-CoA oxidase activity is very low in human tissues and at present it has not been excluded that the low oxidase activity is due to the mitochondrial glutaryl-CoA dehydrogenase, which like other mitochondrial acyl-CoA dehydrogenases [112], may perhaps function as an oxidase when assayed in the absence of suitable electron acceptors.

It is generally believed that the subsequent β-oxidation steps, catalyzed by the multifunctional protein and by the thiolase, are shared by all peroxisomal β-oxidation substrates and that no separate enzymes exist for the degradation of straight chain fatty acids, 2-methyl-branched fatty acids and the bile acid intermediates. Definite proof of this contention awaits the chemical synthesis of the appropriate β-oxidation intermediates, so that the enzyme activities can be measured with the different substrates.

5. Peroxisomal β-oxidation conserves less energy than mitochondrial β-oxidation and does not go to completion

The peroxisomal β-oxidation spiral is not directly coupled to an electron transfer chain that conserves energy by means of oxidative phosphorylation [52, 59]. As a result, peroxisomal β-oxidation is not inhibited by inhibitors of the respiratory chain or oxidative phosphorylation, when assayed in broken cell systems. The energy that is released in the first oxidation step catalyzed by acyl-CoA oxidase (H_2O_2 formation) is lost as heat. The energy that is released in the second oxidation step (NAD^+-linked dehydrogenation) is conserved in the form of the high energy level electrons of NADH. Since isolated peroxisomes and peroxisomes in detergent-permeabilized cells are permeable to small water-soluble molecules such as NAD^+ [115, 117, 120], the NADH formed during β-oxidation can most probably freely diffuse into the cytosol from where its electrons can be shuttled into the mitochondria. Thus, as far as energy conservation is concerned, peroxisomal β-oxidation is only approximately half as efficient as mitochondrial β-oxidation. Because of the heat generation during the first reaction of peroxisomal β-oxidation, it has been proposed that peroxisomal fatty acid oxidation is important to thermogenesis [46]. Its contribution to body heat generation appears to be minor, however.

A special feature of peroxisomal fatty acid oxidation is that it does not go to completion. Unlike mitochondria, which degrade fatty acids completely to their constituent acetyl units, peroxisomes catalyze only a limited number of β-oxidation cycles. The number of cycles catalyzed by isolated peroxisomes *in vitro* is inversely related to the fatty acyl-CoA substrate concentration so that at elevated substrate concentrations only one cycle per acyl-CoA molecule occurs [4, 51, 104]. This suggests that substrate competition may be involved. How many cycles are catalyzed in the intact cell is not clear and may also depend on the chain length of the substrate. What halts peroxisomal β-oxidation in the intact cell is not clear either. Several mechanisms can be envisaged: substrate competition, product inhibition by accumulating intermediates, competition for the shortened acyl-CoAs by carnitine octanoyltransferase [21, 66] and by acyl-CoA hydrolase [1, 7, 75], enzymes that are present in the peroxisomal matrix. In addition, as explained in the preceding section, the acyl-CoA oxidases are less active with and show a lower affinity for medium chain acyl-CoAs. The result of all this is that peroxisomal β-oxidation acts as a chain-shortening system releasing a limited number of acetyl-CoA equivalents and a shortened acyl-CoA that must be metabolized further in other cell compartments.

6. Mitochondria and peroxisomes oxidize different substrates

From a physiologically point of view, the most relevant questions concerning peroxisomal β-oxidation are perhaps the following: Which substrates are oxidized by peroxisomes and which ones by mitochondria and—for substrates

Table IV. Substrate oxidation by mitochondria and peroxisomes. The table indicates by which organelle each substrate is oxidized preferentially[1]

Mitochondria	Peroxisomes
Short chain fatty acids	Very long chain fatty acids
Medium chain fatty acids	Long chain dicarboxylic fatty acids
Long chain fatty acids	2-Methyl-branched fatty acids (e.g. pristanic acid)
	Eicosanoids (prostaglandins, leukotrienes)
	Di- and trihydroxycoprostanic acids
	Carboxyl side chains of xenobiotics

[1] For explanation and references, see text.

that can be degraded by each organelle—what is the contribution of peroxisomes and mitochondria to the overall oxidation of such a substrate? The contribution of each organelle to the oxidation of a specific substrate depends not only on the capability of each organelle to oxidize that substrate but also on kinetic parameters such as V_{max} and affinity and on the relative abundance of each organelle in the cell or tissue.

As already explained for the acyl-CoA oxidases, short chain fatty acids are poor substrates for the peroxisomal enzymes and, as a consequence, they are oxidized in the mitochondria (Table IV). In the intact cell or organism, mitochondria also oxidize the major portion of the medium and long chain fatty acids [23, 59, 95, 104]. It has been estimated that in intact rat hepatocytes incubated with palmitate or oleate, mitochondria generate more than 90% of the acetyl units produced by β-oxidation [59]. The dominant position of mitochondria in the oxidation of long chain fatty acids, which because of their abundance are a major source of metabolic energy, seems to be a logical emanation of the fact that mitochondrial β-oxidation conserves more energy than does peroxisomal β-oxidation. The dominant role of mitochondria in long chain fatty acid oxidation can be explained for an important part by the higher affinity of the mitochondrial system for long chain fatty acids [104].

In contrast, very long chain fatty acids are oxidized predominantly or exclusively by peroxisomes [34, 55, 91, 92]. They are shortened to long chain fatty acids which can then be oxidized further in the mitochondria. The reason why mitochondria are of little importance in the oxidation of very long chain fatty acids appears to be their lack of very long chain acyl-CoA synthetase, as already discussed in one of the preceding sections. Plasma concentrations of very long chain fatty acids are very low and these fatty acids are not important as a source of metabolic fuel. However, their tissue accumulation in disorders of peroxisomal β-oxidation is deleterious, particularly to brain [53, 125].

Long chain dicarboxylic acids are formed from long chain monocarboxylic acids, especially in conditions of excess fatty acid supply to the liver such as uncontrolled diabetes or in conditions in which mitochondrial fatty acid oxidation is deficient or inhibited by poisons [27, 67, 69, 121]. The terminal methyl

group of the monocarboxylic acid is first hydroxylated by lauric acid ω-hydroxylase, a member of the cytochrome P_{450} family present in the endoplasmic reticulum, and the ω-hydroxy fatty acid is then dehydrogenated in the cytosol to the dicarboxylic acid. Following activation by dicarboxylyl-CoA synthetase in the endoplasmic reticulum [108], the dicarboxylyl-CoA is degraded via β-oxidation. Isolated peroxisomes and isolated mitochondria are capable of oxidizing long chain dicarboxylic acids as well as medium chain dicarboxylic acids with a chain length greater than 8 to 10 carbon atoms [45, 68, 102, 109]. Most studies that were aimed at estimating the contribution of mitochondria and peroxisomes to dicarboxylic fatty acid oxidation in rat liver, indicate that the dicarboxylic acids are degraded preferentially in peroxisomes [9, 56, 102]. Nevertheless, one *in vivo* study in the rat suggests a major role for mitochondria [18]. In peroxisomes, long chain dicarboxylic fatty acids are shortened to more polar medium chain dicarboxylic fatty acids, which can then be excreted in the urine. Whether part of the shortened dicarboxylyl-CoAs is oxidized further in the mitochondria remains unknown. Mitochondria degrade dicarboxylyl-CoAs to succinyl-CoA, which enters the Krebs cycle and which can also be used for the synthesis of glucose in gluconeogenic tissues.

Pristanic (2,6,10,14-tetramethylpentadecanoic) acid is a multibranched isoprenoid-derived fatty acid that is degraded via β-oxidation. Because of the methyl substitutions the first, third and fifth round of β-oxidation releases propionyl-CoA instead of acetyl-CoA. Thus, complete β-oxidation of pristanic acid results in the formation of 3 equivalents of propionyl-CoA and of acetyl-CoA, and 1 equivalent of isobutyryl-CoA (Fig. 1). The latter can be metabolized by mitochondria. Pristanic acid originates from phytanic (3,7,11,15-tetramethylhexadecanoic) acid, which, in turn, is a metabolite of phytol, a multibranched fatty alcohol present in esterified form in chlorophyll [99]. Ruminants ingest large amounts of chlorophyll. The bacteria of the rumen liberate phytol, which is then absorbed and converted to phytanic acid in the animal's body. Most probably, humans do not synthesize phytanic acid but ingest it with the diet (dairy products, ruminant fat) [99]. Because of its 3-methyl substitution phytanic acid cannot undergo β-oxidation, but it is first oxidatively decarboxylated—a process called α-oxidation—to pristanic acid [3, 105], which is then further degraded via β-oxidation (Fig. 1). Phytanic acid accumulates in peroxisome deficiency disorders [53, 103, 125], suggesting that peroxisomes may be responsible for α-oxidation. However, subcellular fractionation studies have produced widely conflicting results. Some authors located α-oxidation to the mitochondria [94, 105, 126], mitochondria plus cytosol [70], or the endoplasmic reticulum [32]. A recent publication claims that α-oxidation is mitochondrial in the rat and peroxisomal in the human [93]. A major problem is that α-oxidation, which may consist of several reactions, remains poorly characterized as far as cofactor requirements and identification of putative intermediates are concerned. Rates of α-oxidation obtained in homogenates or subcellular fractions are generally one or even two orders of magnitude lower than the rates obtainable in intact cells [32]. As long as α-oxidation has not been

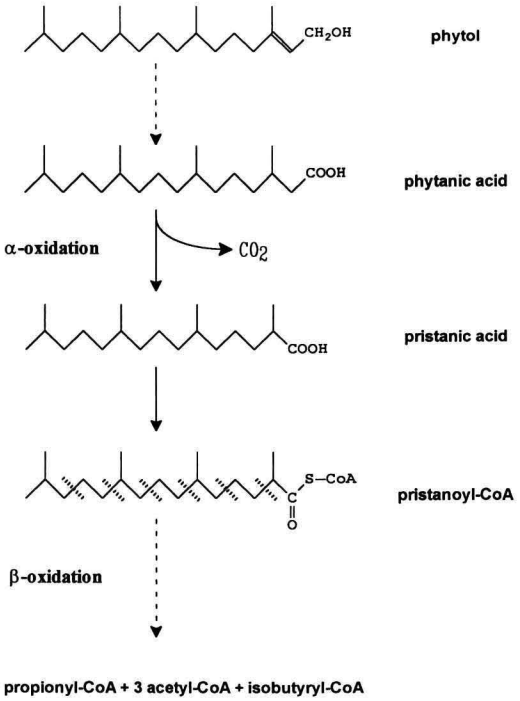

Fig. 1. Degradation of phytanic and pristanic acid.

better characterized, definite proof of its subcellular localization will be difficult to provide.

Pristanic acid, the product of phytanic acid α-oxidation, also accumulates in tissues and body fluids of patients with peroxisome deficiency disorders [53, 103, 125] and it is virtually not oxidized in fibroblasts from such patients [116]. Also experiments on rat liver have confirmed that pristanic acid and other 2-methyl-branched fatty acids such as the synthetic 2-methylpalmitate are oxidized predominantly by peroxisomes [114]. Whether pristanic acid is degraded completely in peroxisomes or only shortened remains unknown. The fact that 2-methylhexanoyl-CoA is oxidized by the human branched chain acyl-CoA oxidase [113] suggests that pristanic acid degradation may go to completion in peroxisomes.

Other molecules with a 2-methyl-branched side chain are the fat-soluble vitamins E and K. They are excreted in the urine partly as metabolites with a carboxyl side chain shortened by an even number of carbon atoms [24], indicating that the side chains undergo β-oxidation after prior ω-oxidation of one of the terminal methyl groups. Retinoic acid possesses a 3-methyl-branched side chain. It is excreted in the urine partly as metabolites with a side chain shortened by an odd number of carbon atoms [24], indicating that in this case β-oxidation follows α-oxidation. The predominant role of peroxisomes in the

oxidation of branched chain fatty acids suggests that the organelles may also be responsible for the β-oxidation of the side chains of these fat-soluble vitamins.

Eicosanoids such as prostaglandins [29], tromboxane [82], prostacyclin [100] and leukotrienes [83] are excreted as urinary dinor- and tetranor metabolites indicating that the (side) chains undergo one or two rounds of β-oxidation. Experiments on rat liver [14, 15, 35, 84], and measurements of urinary metabolite patterns in patients with peroxisome deficiency disorders [16, 63] have established that prostaglandins E_2 and F_{2a} and leukotrienes B_4 and E_4 are oxidized mainly by peroxisomes. These results suggest that the other eicosanoids are also β-oxidized by peroxisomes.

The liver is the sole organ that can degrade cholesterol. In a first series of reactions the steroid nucleus is altered (reduction of the double bond, hydroxylations) and one of the terminal methyl groups of the side chain is oxidized leading to the formation of the C_{27} bile acid intermediates di- and trihydroxycoprostanic acids [78] (Fig. 2). The enzymes involved are present in the endoplasmic reticulum (alterations of the nucleus) and in the mitochondria and cytosol (oxidation of the terminal methyl group). Di- and trihydroxycoprostanic acid are then activated to their CoA esters by trihydroxycoprostanoyl-CoA synthetase [81, 85], present in the endoplasmic reticulum (see one of the preceding sections). Subsequently, the CoA esters of di- and trihydroxycoprostanic acid undergo one cycle of β-oxidation giving rise to propionyl-CoA and chenodeoxycholoyl-CoA and choloyl-CoA, respectively. The CoA esters of the bile acids are then conjugated with taurine or glycine, a reaction that takes place in peroxisomes and the endoplasmic reticulum [38, 41]. The bile acid intermediates di- and trihydroxycoprostanic acids are oxidized exclusively by peroxisomes [39, 40, 80] and they are no substrate for mitochondria. In the rat, trihydroxycoprostanoyl-CoA synthetase and trihydroxycoprostanoyl-CoA oxidase are found only in liver. As mentioned above, in the human, the branched chain acyl-CoA oxidase which oxidizes the CoA esters of branched fatty acids and of the bile acid intermediates, is present in liver but also in extrahepatic tissues. Whether trihydroxycoprostanoyl-CoA synthetase is present in human extrahepatic tissues has not been investigated. If the latter enzyme is also expressed in these tissues, human extrahepatic tissues would have the potential of converting the C_{27} bile acid intermediates into primary bile acids. That this may indeed be the case is suggested by a recent publication, describing the formation of cholic acid from trihydroxycoprostanic acid in human fibroblasts [107].

Xenobiotics with aliphatic side chains are often excreted as metabolites possessing a carboxyl side chain shortened by an even number of carbon atoms. This demonstrates that these metabolites are formed via ω-oxidation of the terminal methyl group of the side chain followed by β-oxidation. Up to now, the degradation of only a few xenobiotics possessing a carboxyl side chain has been studied. In every instance, the compounds were poor substrates for mitochondria but they were reasonably well degraded by peroxisomes [25, 101, 128–130], suggesting that peroxisomes may play an important role in the side chain degradation of xenobiotics.

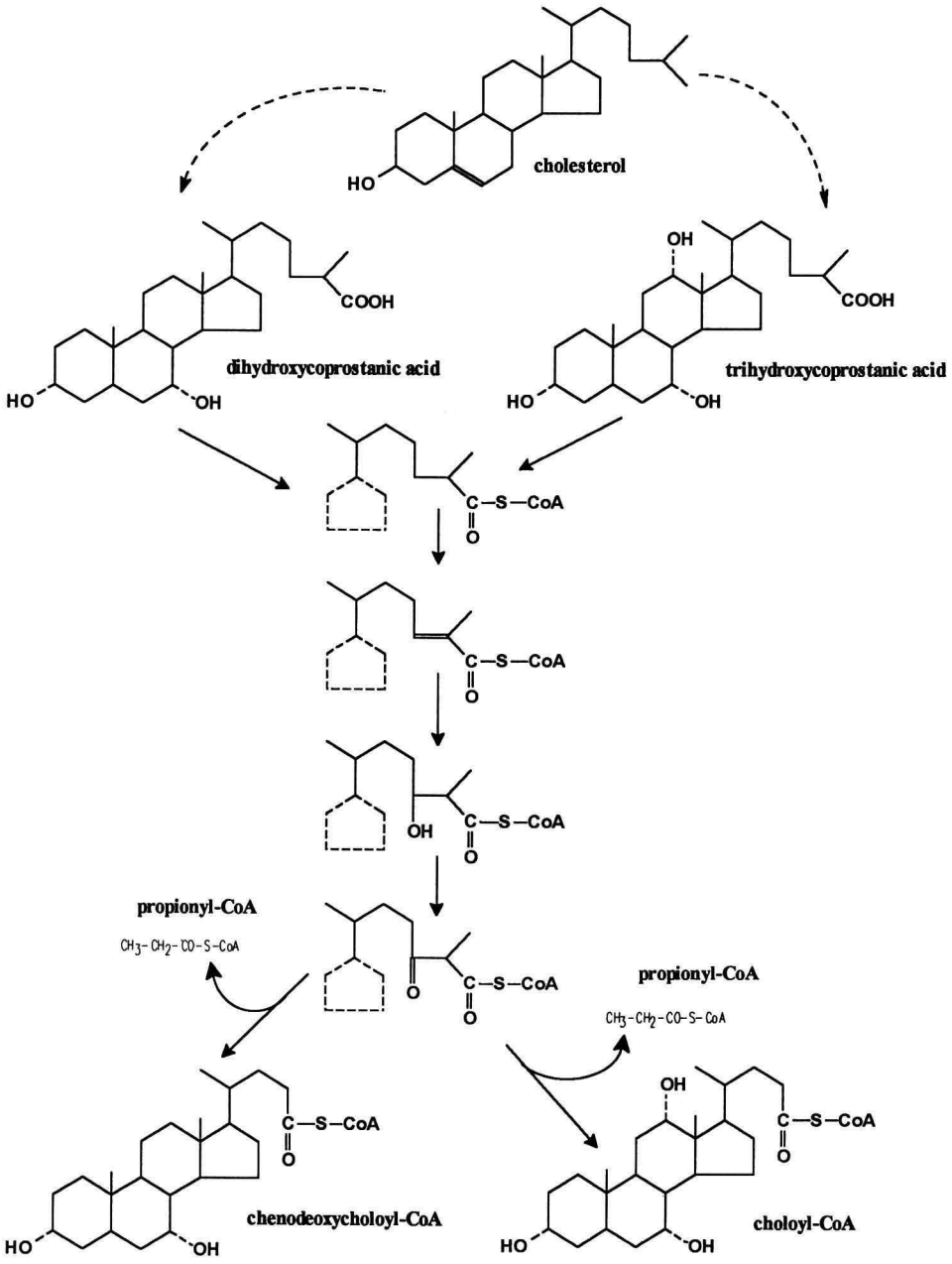

Fig. 2. Degradation of cholesterol to bile acids.

Finally, peroxisomal β-oxidation appears to be involved in the synthesis of polyunsaturated fatty acids considered to be Δ4-desaturation products such as 4,7,10,13,16,19-docosahexaenoic acid. The contents of this fatty acid are seriously decreased in tissues of patients with peroxisome deficiency disorders [62]. There is now evidence that the Δ4-unsaturated bound is not introduced by a Δ4-desaturase. Instead, 7,10,13,16,19-docosapentaenoic acid is elongated, possibly in the endoplasmic reticulum or in peroxisomes, to 9,12,15,18,21-tetracosapentaenoic acid followed by desaturation at position six to 6,9,12,15,18,21-tetracosahexaenoic acid and retroconversion by peroxisomal β-oxidation to 4,7,10,13,16,19-docosahexaenoic acid [122].

From the data summarized in this section, one may conclude that the main function of mitochondrial β-oxidation lies in the generation of energy via degradation of long chain fatty acids, whereas peroxisomes are engaged in the degradation of a wide variety of lipophilic carboxylates that may serve important biological functions but that are unimportant as far as energy provision is concerned.

7. The metabolic fate of the products of peroxisomal β-oxidation is not always clear

Acetyl-CoA generated by mitochondrial β-oxidation enters the Krebs cycle for further oxidation to CO_2 and H_2O. In addition, in ketogenic tissues such as liver, acetyl-CoA can condense to form ketone bodies (acetoacetate and 3-hydroxybutyrate), especially in conditions in which rates of mitochondrial fatty acid oxidation are high (e.g. starvation, uncontrolled diabetes). The ketone bodies diffuse out of the mitochondria and the hepatocytes and serve as oxidizable substrate for extrahepatic tissues such as muscle, heart and brain [64].

Peroxisomes lack the ketogenic and Krebs cycle enzymes [59]. During straight chain fatty acid oxidation they generate acetyl-CoA, acetate and acetoacetyl-CoA [31, 104]. The latter two metabolites are produced by acetyl-CoA hydrolase [1, 7, 75] and by the thiolase, respectively, enzymes that are present in the peroxisomal matrix. When incubated in the presence of fatty acyl-CoAs and carnitine, peroxisomes also form acetylcarnitine [104]. The formation of acetylcarnitine is catalyzed by carnitine acetyltransferase, an enzyme that is not only found in the mitochondrial inner membrane but also in the peroxisomal matrix [21, 61]. The fate of the acetyl units generated by peroxisomal β-oxidation is not always clear. Acetyl-CoA may be used for the acetyl-CoA-dependent fatty acid elongation system that has been described in rat liver peroxisomes [30] or it may diffuse to the cytosol where it can be used for cholesterol and dolichol synthesis (after condensation to acetoacetyl-CoA) or for fatty acid synthesis (after carboxylation to malonyl-CoA). Acetyl-CoA cannot cross the mitochondrial inner membrane. Before entering the mitochondria, it must first be converted to acetylcarnitine by carnitine acetyltransferase (see

above). Evidently, acetylcarnitine formed in the peroxisome can enter the mitochondria directly. After conversion to acetyl-CoA, it can join the Krebs cycle or in liver the ketogenic pathway. It remains unknown, however, to which extent acetyl units produced by peroxisomes are metabolized further in the mitochondria. In the intact liver cell, acetate appears to be the major end product of peroxisomal β-oxidation [56]. Although acetate can enter the mitochondria without prior conversion, the major portion of the peroxisomal acetate leaves the hepatocyte. Acetate can be used as oxidizable substrate by extrahepatic tissues. Acetoacetyl-CoA may enter the mevalonate pathway, which results in the formation of farnesyldiphosphate and geranyl geranyldiphosphate used for protein prenylation and in the formation of cholesterol, dolichols and the side chain of ubiquinone [28]. Interestingly, several enzymes involved in cholesterol and dolichol synthesis (e.g. 3-hydroxy-3-methylglutaryl-CoA reductase [43, 44], mevalonate kinase [98], cis-prenyltransferase [20] and the enzymes converting squalene into cholesterol [2]) are present also in the peroxisome. Whether acetoacetyl-CoA formed in the peroxisome is used preferentially over that formed in the cytosol for peroxisomal cholesterol or dolichol synthesis is not known.

The oxidation of 2-methyl-branched fatty acids and of the 2-methyl-branched side chain of di- and trihydroxycoprostanic acid yields propionyl-CoA instead of acetyl-CoA. In isolated rat hepatocytes incubated with trihydroxycoprostanic acid or 2-methyl-branched fatty acids, approximately half of the propionyl units produced is oxidized to CO_2 [8, 114], indicating that at least this portion enters the mitochondria, in which it joins the Krebs cycle after conversion to methylmalonyl-CoA and succinyl-CoA. Propionyl-CoA can enter the mitochondria as propionate or propionylcarnitine. The fate of the remaining propionyl units is unknown (release from the cells as propionate; conversion of succinyl-CoA to glucose?).

What exactly happens with the shortened acyl-CoAs is not always clear either. The shortened eicosanoids and the partially degraded fat-soluble vitamins and xenobiotics are excreted. After conjugation with taurine or glycine the bile acids chenodeoxycholic and cholic acids are excreted in the bile. A major portion of the shortened dicarboxylic fatty acids is probably excreted in the urine. In how far shortened dicarboxylic fatty acids are oxidized further in the mitochondria remains unknown. It has already been mentioned that the possibility exists that multibranched fatty acids such as pristanic acid are degraded completely by peroxisomes. Proof of this remains to be offered, however. Shortened straight chain acyl-CoAs have to be either oxidized in the mitochondria (medium or long chain acyl-CoAs) or esterified in the peroxisome itself (long chain acyl-CoAs: dihydroxyacetone-phosphate acylation, the first step in ether lipid synthesis) or in the endoplasmic reticulum (long chain acyl-CoAs: glycerophosphate acylation, the first step in 'ester' glycerolipid synthesis). Theoretically, a shortened straight chain acyl-CoA can leave the peroxisome in unchanged form or it can be converted within the peroxisome to an acylcarnitine or a fatty acid by the peroxisomal matrix enzymes carnitine octanoyltransferase [21, 66] and acyl-

CoA hydrolase [1, 7,7 5], respectively. Acylcarnitines can directly enter the mitochondria for further oxidation. Acyl-CoAs first need to be converted to acylcarnitines by the mitochondrial carnitine palmitoyltransferase I before they can enter the mitochondria, but they can be used for glycerolipid synthesis in the endoplasmic reticulum without further conversion (long chain acyl-CoAs). Fatty acids leaving the peroxisome need re-activation to their CoA esters—at the expense of 2 ATP equivalents—before they can be metabolized further.

8. Peroxisomal fatty acid oxidation is not regulated by malonyl-CoA and by feeding as opposed to starvation

We have already explained that in order to cross the mitochondrial inner membrane long chain acyl-CoAs have to be converted to acylcarnitines, a reaction catalyzed by carnitine palmitoyltransferase I. Malonyl-CoA, the first intermediate committed to lipogenesis is a potent inhibitor of carnitine palmitoyltransferase I [64]. In the fed state, hepatic lipogenesis is high and hepatic malonyl-CoA levels are elevated. As a result, carnitine palmitoyltransferase I activity and fatty acid entry into the mitochondria are suppressed so that the newly synthesized fatty acids are prevented from being oxidized. They are thereby directed to triacylglycerol synthesis. In the starved state, rates of lipogenesis and malonyl-CoA levels decline, so that the inhibition of mitochondrial fatty acid oxidation is released. In addition, activation of lipolysis in adipose tissue provides the liver with an increased fatty acid supply, which further enhances the rates of oxidation, allowing the liver to generate energy for gluconeogenesis and to produce ketone bodies, which serve as glucose-sparing substrate for extrahepatic tissues [64]. The rates of hepatic lipogenesis and adipose tissue lipolysis in feeding and starvation are controlled mainly by the balance of insulin and glucagon. Malonyl-CoA inhibits carnitine palmitoyltransferase I also in extrahepatic tissues. It is not known, however, in how far fluctuations in malonyl-CoA regulate mitochondrial fatty acid oxidation also in these tissues [11, 22, 42, 64].

The peroxisomal membrane does not contain carnitine palmitoyltransferase [59, 66]. Fatty acyl-CoA entry into the peroxisome is not carnitine-dependent and not inhibited by malonyl-CoA [59] and peroxisomal fatty acid oxidation is not regulated by feeding as opposed to starvation, except in an indirect way via alterations in fatty acid supply (see later). A direct regulation of peroxisomal β-oxidation by feeding as opposed to starvation appears to be of little importance, since peroxisomal fatty acid oxidation is not primarily concerned with energy provision. The peroxisomal matrix contains carnitine octanoyltransferase [21, 66], which probably converts shortened acyl-CoAs into acylcarnitines. After leaving the peroxisome, the acylcarnitines can penetrate the mitochondria for further oxidation. Interestingly, peroxisomal carnitine octanoyltransferase is also inhibited by malonyl-CoA [13, 72]. In the fed state, this prevents fatty acyl-CoAs from being converted to acylcarnitines in the peroxisome. Such conversion would circumvent the block imposed on carnitine palmitoyltransferase I.

It has already been mentioned that the mechanism of penetration of acyl-CoAs into the peroxisome remains unknown, except for the fact that carnitine and malonyl-CoA are not involved. It is not clear whether fatty acyl-CoA entry into the peroxisome is regulated by other effectors or mechanisms.

Perhaps the major factor regulating peroxisomal β-oxidation is substrate supply. Indeed, the apparent Kms of the different acyl-CoA oxidases are generally one to two orders of magnitude higher than the unbound acyl-CoA concentrations found in the cell [113, 118]. This suggests that peroxisomal β-oxidation increases linearly with increasing substrate concentration. Increased fatty acid supply due to activation of adipose tissue lipolysis is therefore most probably the reason why the flux through peroxisomal β-oxidation is higher in the starved than in the fed state [111].

Although peroxisomal β-oxidation is not acutely regulated by feeding as opposed to starvation, treatment of rodents with a high-fat diet, especially when the diet contains a large percentage of very long chain and/or *trans* fatty acids, results in a 2 to 3-fold increase in the activity and concentration of three hepatic peroxisomal β-oxidation enzymes: palmitoyl-CoA oxidase, the multifunctional protein and thiolase [76]. Pristanoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase are not inducible [86, 119]. Smaller increases in enzyme activity are observed in heart, kidney and intestinal mucosa. 10 to 15-fold increases in hepatic peroxisomal β-oxidation enzymes (except pristanoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase) and 3 to 4-fold increases in those of heart, kidney and intestinal mucosa can be seen when rodents are treated with so-called peroxisome proliferators, a class of structurally diverse xenobiotics that include the hypolipidemic fibrates [58]. Along with the induction of the peroxisomal β-oxidation enzymes, these compounds cause a proliferation of peroxisomes in the mentioned tissues. As detailed in another chapter of this book, the peroxisome proliferators activate the peroxisome proliferator-activated receptor, a nuclear transcription factor that belongs to the thyroid/retinoid hormone subclass of ligand-dependent transcription factors [19, 33]. The putative ligand (a metabolite of the peroxisome proliferator?) and the putative endogenous ligand (a lipid intermediate which is possibly involved in the induction of peroxisomal enzymes by high fat diets?) remain to be identified. The activated receptors bind to direct repeats of specific base sequences in the promoter region of the responsive genes and stimulate thereby the transcription of these genes [106]. Peroxisome proliferation and peroxisomal enzyme induction do not seem to occur in the human, despite the fact that the peroxisome proliferator-activated receptor is expressed in human tissues [87]. This suggests that human tissues may contain a silencer that neutralizes the action of the peroxisome proliferator-activated receptor.

Treatment of rats with high doses of thyroxine for several days results in a 2.5-fold increase in peroxisomal β-oxidation [36]. Acute treatment of rats with glucagon does not affect the peroxisomal β-oxidizing activity [97].

Addition of ATP to isolated peroxisomes oxidizing fatty acyl-CoA esters stimulates oxidation [104]; addition of CoA or NADH is inhibitory [73]. The

stimulatory effect of ATP appears to be exerted at the level of palmitoyl-CoA oxidase (which is probably the rate-limiting enzyme of peroxisomal fatty acid oxidation), since peroxisomal fatty acyl-CoA oxidation is stimulated to the same extent as the enzyme [8]. The inhibitory effect of CoA is observed in intact peroxisomes but not in broken peroxisomes [74]. This led the authors to conclude that CoA affects fatty acyl-CoA entry into peroxisomes. However, direct proof of such a mechanism remains to be given. The inhibitory effect of NADH is exerted at the level of the β-hydroxyacyl-CoA dehydrogenase reaction. It would appear doubtful, however, that in the intact cell peroxisomal β-oxidation is regulated by fluctuations in ATP or CoA. Likewise, it seems doubtful that the intraperoxisomal redox state of the pyridine nucleotides would constitute a regulatory mechanism. As already mentioned, isolated peroxisomes and peroxisomes in permeabilized hepatocytes are permeable to small water soluble molecules of the size of NADH. It is probable, therefore, that the intraperoxisomal redox state is governed by that of the cytosol. Summarizing the data described in this section, it would appear that peroxisomal β-oxidation is regulated mainly by substrate supply. Since peroxisomal β-oxidation seems to function primarily as a detoxification system, degrading a variety of lipophilic carboxylates to shorter and more polar metabolites that can be excreted, regulation by substrate supply would seem both sufficient and adequate.

References

1. Alexson, S.E.H., H. Osmundsen and R.K. Berge. The presence of acyl-CoA hydrolase in rat brown-adipose tissue peroxisomes. *Biochem. J.* **262**, 41–46, 1989.
2. Appelkvist, E.-L., M. Reinhart, R. Fischer, J. Billheimer and G. Dallner. Presence of individual enzymes of cholesterol biosynthesis in rat liver peroxisomes. *Arch. Biochem. Biophys.* **282**, 318–325, 1990.
3. Avigan, J., D. Steinberg, A. Gutman, C.E. Mize and G.W. Milne. Alpha decarboxylation, an important pathway for degradation of phytanic acid in animals. *Biochem. Biophys. Res. Commun.* **24**, 838–844, 1966.
4. Bartlett, K., R. Hovik, S. Eaton, N.J. Watmough and H. Osmundsen. Intermediates of peroxisomal β-oxidation. A study of the fatty acyl-CoA esters which accumulate during peroxisomal β-oxidation of [U-^{14}C] hexadecanoate. *Biochem. J.* **270**, 175–180, 1990.
5. Baudhuin, P. Liver peroxisomes, cytology and function. *Ann. N.Y. Acad Sci.* **168**, 214–228, 1969.
6. Bennett, M.J., R.J. Pollitt, S.I. Goodman, D.E. Hale and J. Vamecq. Atypical riboflavin-responsive glutaric aciduria, and deficient peroxisomal glutaryl-CoA oxidase activity: a new peroxisomal disorder. *J. Inher. Metab. Dis.* **14**, 165–173, 1991.

7. Berge, R.K., T. Flatmark and H. Osmundsen. Enhancement of long-chain acyl-CoA hydrolase activity in peroxisomes and mitochondria of rat liver by peroxisomal proliferators. *Eur. J. Biochem.* **141**, 637–644, 1984.
8. Casteels, M., L. Schepers, J. van Eldere, H.J. Eyssen and G.P. Mannaerts. Inhibition of 3α, 7α, 12α-trihydroxy-5β-cholestanoic acid oxidation and of bile acid secretion in rat liver by fatty acids. *J. Biol. Chem.* **263**, 4654–4661, 1988.
9. Cerdan, S., B. Künnecke, A. Dölle and J. Seelig. *In situ* metabolism of 1, ω medium chain dicarboxylic acids in the liver of intact rats as detected by ^{13}C and ^{1}H NMR. *J. Biol. Chem.* **263**, 11664–11674, 1988.
10. Cooper, T.G., H. Beevers. β-Oxidation in glyoxysomes from Castor bean endosperm. *J. Biol. Chem.* **244**, 3514–3520, 1969.
11. Corkey, B.E., M.C. Glennon, K.S. Chen, J.T. Deeney, F.M. Matschinsky and M. Prentki. A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic β-cells. *J. Biol. Chem.* **264**, 21608–21612, 1989.
12. Declercq, P.E., H.P. Haagsman, P.P. van Veldhoven, L.J. Debeer, L.M.G. van Golde and G.P. Mannaerts. Rat liver dihydroxyacetone-phosphate acyltransferases and their contribution to glycerolipid synthesis. *J. Biol. Chem.* **259**, 9064-9075, 1984.
13. Derrick, J.R. and R.R. Ramsay. L-Carnitine acyltransferase in intact peroxisomes is inhibited by malonyl-CoA. *Biochem. J.* **262**, 801–806, 1989.
14. Diczfalusy, U. and S.E.H. Alexon. Peroxisomal chain-shortening of prostaglandin F2α. *J. Lipid Res.* **29**, 1629–1636, 1988.
15. Diczfalusy, U. and S.E.H. Alexson. Identification of metabolites from peroxisomal β-oxidation of prostaglandins. *J. Lipid Res.* **31**, 307–314, 1990.
16. Diczfalusy, U., B.F. Kase, S.E.H. Alexson and I. Björkhem. Metabolism of prostaglandin $F_{2\alpha}$ in Zellweger syndrome. Peroxisomal β-oxidation is of major importance for *in vivo* degradation of prostaglandins in humans. J. Clin. Invest. **88**, 978–984, 1991.
17. Dieuaide, M., Brouquisse, A. Pradet and P. Raymond. Increased fatty acid β-oxidation after glucose starvation in maize root tips. *Plant Physiol.* **99**, 595–600, 1992.
18. Draye, J.P., K. Veitch, J. Vamecq and F. van Hoof. Comparison on the metabolism of dodecanedioic acid *in vivo* in control, riboflavin-deficient and clofibrate-treated rats. *Eur. J. Biochem.* **178,** 183–189, 1988.
19. Dreyer, C., G. Krey, H. Keller, F. Givel, G. Helftenbein and W. Wahli. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **68**, 879–887, 1992.
20. Ericsson, J., E.L. Appelkvist, A. Thelin, T. Chojnacki and G. Dallner. Isoprenoid synthesis in rat liver peroxisomes. Characterization of *cis*-prenyltransferase and squalene synthetase. *J. Biol. Chem.* **267**, 18708–18714, 1992.
21. Farrell, S.O., C.J. Fiol, J.K. Reddy and L.L. Bieber. Properties of purified carnitine acyltransferases of mouse liver peroxisomes. *J. Biol. Chem.* **259**, 13089–13095, 1984.

22. Fiol, C.J., J. Kerner and L.L. Bieber. Effect of malonyl-CoA on the kinetics and substrate cooperativity of membrane-bound carnitine palmitoyltransferase of rat heart mitochondria. *Biochim. Biophysica Acta* **916**, 482–492, 1987.
23. Foerster, E.-C., T. Fährenkemper, U. Rabe, P. Graf and H. Sies. Peroxisomal fatty acid oxidation as detected by H_2O_2 production in intact perfused liver. *Biochem. J.* **196**, 705–712, 1981.
24. Friedrich, W. **In**: W. de Gruyter (ed.), *Vitamins*, Berlin, 1988.
25. Gatt, S., J. Bremer and H. Osmundsen. Pyrene dodecanoic acid coenzyme A ester: peroxisomal oxidation and chain shortening. *Biochim. Biophys. Acta* **958**, 130–133, 1988.
26. Gerhardt, B. Higher plant peroxisomes and fatty acid degradation. **In**: Fahimi, H.D. and H. Sies (eds.), *Peroxisomes in Biology and Medicine*, 141–151, Springer-Verlag, Berlin, Heidelberg, 1987.
27. Golden, K.D. and E.A. Kean. The biogenesis of dicarboxylic acids in rats given hypoglycin. *Biochim. Biophys. Acta* **794**, 83–88, 1984.
28. Goldstein, J.L. and M.S. Brown. Regulation of mevalonate pathway. *Nature* **343**, 425–430, 1990.
29. Hamberg, M. and B. Samuelson. On the metabolism of prostaglandins E_1 and E_2 in man. *J. Biol. Chem.* **246**, 6713–6721, 1971.
30. Horie, S., T. Suzuki and T. Suga. Existence of acetyl-CoA-dependent chain elongation system in hepatic peroxisomes of rat: effects of clofibrate and di-(2-ethylhexyl) phthalate on the activity. *Arch. Biochem. Biophys.* **274**, 64–73, 1989.
31. Hovik, R., B. Brodal, K. Bartlett and H. Osmundsen. Metabolism of acetyl-CoA by isolated peroxisomal fractions: formation of acetate and acetoacetyl-CoA. *J. Lipid Res.* **32**, 993–999, 1991.
32. Huang, S., P.P. van Veldhoven, F. Vanhoutte, G. Parmentier, H.J. Eyssen and G.P. Mannaerts. α-Oxidation of 3-methyl-substituted fatty acids in rat liver. *Arch. Biochem. Biophys.* **296**, 214–223, 1992.
33. Isseman, I. and S. Green. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645–649, 1990.
34. Jakobs, B.S. and R.J.A. Wanders. Conclusive evidence that very long chain fatty acids are oxidized exclusively in peroxisomes in human skin fibroblasts. *Biochem. Biophys. Res. Commun.* **178**, 842–847, 1991.
35. Jedlitschky, G., M. Huber, A. Völkl, M. Müller, I. Leier, J. Müller, W.D. Lehmann, H.D. Fahimi and D. Keppler. Peroxisomal degradation of leukotrienes by β-oxidation from the ω-end. *J. Biol. Chem.* **266**, 24763–24772, 1991.
36. Just, W.W. and F.U.Hartl. Rat liver peroxisomes, II. Stimulation of peroxisomal fatty acid β-oxidation by thyroid hormones. *Hoppe-Seyler's Z. Physiol. Chem.* **364**, 1541–1547, 1983.
37. Kamijo, K., S. Taketani, S. Yokota, T. Osumi and T. Hashimoto. The 70 kDa peroxisomal membrane protein is a member of the Mdr (P-glycoprotein)-related ATP-binding protein superfamily. *J. Biol. Chem.* **265**, 4534–4540, 1990.

38. Kase, B.F. and I. Björkhem. Peroxisomal bile acid-CoA: amino-acid N-acyltransferase in rat liver. *J. Biol. Chem.* **264**, 9220–9223, 1989.
39. Kase, F., I. Björkhem and J.I. Pedersen. Formation of cholic acid from 3α, 7α, 12α-trihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **24**, 1560–1567, 1983.
40. Kase, B.F., K. Prydz, I. Björkhem and J.I. Pedersen. *In vitro* formation of bile acids from di- and trihydroxy-5β-cholestanoic acid in human liver peroxisomes. *Biochim. Biophys. Acta* **877**, 37–42, 1986.
41. Kase, B.F., K. Prydz, I. Björkhem and J.I. Pedersen. Conjugation of cholic acid with taurine and glycine by rat liver peroxisomes. *Biochem. Biophys. Res. Commun.* **138**, 167–173, 1986.
42. Kashfi, K., L. Dory and G.A. Cook. Effects of cholesterol loading of mouse macrophages on palmitoyltransferase activity and sensitivity to inhibition by malonyl-CoA. *Biochim. Biophys. Acta* **117**, 1121–1126, 1991.
43. Keller, G.A., M.C. Barton, D.J. Shapiro and S.J. Singer. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase is present in peroxisomes in normal rat liver cells. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 770–774, 1985.
44. Keller, G.A., M. Pazirandeh and S. Krisans. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-traeted animals: quantitative biochemical and immunoelectron microscopic analyses. *J. Cell. Biol.* **103**, 875–886, 1986.
45. Kølvraa, S. and N. Gregersen. *In vitro* studies on the oxidation of medium-chain dicarboxylic acids in rat liver. *Biochem. Biophys. Acta* **876**, 515–525, 1986.
46. Kramar, R., M. Hüttinger, B. Gmeiner and H. Goldenberg. β-Oxidation in peroxisomes of brown adipose tissue. *Biochim. Biophys. Acta* **531**, 353–356, 1978.
47. Krisans, S.K., R.M. Mortensen and P.B. Lazarow. Acyl-CoA synthetase in rat liver peroxisomes. *J. Biol. Chem.* **255**, 9599–9607, 1980.
48. Kunau, W.-H., C. Kionka, A. Ledebur, M. Mateblowski, M. Moreno de la Garza M, U. Schultz-Borchard, R. Thieringer and M. Veenhuis. β-Oxidation systems in eukaryotic microorganisms. **In**: Fahimi, H.D. and H. Sies (eds.). *Peroxisomes in Biology and Medicine*, 128–140, 1987. Springer-Verlag, Berlin, Heidelberg.
49. Labarca, P., D. Wolff, U. Soto, C. Necochea and F. Leighton. Large cation-selective pores from rat liver peroxisomal membranes incorporated to plasma lipid bilayers. *J. Membr. Biol.* **94**, 285–291, 1986.
50. Lageweg, W., J.M. Tager and R.J.A. Wanders. Topography of very long chain fatty acid activating activity in peroxisomes from rat liver. *Biochem. J.* **276**, 53–56, 1991.
51. Lazarow, P.B. Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J. Biol. Chem.* **253**, 1522–1528, 1978.
52. Lazarow, P.B. and C. de Duve. A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046, 1976.

53. Lazarow, P.B. and H.W. Moser. Disorders of peroxisome biogenesis. **In**: Scriver, C.R., A.L. Beaudet, W.S. Sly and D. Valle, D. (eds.). *The metabolic and molecular bases of inherited disease*, 7th. edn., 2287–2325, McGraw-Hill, New York, 1995.
54. Lazo, O., M. Contreras and I. Singh. Topographical localization of peroxisomal acyl-CoA ligases: differential localization of palmitoyl-CoA and lignoceroyl-CoA ligases. *Biochemistry* **29**, 3981–3986, 1990.
55. Lazo, O., M. Contreras, Y. Yoshida, A.K. Singh, W. Stanley, M. Weise and I. Singh. Cellular oxidation of lignoceric acid is regulated by the subcellular localization of lignoceroyl-CoA ligases. *J. Lipid Res.* **31**, 583–595, 1990.
56. Leighton, F., S. Bergseth, T. Rørtveit, F.N. Christiansen and J. Bremer. Free acetate production by rat hepatocytes during peroxisomal fatty acid and dicarboxylic acid oxidation. *J. Biol. Chem.* **264**, 10347–10350, 1989.
57. Lemmens, M., K. Verheyden, P. van Veldhoven, J. Vereecke, G.P. Mannaerts and E. Carmeliet. Single-channel analysis of a large conductance channel in peroxisomes from rat liver. *Biochim. Biophys. Acta* **984**, 351–359, 1989.
58. Lock, E.A., A.M. Mitchell and C.R. Elcombe. Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu. Rev. Pharmacol. Toxicol.* **29**, 145–163, 1989.
59. Mannaerts, G.P., L.J. Debeer, J. Thomas and P.J. de Schepper. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J. Biol. Chem.* **254**, 4585–4595, 1979.
60. Mannaerts, G.P., P.P. van Veldhoven, A. van Broekhoven, G. Vandebroek and L.J. Debeer L.J. Evidence that peroxisomal acyl-CoA synthetase is located at the cytoplasmic side of the peroxisomal membrane. *Biochem. J.* **204**, 17–23, 1982.
61. Markwell, M.A.K., N.E. Tolbert and L.L. Bieber. Comparison of the carnitine acyltransferase activities from rat liver peroxisomes and microsomes. *Arch. Biochem. Biophys.* **176**, 479–488, 1976.
62. Martinez, M. Polyunsaturated fatty acid changes suggesting a new enzymatic defect in Zellweger Syndrome. *Lipids* **24**, 261–265, 1989.
63. Mayatepek, E., W.D. Lehman, J. Fauler, D. Tsikas, J.C Frölich, R.B.H. Schutgens, R.J.A. Wanders and D. Keppler. Impaired degradation of leukotrienes in patients with peroxisome deficiency disorders. *J. Clin. Invest.* **91,** 881–888, 1993.
64. McGarry, J.D. and D.W. Foster. Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.* **49**, 395–420, 1980.
65. Miyazawa, S., T. Hashimoto and S. Yokota. Identity of long-chain acyl-coenzyme A synthetase of microsomes, mitochondria, and peroxisomes in rat liver. *J. Biochem.* (Tokyo) **98**, 723–733, 1985.
66. Miyazawa, S., H. Ozasa, T. Osumi and T. Hashimoto. Purification and properties of carnitine octanoyltransferase and carnitine palmitoyltransferase from rat liver. *J. Biochem.* (Tokyo) **94**, 529 542, 1993.

67. Mortensen, P.B. C_6-C_{10}-dicarboxylic acids in liver and kidney tissue in normal, diabetic ketotic and clofibrate-treated rats. *Biochim. Biophys. Acta* **878**, 14–19, 1986.
68. Mortensen, P.B. Formation and degradation of dicarboxylic acids in relation to alterations in fatty acid oxidation in rats. *Biochim. Biophys. Acta* **1124**, 71–79, 1992.
69. Mortensen, P.B. and N. Gregersen. The biological origin of ketotic dicarboxylic aciduria. *Biochim. Biophys. Acta* **666**, 394–404, 1981.
70. Muralidharan, V.B. and Y. Kishimoto. Phytanic acid α-oxidation in rat liver. *J. Biol. Chem.* **259**, 13021–13026, 1984.
71. Murthy, M.S.R. and S.V. Pande. Malonyl-CoA binding site and the overt carnitine palmitoyltransferase activity reside on the opposite sites of the outer mitochondrial membrane. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 378–382, 1987.
72. Nic Á Bháird, N. and R.R. Ramsay. Malonyl-CoA inhibition of peroxisomal carnitine octanoyltransferase. *Biochem. J.* **286**, 637–640, 1992.
73. Osmundsen, H. Factors which can influence β-oxidation by peroxisomes isolated from livers of clofibrate treated rats. Some properties of peroxisomal fractions isolated in a self-generated percoll gradient by vertical rotor centrifugation. *Int. J. Biochem.* **14**, 1982, 905–914.
74. Osmundsen, H. and C.E. Neat. Regulation of peroxisomal fatty acid oxidation. *FEBS Lett.* **107**, 81–85, 1979.
75. Osmundsen, H., C.E. Neat and B. Borrebaek. Fatty acid products of peroxisomal β-oxidation. *Int. J. Biochem.* **12,** 625–630, 1980.
76. Osmundsen, H., M.S. Thomassen, J.K. Hiltunen and R.K. Berge. Physiological role of peroxisomal β-oxidation. **In**: Fahimi, H.D. and H. Sies, (eds.). *Peroxisomes in Biology and Medicine*, 152–165, 1987. Springer-Verlag, Berlin, Heidelberg.
77. Pahan, K., J. Cofer, P. Baliga and I. Singh. Identification of phytanoyl-CoA ligase as a distinct acyl-CoA ligase in peroxisomes from cultured human skin fibroblasts. *FEBS Lett.* **322**, 101–104, 1993.
78. Pedersen, J.L., B.F. Kase, K. Prydz and I. Björkhem. Liver peroxisomes and bile acid formation. **In**: Fahimi, H.D. and H. Sies, (eds.). *Peroxisomes in Biology and Medicine*, 67–77, 1987. Springer-Verlag, Berlin, Heidelberg.
79. Poosch, M.S. and R.K. Yamazaki. The oxidation of dicarboxylic acid CoA esters via peroxisomal fatty acyl-CoA oxidase. *Biochim. Biophys. Acta* **1006**, 291–298, 1989.
80. Prydz, K., B.F. Kase, I. Björkhem and J.I. Pedersen. Formation of chenodeoxycholic acid from 3α, 7α-dihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **27**, 622–628, 1986.
81. Prydz, K., B.F. Kase, I. Björkhem and J.I. Pedersen. Subcellular localization of 3α, 7α-dihydroxy and 3α, 7α, 12α-trihydroxy-5β-cholestanoyl-coenzyme A ligase(s) in rat liver. *J. Lipid Res.* **29**, 992–1004, 1988.
82. Roberts, L.J. II, B.J. Sweetman, N.A. Payne and J.A. Oates. Metabolism of thromboxane B_2 in man. *J. Biol. Chem.* **252**, 7415–7417, 1977.

83. Sala, A., N. Voelkel, J. Maclouf and R.C. Murphy. Leukotriene E_4 elimination and metabolism in normal human subjects. *J. Biol. Chem.* **265**, 21771–21778, 1990.
84. Schepers, L., M. Casteels, J. Vamecq, G. Parmentier, P.P. van Veldhoven and G.P. Mannaerts. β-Oxidation of the carboxyl side chain of prostaglandin E_2 in rat liver peroxisomes and mitochondria. *J. Biol. Chem.* **263**, 2724–2731, 1988.
85. Schepers, L., M. Casteels, K. Verheyden, G. Parmentier, S. Asselberghs, H.J. Eyssen, and G.P. Mannaerts. Subcellular distribution and characteristics of trihydroxycoprostanoyl-CoA synthetase in rat liver. *Biochem. J.* **257**, 221–229, 1989.
86. Schepers, L., P.P. van Veldhoven, M. Casteels, H.J. Eyssen and G.P. Mannaerts. Presence of three acyl-CoA oxidases in rat liver peroxisomes: an inducible fatty acyl-CoA oxidase, a noninducible fatty acyl-CoA oxidase and a noninducible trihydroxycoprostanoyl-CoA oxidase. *J. Biol. Chem.* **265**, 5242–5246, 1990.
87. Sher, T., H.F. Yi, O.W. McBride and F.J. Gonzalez. cDNA cloning, chromosomal mapping and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* **32**, 5598–5604, 1993.
88. Shindo, Y. and T. Hashimoto. Acyl-Coenzyme A synthetase and fatty acid oxidation in rat liver peroxisomes. *J. Biochem.* (Tokyo) **84**, 1177–1181, 1978.
89. Singh, H. and A. Poulos. Distinct long chain and very long chain fatty acyl-CoA synthetase in rat liver peroxisomes and microsomes. *Arch. Biochem. Biophys.* **266**, 486–495, 1988.
90. Singh, I., O. Lazo, G. Dhaunsi and H. Contreras. Transport of fatty acids into human and rat peroxisomes. *J. Biol. Chem.* **267**, 13306–13313, 1992.
91. Singh, I., A.E. Moser, S. Goldfischer and H.W. Moser. Lignoceric acid is oxidized in the peroxisome: implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4203–4207, 1984.
92. Singh, I., H.W. Moser, A.B. Moser and Y. Kishimoto. Adrenoleukodystrophy: impaired oxidation of long chain fatty acids in cultured skin fibroblasts and adrenal cortex. *Biochem. Biophys. Res. Commun.* **102**, 1223–1229, 1981.
93. Singh, I., K. Pahan, G.S. Dhaunsi, O. Lazo and P. Ozand. Phytanic acid α-oxidation. Differential subcellular localization in rat and human tissues and its inhibition by Nycodenz. *J. Biol. Chem.* **268**, 9972–9979, 1993.
94. Skjeldal, O.H. and O. Stokke. The subcellular localization of phytanic acid oxidase in rat liver. *Biochim. Biophys. Acta* **921**, 38–42, 1987.
95. Skorin, C., C. Necochea, V. Jokow, U. Soto, A.M. Grau, J. Bremer and F. Leighton. Peroxisomal fatty acid oxidation and inhibition of the mitochondrial carnitine palmitoyltransferase I in isolated rat hepatocytes. *Biochem. J.* **281**, 561–567, 1992.

96. Skrede, S. and J. Bremer. The compartmentation of CoA and fatty acid activating enzymes in rat liver mitochondria. *Eur. J. Biochem.* **14**, 465–472, 1970.
97. Slauter, R.W. and R.K. Yamazaki. Glucagon and fasting do not activate peroxisomal fatty acid β-oxidation in rat liver. *Arch. Biochem. Biophys.* **233**, 197–202, 1984.
98. Stamellos, K.D., J.E. Shackelford, R.D. Tanaka and S.K. Krisans. Mevalonate kinase is localized in rat liver peroxisomes. *J. Biol. Chem.* **267**, 5560–5568, 1992.
99. Steinberg, D. Refsum disease. **In**: Scriver, C.R., A.L. Beaudet, W.S. Sly and D. Valle, (eds.). *The metabolic basis of inherited disease*, 6th. edn., 1533–1550, 1989. McGraw-Hill, New York.
100. Sun, F.F., and B.M. Taylor. Metabolism of prostacyclin in rat. *Biochemistry* **19**, 4096–4101, 1978.
101. Suzuki, H., K. Mori, J. Yamada and T. Suga. Contribution of peroxisomal β-oxidation system to the chain-shortening of N-(α-methylbenzyl) azelaamic acid in rat liver. *Biochem. Pharmacol.* **39**, 1975–1981, 1990.
102. Suzuki, H., J. Yamada, T. Watanabe and T. Suga. Compartmentation of dicarboxylic β-oxidation in rat liver: importance of peroxisomes in the metabolism of dicarboxylic acids. *Biochim. Biophys. Acta* **990**, 25–30, 1989.
103. Ten Brink, H.J., F. Stellaard, C.M.M. van den Heuvel, R.M. Kok, D.S.M. Schor, R.J.A. Wanders and C. Jakobs. Pristanic acid and phytanic acid in plasma from patients with peroxisomal disorders: stable isotope dilution analysis with electron capture negative ion fragmentography. *J. Lipid Res.* **33**, 41–47, 1992.
104. Thomas, J., L.J. Debeer, P.J. de Schepper and G.P. Mannaerts. Factors influencing palmitoyl-CoA oxidation by rat liver peroxisomal fractions. *Biochem. J.* **190**, 485–494, 1980.
105. Tsai, S.C., J. Avigan and D. Steinberg. Studies on the α-oxidation of phytanic acid by rat liver mitochondria. *J. Biol. Chem.* **244**, 2682–2692, 1969.
106. Tugwood, J.D., F. Issemann, R.G. Anderson, K.R. Bundell, W.L. McPheat and S. Green. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl-CoA oxidase gene. *EMBO J.* **11**, 433–439, 1992.
107. Une, M., N. Isumi, T. Imanaka and T. Hoshita. Formation of cholic acid from 3α, 7α, 12α-trihydroxy-5β-cholestanoic acid in human skin fibroblasts. *J. Biochem.* (Tokyo) **112**, 576–577, 1992.
108. Vamecq, J., E. de Hoffmann and F. van Hoof. The microsomal dicarboxylyl-CoA synthetase. *Biochem. J.* **230**, 683–693, 1985.
109. Vamecq, J. and J.P. Draye. Peroxisomal and mitochondrial β-oxidation of monocarboxylyl-CoA, ω-hydroxymonocarboxylyl-CoA and dicarboxylyl-CoA ester in tissues from untreated and clofibratetreated rats. *J. Biochem.* (Tokyo) **106**, 216–222 1989.
110. Vamecq, J. and F. van Hoof. Implication of a peroxisomal enzyme in the catabolism of glutaryl-CoA. *Biochem. J.* **221**, 203–211, 1984.

111. Van den Branden, C., I. Kerckaert and F. Roels. Peroxisomal β-oxidation from endogenous substrates. Demonstration through H_2O_2 production in the unanaesthetized mouse. *Biochem. J.* **218**, 697–702, 1984.
112. Vanhove, G., P.P. van Veldhoven, H.J. Eyssen and G.P. Mannaerts. Mitochondrial short chain acyl-CoA dehydrogenase of human liver and kidney can function as an oxidase. *Biochem. J.* **292**, 23–30, 1993.
113. Vanhove, G.F., P.P. van Veldhoven, M. Fransen, S. Denis, H.J. Eyssen, R.J.A. Wanders and G.P. Mannaerts. The CoA esters of 2-methyl-branched chain fatty acids and of the bile acid intermediates di- and trihydroxycoprostanic acids are oxidized by one single peroxisomal branched chain acyl-CoA oxidase in human liver and kidney. *J. Biol. Chem.* **268**, 10335–10344, 1993.
114. Vanhove, G., P.P. van Veldhoven, F. Vanhoutte, G. Parmentier, H.J. Eyssen and G.P. Mannaerts. Mitochondrial andperoxisomal β-oxidation of the branched chain fatty acid 2-methylpalmitate in rat liver. *J. Biol. Chem.* **266**, 24670–24675, 1991.
115. Van Veldhoven, P., L.J. Debeer and G.P. Mannaerts. Water- and solute-accessible spaces of purified peroxisomes. Evidence that peroxisomes are permeable to NAD^+. *Biochem. J.* **210**, 685–693, 1983.
116. Van Veldhoven, P.P., S. Huang, H.J. Eyssen and G.P. Mannaerts. The deficient degradation of synthetic 2- and 3-methyl-branched fatty acids in fibroblasts from patients with peroxisomal disorders. *J. Inher. Metab. Dis.* **16**, 381–391, 1993.
117. Van Veldhoven, P.P., W.W. Just and G.P. Mannaerts. Permeability of the peroxisomal membrane to cofactors of β-oxidation. Evidence for the presence of a pore-forming protein. *J. Biol. Chem.* **262**, 4310–4318, 1987.
118. Van Veldhoven, P.P., G. Vanhove, S. Asselberghs, H.J. Eyssen and G.P. Mannaerts. Substrate specificities of rat liver peroxisomal acyl-CoA oxidases: palmitoyl-CoA oxidase (inducible acyl-CoA oxidase), pristanoyl-CoA oxidase (non-inducible acyl-CoA oxidase) and trihydroxycoprostanoyl-CoA oxidase. *J. Biol. Chem.* **267**, 20065–20074, 1992.
119. Van Veldhoven, P.P., G. Vanhove, F. Vanhoutte, G. Dacremont, G. Parmentier, H.J. Eyssen and G.P. Mannaerts. Identification and purification of a peroxisomal branched chain fatty acyl-CoA oxidase. *J. Biol. Chem.* **266**, 24676–24683, 1991.
120. Verleur, N. and R.J.A. Wanders. Permeability properties of peroxisomes in rat liver. *Biol. Cell* **77**, 109, 1993.
121. Viaeney-Liaud, C., P. Divry, N. Gregersen and M. Mathieu. The inborn errors of mitochondrial fatty acid oxidation. *J. Inher. Metabol. Dis.* **10**, suppl 1, 159–198, 1987.
122. Voss, A., M. Reinhart, S. Sankarappa and H. Sprecher. The metabolism of 7, 10, 13, 16, 19-docosapentaenoic acid to 4, 7, 10, 13, 16, 19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J. Biol. Chem.* **266**, 19995–20000, 1991.
123. Wanders, R.J.A., S.W. Denis and G. Dacremont. Studies on the substrate

specificity of the inducible and non-inducible acyl-CoA oxidases from kidney peroxisomes. *J. Biochem.* (Tokyo) **43**, 577–582, 1993.
124. Wanders, R.J.A., S. Denis, C.W.T. van Roermund, C. Jakobs and H.J. ten Brink. Characteristics and subcellular localization of pristanoyl-CoA synthetase in rat liver. *Biochim. Biophys. Acta* **1125**, 274–279, 1992.
125. Wanders, R.J.A., C.W.T. van Roermund, R.B.H. Schutgens, P.G. Barth, H.S.A. Heymans, H. van den Bosch and J.M. Tager. The inborn errors of peroxisomal β-oxidation: a review. *J. Inher. Metabol. Dis.* **13**, 4–36, 1990.
126. Watkins, P.A., S.J. Mihalik and O.H. Skjeldal. Mitochondrial oxidation of phytanic acid in human and monkey liver: implication that Refsum's disease is not a peroxisomal disorder. *Biochem. Biophys. Res. Commun.* **167**, 580–586, 1990.
127. Wolvetang, E.J., J.M. Tager and R.J.A. Wanders. Latency of the peroxisomal enzyme acyl-CoA: dihydroxyacetone phosphate acyltransferase in digotinin-permeabilized fibroblasts: the effect of ATP and ATPase inhibitors. *Biochem. Biophys. Res. Commun.* **170**, 1135–1143, 1990.
128. Yamada, J., S. Horie, T. Watanabe and T. Suga. Participation of peroxisomal β-oxidation system in the chain-shortening of a xenobiotic acyl compound. *Biochem. Biophys. Res. Commun.* **125**, 123–128, 1984.
129. Yamada, J., S. Ogawa, S. Horie, T. Watanabe and T. Suga. Participation of peroxisome in the metabolism of xenobiotic acyl compounds: comparison between peroxisomal and mitochondrial β-oxidation of ω-phenyl fatty acids in rat liver. *Biochim. Biophys. Acta* **921**, 292–301, 1987.
130. Yoshida, Y., J. Yamada, T. Watanabe, T. Suga and H. Takayama. Participation of the peroxisomal β-oxidation system in the chain-shortening of PCA_{16}, a metabolite of the cytosine arabinoside prodrug, YNKO1, in rat liver. *Biochem. Pharmacol.* **39**, 1505–1512, 1990.

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