**PEROXISOMES AND THEIR CENTRAL ROLE IN METABOLIC INTERACTION NETWORKS IN HUMANS**

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*Running title: Peroxisomes and metabolic networks in humans*

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**Summary (Abstract)**: Peroxisomes catalyze a number of essential metabolic functions and impairments in any of these are usually associated with major clinical signs and symptoms. In contrast to mitochondria which are autonomous organelles which can catalyze the degradation of fatty acids, certain amino acids and other compounds all by themselves, peroxisomes are non-autonomous organelles which very much depend on the interaction with other organelles and compartments to fulfill their role in metabolism. This includes mitochondria, the endoplasmic reticulum, lysosomes, and the cytosol. In this paper we will discuss the central role of peroxisomes in different metabolic interaction networks in humans, including fatty acid oxidation, ether phospholipid biosynthesis, bile acid synthesis, fatty acid alpha-oxidation and glyoxylate metabolism.

**Keywords**: Peroxisomes – fatty acids – ether phospholipids – docosahexaenoic acid – genetic diseases – Refsum disease – phytanic acid – tethering proteins – metabolic networks

**INTRODUCTION**

Peroxisomes are dynamic subcellular organelles, which play an essential role in a variety of different metabolic pathways, including the alpha- and beta-oxidation of a distinct series of fatty acids (FAs), the biosynthesis of bile acids and ether phospholipids (EPL) including plasmalogens, and the detoxification of glyoxylate [1-3]. In order to fulfill their role in each of these metabolic pathways, peroxisomes need to interact with other subcellular organelles, because none of the metabolic pathways in which peroxisomes are involved, can be fully executed by peroxisomes on their own. A good example of the dependence of peroxisomes on other organelles concerns the degradation of FAs like C26:0 in peroxisomes. For this metabolic pathway peroxisomes first require the active interaction with the endoplasmic reticulum (ER) since the ER is the main site of synthesis of C26:0-CoA from shorter-chain FAs first via the FAS-complex in the cytosol, followed by chain elongation to C26:0-CoA via the chain-elongation system in the ER. Very long-chain FAs (VLCFAs) like C26:0 are also derived from dietary sources albeit to a limited extent. In addition, VLCFAs like C26:0 are also released from lysosomes, following the intra-lysosomal degradation of (complex) lipids like C26:0-containing ceramides [4]. Whatever the source of C26:0-CoA, the C26:0-CoA needs to be transported to the peroxisomal membrane, followed by the transport of C26:0-CoA across the peroxisomal membrane by ABCD1. The products of beta-oxidation of C26:0-CoA in peroxisomes include acetyl-CoA, a medium-chain acyl-CoA and NADH, which all need to be shuttled to the mitochondria for full oxidation to CO2 and H2O and reoxidation of NADH back to NAD+, respectively. This example shows that with respect to fatty acid oxidation, peroxisomes are non-autonomous organelles in contrast to mitochondria which can catalyze the oxidation of long, medium and short fatty acids down to CO2 and H2O all by themselves.

In this paper we will present the current state of knowledge about the interaction between peroxisomes and other organelles with respect to the major metabolic pathways of peroxisomes, at least in humans, including the alpha- and beta-oxidation of FAs, the biosynthesis of EPL and bile acids and the detoxification of glyoxylate. For the role of peroxisomes in ROS/RNS metabolism the reader is referred to the Chapters by Fransen et al. and Corpas et al. in this issue.

1. **EFFECTIVE PEROXISOMAL BETA-OXIDATION REQUIRES A HIGHLY INTERCONNECTED METABOLIC NETWORK INVOLVING MITOCHONDRIA AND THE ER**

Peroxisomes catalyze the beta-oxidation of a range of different substrates some of which are unique to peroxisomes since they cannot be degraded by mitochondria. These include: (1.) saturated very long-chain fatty acids (C≥ 22 carbon atoms) including hexacosanoic acid (C26:0); (2.) the branched-chain fatty acid pristanic acid (2,6,10,14-tetramethylpentadecanoic acid); (3.) the bile acid intermediates di- and trihydroxycholestanoic acid (DHCA and THCA), and (4.) long-chain dicarboxylic acids [1, 3].

Oxidation of these fatty acids requires the interaction with other organelles, which are different for each of the different fatty acids as outlined below.

1. *Hexacosanoic acid (C26:0)*: it was originally thought that VLCFAs like C26:0 would be derived from dietary products, but early studies, notably by Kishimoto and coworkers [5] already established that the bulk of VLCFAs is not derived from exogenous sources but are synthesized from shorter-chain fatty acids like palmitic acid which itself may be synthesized endogenously via the FAS-complex from acetyl-CoA or can be derived from dietary sources (see Fig. 1). Chain elongation of fatty acids is performed by a multi-enzyme chain elongation complex localized in the ER-membrane with each of the four steps catalyzed by multiple enzymes [6]. The first step in the elongation of fatty acids or rather of the corresponding acyl-CoAs, is brought about by seven different so-called ELOVLs, which catalyze the malonyl-CoA driven production of 3-ketoacyl-CoAs and differ in their tissue distribution and substrate specificities (see [6] [7] for review and Fig. 1). Work from our own laboratory has shown that ELOVL1 is the major enzyme responsible for the formation of C26:0-CoA [8].
Once formed at the ER membrane, the C26:0-CoA needs to traverse the cytosol to reach the peroxisomal membrane. Spontaneous, non-assisted transfer of acyl-CoAs is very unlikely, especially since long-chain acyl-CoAs are only partially soluble in aqueous environments and are, in fact, predominantly membrane-bound [9, 10]. Eukaryotic cells express different members of at least three separate families of cytoplasmic acyl-CoA binding proteins. These include: (1.) acyl-CoA binding protein (ACBP) [11, 12]; (2.) fatty acid binding proteins (FABP) [13, 14], and (3.) sterol-carrier-protein-2 (SCP2) [15, 16]. The affinity of C26:0-CoA for any of these proteins has never been tested experimentally and awaits further studies. Recently, however it has been shown that ACBD5, which is a tail-anchored protein localized in the peroxisomal membrane and equipped with an acyl-CoA binding site, is involved in the transfer of C26:0-CoA from the ER to the peroxisome. The first indication supporting this notion, has come from studies on a patient with clinical signs and symptoms suggestive for a peroxisomal disorder who had elevated VLCFAs which turned out to be caused by mutations in the *ACBD5* gene [17]. This report was soon followed by subsequent work, showing that ACBD5 interacts with the ER-resident VAMP-associated proteins A and B (VAPA and VAPB) and that this interaction is required to tether the two organelles together, thereby facilitating the exchange of metabolites between the two organelles which probably includes C26:0-CoA (Fig. 1). The two VAP proteins are also tail-anchored proteins, are both localized in the ER membrane and contain so-called MSP domains which recognize the FFAT-like motif (two phenylalanines (FF) in an acidic track (AT)) as present in ACBD5 (see Fig. 1). When the interaction of the VAPs with ACBD5 is lost, peroxisome mobility is increased and lipid homeostasis disturbed [18, 19]. Once inside the peroxisome, C26:0-CoA undergoes beta-oxidation via acyl-CoA oxidase 1, D-bifunctional protein, and the two thiolases ACAA1 and sterol carrier protein X (SCPx), respectively (see Fig. 2).

Although not rigorously established, the generally accepted notion holds that C26:0-CoA undergoes a number of cycles of beta-oxidation within peroxisomes until beta-oxidation stops at the level of a C6/C8-medium-chain acyl-CoA. Indeed, in 1978 Lazarow [20] already showed that rat liver peroxisomes were only able to oxidize acyl-CoAs having a chain length of 8 carbon atoms or more. Later work has shown that rat as well as human ACOX1 has two different isoforms produced by the alternative use of two exons 3 which differ in substrate specificity. Rat Acox1-exon3I was found to be most reactive with C10:0-CoA whereas Acox1-exon3II was most reactive with C14:0-CoA. A somewhat different substrate specificity was found for human ACOX1-exon3I and ACOX1-exon3II with the 3I-isoform being most active with C8-C12 acyl-CoAs but not with acyl-CoAs ≥ C16 whereas the 3II-isoform was active with all acyl-CoAs with a chain length of 8 carbon atoms or more up to C24 with C12:0-CoA as best substrate. Both isoforms showed virtually no activity with hexanoyl-CoA [21].

The CoA-esters generated by beta-oxidation in peroxisomes, can be shuttled across the peroxisomal membrane to the cytosol by means of two different mechanisms, including: (1.) a carnitine-mediated pathway involving a carnitine acyl-transferase, and (2.) a free acid route. Whereas the carnitine-mediated export of acyl-CoAs out of the peroxisome is comparable between species, the situation is different for the free-acid route mediated by different acyl-CoA thioesterases. Indeed, in the mouse, four different peroxisomal acyl-CoA thioesterases (Acot3, Acot4, Acot5, and Acot6) were identified as part of a gene cluster spanning 120 kb on mouse chromosome 12D3 which consists of 6 thioesterase genes in total, whereas in humans a similar gene cluster was identified on chromosome 14q24.3 spanning 80 kb which contains only 4 thioesterase genes [22]. Remarkably, only one of these 4 genes turned out to code for a peroxisomal thioesterase [22]. The gene involved (*ACOT4*) showed the highest similarity to the mouse *Acot4* gene but surprisingly the kinetic parameters of the human enzyme were quite different from that of mouse Acot4. Both mouse Acot4 and human ACOT4 show highest activity with succinyl-CoA, but the human enzyme is also active with a range of long-chain acyl-CoAs of > 8 carbon atoms and is also active with several unsaturated acyl-CoAs including C18:1-CoA, C18:2-CoA, and C20:4-CoA [22] which is in marked contrast to the mouse enzyme [23]. Since the pattern of activity of human ACOT4 mirrors the combined activity of mouse Acot3, Acot4 and Acot5, Hunt et al. [24] concluded that the human peroxisomal ACOT4 can replace the function of the three distinct peroxisomal thioesterase enzymes in the mouse.

Interestingly, the expression of carnitine octanoyltransferase (Crot), carnitine acetyltransferase (Crat), and the different acyl-CoA thioesterases was found to differ markedly between mouse tissues as shown by Westin et al. [25]. This was not only true for Crot and peroxisomal Crat, but also for the different Acots. In mice, Crot showed highest expression in the liver and proximal intestine followed by kidney and the distal intestine with low expression in all other tissues, whereas peroxisomal Crat showed low expression in all tissues including liver and the proximal intestine, but showed highest activities in brown adipose tissue (BAT) followed by white adipose tissue (WAT) and heart. Based on these findings Westin et al. [25] concluded that the fate of the medium-chain acyl-CoAs as well as short-chain acyl-CoAs, including acetyl-CoA and propionyl-CoA is different among tissues with: (1.) acetate and propionate being the predominant products in liver, kidney and intestine; (2.) acetyl- and propionylcarnitine in BAT, WAT, and the heart; (3.) medium-chain FAs in brain and WAT, and (4.) medium-chain acylcarnitines in liver, kidney and intestine (see Fig. 3). The underlying physiological relevance of this remarkable difference in expression of the various enzymes has remained unresolved so far (see [25] for discussion). One logical option would be that the acylcarnitines produced in peroxisomes are primarily shuttled to mitochondria for full oxidation to CO2 and H2O as mediated by the mitochondrial carnitine acylcarnitine carrier (CACT; SLC25A20) whereas the short-chain and medium-chain FAs released from peroxisomes, may be shuttled to mitochondria, but can also move to the extracellular space. In line with this, it has long been established that acetate is the primary product of peroxisomal beta-oxidation in the liver as first shown by Leighton and coworkers [26] and is readily released from the liver to be oxidized in other tissues.

1. *Pristanic acid (2,4,10,14-tetramethylpentadecanoic acid)*: Pristanic acid is not synthesized endogenously but is solely derived from exogenous sources either directly or indirectly from phytanic acid after one round of alpha-oxidation. Since the alpha-oxidation machinery is strictly peroxisomal, activation of pristanic acid generated from phytanic acid is believed to occur within the peroxisome whereas the pristanic acid derived from the diet is probably activated by extra-peroxisomal synthetase(s) after which pristanoyl-CoA enters the peroxisome via one of the half-ABC transporters, notably ABCD3 (PMP70) in order to be beta-oxidized. Although not proven definitively, activation of intra-peroxisomal pristanic acid as generated from phytanic acid is probably mediated by the enzyme very long-chain acyl-CoA synthetase encoded by *SLC27A2* which also codes for an isoform in the ER [27]. The identity of the extra-peroxisomal pristanoyl-CoA synthetase has not been identified with certainty (see Fig. 4.). Nevertheless, our own work has shown that peroxisomes as well as mitochondria and microsomes contain pristanoyl-CoA synthetase activity [28].

Intraperoxisomal pristanoyl-CoA undergoes three cycles of beta-oxidation in the peroxisome after which the different products including 4,8-dimethylnonanoyl-CoA, propionyl-CoA and acetyl-CoA are exported out of the peroxisome via the carnitine-mediated route and/or the free-acid route. Indeed, making clever use of fibroblasts with a genetic deficiency of the mitochondrial carnitine acyl-carnitine carrier (CACT/SLC25A20), Verhoeven et al. [29] discovered the accumulation of 4,8-dimethylnonanoyl-carnitine as main product of peroxisomal beta-oxidation next to acetyl- and propionylcarnitine. Subsequent work revealed that CROT is the enzyme which generates 4,8-dimethylnonanoyl-carnitine from the corresponding CoA-ester (Fig. 4) [30]. Furthermore, we established that peroxisomes also contain 4,8-dimethylnonanoyl-CoA thioesterase activity, at least in rat liver peroxisomes [31]. The identity of the thioesterase involved has been determined to be Acot8 (see Hunt and Alexson [32]). It should be noted that *in vivo* pristanic acid occurs in two different stereoisomers, including (2*R,*6*R,*10*R,*14)-tetramethylpentadecanoic acid and (2*S,*6*R,*10*R,*14)-tetramethylpentadecanoic acid. Since acyl-CoA oxidases in general only accept (2*S*)-branched-chain acyl-CoAs as substrate, conversion of (2*R*)- to (2*S*)-acyl-CoAs must occur which is mediated by the enzyme 2-methylacyl-CoA racemase (AMACR). The enzyme involved is a monomeric protein of 43 kDa present in peroxisomes and mitochondria which catalyzes the interconversion of a range of different 2-methyl-branched-chain acyl-CoAs. Interestingly, the peroxisomal and mitochondrial forms of AMACR are the products of a single gene which is targeted to the different organelles by virtue of an amino terminal mitochondrial targeting signal (MTS) and carboxy terminal PTS1 (-KASL in human AMACR).

1. *Long-chain dicarboxylic acids*Dicarboxylic acids are generated from monocarboxylic acids by omega-oxidation via a 3-step mechanism which involves hydroxylation, followed by two subsequent steps of dehydrogenation. Hydroxylation of monocarboxylic acids is catalyzed by one of a variety of different cytochrome P450 enzymes localized at the ER membrane after which the omega-hydroxymonocarboxylic acids are converted into the corresponding dicarboxylic acid through the sequential action of cytosolic long-chain alcohol and aldehyde dehydrogenases. After activation by a specific dicarboxylyl-CoA synthetase, present in microsomes, at least in rat liver [33], the dicarboxylyl-CoA esters are shortened via beta-oxidation. Although *in vitro* studies with isolated organelles have shown that peroxisomes as well as mitochondria can catalyze the oxidation of dicarboxylic acids, studies in intact cells have shown that at least C16DCA is exclusively oxidized in peroxisomes [34]. Although not tested experimentally the C16DCA probably undergoes a number of cycles of beta-oxidation in the peroxisome until a medium-chain dicarboxylyl-CoA is formed which is then transported out of the peroxisome for further oxidation in mitochondria either via the carnitine- or free acid route (see Fig. 5).
2. *Docosahexaenoic acid synthesis (C22:6n-3)*
Docosahexaenoic acid (DHA) is one of the major n-3 polyunsaturated fatty acids (PUFAs) in adult mammalian brain and retina and a deficiency of DHA is associated with memory loss, learning disabilities and impaired vision acuity [35]. DHA can be derived from dietary sources but can also be synthesized endogenously from linolenic acid (C18:3 n-3) via a series of chain-elongation and desaturation reactions. Pioneering work by Sprecher and coworkers [36] has revealed that this pathway cannot synthesize DHA directly since this would require the active participation of a delta-4-desaturase which turned out not to exist in mammalian cells. Instead, C18:3 n-3 is first elongated and desaturated up to C24:6 n-3 which then undergoes one cycle of beta-oxidation in the peroxisome followed by transfer back to the ER for incorporation in different lipid species (Fig. 6) [37, 38].
3. **EFFECTIVE BILE ACID SYNTHESIS REQUIRES A HIGHLY INTERCONNECTED METABOLIC NETWORK INVOLVING THE CYTOSOL, MITOCHONDRIA, ER AND PEROXISOMES**

Although bile acid synthesis is not strictly a “biosynthetic” process in the sense of an anabolic pathway but is in fact a catabolic pathway in which cholesterol, which contains 27 carbon atoms, is ultimately split into two parts, including the primary bile acids, cholic acid and chenodeoxycholic acid - which both contain 24 carbon atoms - and propionic acid, we will discuss bile acid synthesis separately. Formation of cholic acid and chenodeoxycholic acid from cholesterol involves the complicated interaction between a large variety of enzymes localized in different compartments (Fig. 7) which can be separated into 5 distinct parts including: (1.) the conversion of cholesterol to the CoA-esters of DHCA and THCA; (2.) the transport of DHC-CoA and THC-CoA from the ER membrane to the peroxisome, followed by uptake into the peroxisome interior; (3.) the cleavage of the side-chain of DHC-CoA and THC-CoA by beta-oxidation to produce chenodeoxycholoyl-CoA and choloyl-CoA; (4.) the formation of the taurine or glycine esters, and (5.) the export of tauro/glycocholate and tauro/glycochenodeoxycholate out of the peroxisomes to the canalicular membrane followed by extrusion into bile.

Without going into full details about the bile acid synthesis pathway (see [39, 40] for reviews), it is important to emphasize that DHCA and THCA are produced from cholesterol via a series of different enzymes located in the ER, the cytosol and mitochondria and are then activated at the site of the ER membrane. Both Schepers et al. [41] and Prydz et al. [42] resolved that activation of DHCA and THCA is catalyzed by microsomes and not peroxisomes. The identity of the acyl-CoA synthetase involved has not been established definitively although ACSVL1 has been claimed to be the responsible enzyme [43]. This conclusion remains controversial, however, giving the normal levels of the bile acid intermediates in *Acsvl1 -/-* mice [44]. After activation at the ER membrane, DHC-CoA and THC-CoA have to move from the ER to the peroxisomal membrane. Whether this involves one of the acyl-CoA binding proteins like SCP2 has not been established and the same is true for the potential involvement of the VAPB-ACBD5-tether as discussed above for C26:0-CoA. What has been established is that DHC-CoA and THC-CoA enter the peroxisome via the half ABC transporter ABCD3 (PMP70) as recently concluded following the identification of a patient with a full deficiency of ABCD3 (PMP70) in whom there was marked accumulation of the bile acid intermediates combined with similar findings in an Abcd3 knockout mouse [45]. The actual beta-oxidation of DHC-CoA and THC-CoA proceeds via the branched-chain acyl-CoA oxidase (ACOX2), D-bifunctional protein, and SCPx as shown schematically in Fig. 2. Subsequent exchange of the CoA unit present in DHC-CoA and THC-CoA for taurine or glycine is mediated by the enzyme bile acid-CoA:amino acid transferase (BAAT) which was originally thought to be localized both in the cytosol and in peroxisomes, but has been shown to be fully peroxisomal, at least in rat hepatocytes [46]. After the export from the peroxisome, the taurine/glycine esters are shuttled to the bile canalicular membrane which contains a number of different transporters, including the Bile Salt Export Pump (BSEP; ABCB11) which catalyzes the ATP-driven export of tauro/glycocholate and tauro/glycochenodeoxycholate from the hepatocyte into bile.

1. **GLYOXYLATE METABOLISM REQUIRES A HIGHLY INTERCONNECTED METABOLIC NETWORK INVOLVING MITOCHONDRIA, PEROXISOMES AND THE CYTOSOL TO BE EFFECTIVE**

Glyoxylate is a highly toxic substance by virtue of the fact that it may undergo rapid oxidation to oxalate which forms insoluble crystals with calcium. These calcium oxalate crystals precipitate in various organs, notably the kidneys, causing renal failure. This implies that glyoxylate needs to be detoxified rapidly since otherwise glyoxylate will be converted into oxalate via the enzyme lactate dehydrogenase which is an ubiquitous enzyme expressed in all eukaryotic cells. Although not all aspects of glyoxylate metabolism have been resolved definitively it is clear that glyoxylate is produced in different compartments, including the mitochondria (from 4-hydroxyproline, a degradation product of collagen), the cytosol, and in peroxisomes (see Fig. 8). The glyoxylate generated within mitochondria and the cytosol, is rapidly converted into glycolate by the enzyme glyoxylate reductase (GR/HPR) which has a mitochondrial and cytosolic isoform. The high NADPH/NADP+ ratios in both the mitochondrial and cytosolic space drive the GR/HPR reaction into the direction of glycolate formation which ensures that glyoxylate will be rapidly detoxified at least under energized conditions when the NADPH/NADP+ ratio is high (> 100). The peroxisome plays a unique role in the detoxification of glyoxylate since the only way to get rid of the glycolate generated in the mitochondrion and cytosol definitively is to convert glyoxylate into glycine within the peroxisome via the concerted action of the two peroxisomal enzymes glycolate oxidase (HAO1) and alanine glyoxylate aminotransferase (AGXT). The crucial role of GR/HPR and AGXT in the detoxification of glyoxylate as depicted in Fig. 8 is exemplified by the existence of two different forms of hyperoxaluria in man in which glyoxylate metabolism is disturbed due to mutations in the genes coding for *GR/HPR* and *AGXT* [47]. The hyperoxaluria in these two genetic disorders is associated with usually very severe clinical signs and symptoms including the loss of kidney function which in many cases can only be resolved by a combined liver-kidney transplantation.

1. **EFFECTIVE PHYTANIC ALPHA-OXIDATION REQUIRES A HIGHLY INTERCONNECTED METABOLIC NETWORK INVOLVING PEROXISOMES AND MITOCHONDRIA**

Another unique function of peroxisomes involves the alpha-oxidation of 3-methyl branched-chain fatty acids. The latter FAs can only be degraded by beta-oxidation if the terminal carboxy group is first released by alpha-oxidation to generate the corresponding 2-methyl FA which can then be oxidized by beta-oxidation. Like all FAs 3-methyl FAs can also undergo omega oxidation although this is usually regarded as a minor metabolic pathway [48]. Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is the most predominant 3-methyl FA present in our daily diet and as such it is taken up into cells either in its free acid form or in an esterified form since phytanic acid is known to occur in multiple different lipid species, including lipoproteins [49].

After release of phytanic acid from these different lipid species which (mainly) occurs in the lysosome, phytanic acid needs to be converted into phytanoyl-CoA via one of the many members of the ACS family. Although the identity of the enzymes involved has not been established with certainty, enzymatic studies have shown that activation of phytanic acid can occur in mitochondria, peroxisomes and the ER (see [48]). After activation, phytanoyl-CoA has to be transported to the peroxisome, followed by transport into the organelle, probably mediated by ABCD3 (PMP70) [45]. Once inside, the alpha-oxidation machinery consisting of phytanoyl-CoA 2-hydroxylase, 2-hydroxyacyl-CoA lyase, pristanal dehydrogenase, and pristanoyl-CoA synthetase takes care of the oxidative decarboxylation of phytanoyl-CoA to produce pristanoyl-CoA which then undergoes beta-oxidation as described above. As described in detail in our 2011 review [48], alpha-oxidation in peroxisomes heavily relies on the proper interaction with mitochondria for the provision of 2-oxoglutarate required in the phytanoyl-CoA hydroxylase reaction, the reoxidation of the NADH generated in the pristanal dehydrogenase step and the provision of ATP as required in the pristanoyl-CoA synthetase step (see Fig. 9). Although all these molecules are in principle water-soluble, future work has to reveal whether the close interaction between peroxisomes and mitochondria with respect to alpha-oxidation requires tethering of the organelles or not.

1. **EFFECTIVE ETHER PHOSPHOLIPID SYNTHESIS REQUIRES A HIGHLY INTERCONNECTED METABOLIC NETWORK INVOLVING PEROXISOMES AND THE ER**

Another major function of peroxisomes concerns the biosynthesis of ether phospholipids of which at least the first few steps are unique to peroxisomes. The underlying basis for the unique role of peroxisomes in EPL synthesis is the fact that higher eukaryotes only contain a single enzyme able to catalyze the formation of the characteristic ether-bond in ether phospholipids. This unique enzyme is alkylglycerone phosphate synthase (AGPS), which catalyzes the formation of alkylglycerone phosphate from acylglycerone-3-phosphate. The acylglycerone-3-phosphate required in the AGPS reaction is provided by the enzyme glycerone-3-phosphate acyltransferase (GNPAT) from glycerone-3-phosphate and an acyl-CoA ester whereas the long-chain alcohol is provided by one of two different acyl-CoA reductases encoded by *FAR1* and *FAR2*. Interestingly, GNPAT and AGPS form a heterotrimeric enzyme complex attached to the peroxisomal membrane facing the peroxisome interior whereas the acyl-CoA reductase is tail-anchored into the peroxisomal membrane facing the cytosol. All three enzymes play an indispensable role in EPL biosynthesis as exemplified by the finding that EPL biosynthesis is fully deficient in patients with a deficiency of AGPS, GNPAT and FAR1 (see [50-52]). The product of the concerted action of AGPS, GNPAT and FAR1/2 is alkylglycerone-3-phosphate which is then converted into alkylglycerol-3-phosphate followed by a series of reactions not specified here but all localized in the ER [53-55]. The identity of the enzyme which catalyzes the reduction of alkylglycerone-3-phosphate into alkylglycerol-3-phosphate has long remained an enigma until recently [56], although the enzyme was already purified by LaBelle and Hajra in 1974 [57]. The gene involved is *Dhrs7b* and the protein is named PexRAP (for peroxisomal reductase activating PPARgamma) [56]. In contrast to the findings in the *Gnpat (-/-)* mouse in which EPLs were found to be completely deficient [58], EPLs were only partially deficient (50- 80%) in the *Dhrs7b (-/-)* mice. Apparently, PexRAP is not fully obligatory for EPL biosynthesis like FAR1, AGPS, and GNPAT, which may either point to the existence of a second reductase or an alternative pathway which allows formation of alkylglycerone-3-phosphate via some bypass. It has been suggested that this bypass involves dephosphorylation of alkylglycerone-3-phosphate to alkylglycerone, followed by its reduction to alkylglycerol and phosphorylation to produce alkylglycerol-3-phosphate [59]. Whatever the mechanism, the alkylglycerone-3-phosphate needs to be shuttled from the peroxisome to the ER where all subsequent steps take place. It has been suggested that the VAPB-ACBD5 tether plays a role in this process [19].

**Conclusions**: peroxisomes play a crucial role in cellular metabolism as exemplified by the existence of many different genetic diseases in which one or more peroxisomal functions are impaired. Most of these metabolic functions of peroxisomes are catabolic which implies the formation of degradation products. Ultimate degradation of these products to CO2 and H2O requires the active participation of mitochondria since the citric acid cycle and oxidative phosphorylation system which are needed for this purpose are solely confined to mitochondria. The interdependence between peroxisomes and mitochondria is true for all major functions of peroxisomes including fatty acid alpha- and beta-oxidation, glyoxylate detoxification but not ether phospholipid biosynthesis. Apart from the obligatory connection between peroxisomes and mitochondria, peroxisomes also require interaction with the ER for EPL synthesis, bile acid formation and fatty acid beta-oxidation and the same is true for the interaction between peroxisomes and lysosomes since several of the FAs degraded by alpha- and/or beta-oxidation in the peroxisomes are generated within lysosomes. At present, we are only at the very beginning of the understanding of the mechanisms behind these interactions and future will tell the exact extent and nature of these inter-organellar interactions.

**ACKNOWLEDGEMENT**

Furthermore, the authors would like to thank Jos Ruiter for preparation of the figures and Maddy Festen for preparation of the manuscript.

**LEGENDS TO FIGURES**

**Figure 1.** Very long-chain fatty acid homeostasis in human cells and the complicated interplay between a variety of different subcellular organelles including peroxisomes, lysosomes, mitochondria, the cytosol, and the endoplasmic reticulum (ER). Very long-chain fatty acids like C26:0 start their life as acetyl-CoA in the cytosol followed by their conversion into C16:0-CoA by the Fatty Acid Synthase (FAS)-complex in the cytosol. Subsequently, C16:0-CoA undergoes chain-elongation at the endoplasmic reticulum membrane via repetitive cycles of chain elongation after which the C26:0-CoA produced at the site of the endoplasmic reticulum (ER) is shuttled to the peroxisomal membrane probably mediated by the VAPB-ACBD5 peroxisome-ER tethering complex. After transport of C26:0-CoA across the peroxisomal membrane via ABCD1, beta-oxidation occurs within the peroxisome, followed by full oxidation of the end products of peroxisomal beta-oxidation in mitochondria. Apart from *de novo* formation of VLCFAs via the concerted action of the FAS-complex and the chain elongation system in the ER, VLCFAs can also be generated within lysosomes following the degradation of VLCFA-containing (complex) lipids.

Figure 2. Schematic diagram, showing the identity of the different peroxisomal beta-oxidation enzymes, involved in the oxidation of C26:0, pristanic acid, di-and trihydroxycholestanoic acid, and long-chain dicarboxylic acids.

Figure 3. Schematic diagram, depicting the basic principle of C26:0-CoA beta-oxidation in peroxisomes and the transfer of the products of peroxisomal beta-oxidation of C26:0-CoA including acetyl-CoA and a medium-chain acyl-CoA to the cytosol, either as free acid or as carnitine ester and their different fates depending upon the type of tissue.

Figure 4. Schematic diagram depicting the oxidation of pristanic acid in peroxisomes. Pristanic acid is derived from phytanic acid (3,7,11,15)-tetramethylhexadecanoic acid which occurs in two different isoforms including (3*R*, 7*R,* 11*R,* 15)-tetramethylhexadecanoic acid and (3*S*, 7*R,* 11*R,* 15)-tetramethylhexadecanoic acid. The two different isomers can be activated by one of a variety of different acyl-CoA synthetases and the two phytanoyl-CoA esters are then transported across the peroxisomal membrane by ABCD3. Once inside, (3*S*)- and (3*R*)-phytanoyl-CoA undergo alpha-oxidation to produce ((3*S*, 6*R,*10*R,*14)-pentadecanoyl-CoA and (2*R*, 6*R,* 10*R,*14)-tetramethyl pentadecanoyl-CoA which then undergo three rounds of beta-oxidation in peroxisomes to produce 4,8-dimethylnonanoyl-CoA (4,8-dimethyl C9-CoA), propionyl-CoA and acetyl-CoA which are then transported out of the peroxisome via two different routes including the free-acid route or the carnitine-mediated route. (2*R*, 6*R,* 10*R,*14)-pristanoyl-CoA can only undergo beta-oxidation if the (2*R*)-group is converted into the (2*S*)-group by means of the enzyme 2-methylacyl-CoA racemase (AMACR).

Figure 5. Synthesis and degradation of long-chain dicarboxylic acids and the complicated interplay between different subcellular organelles. A typical long-chain dicarboxylic acid like hexadecadioic acid can either be formed *de novo* from acetyl-CoA as mediated by the FAS-complex or can be produced directly from palmitic acid via the omega-oxidation system in the endoplasmic reticulum with CYP4A and/or CYP4F as the main cytochrome P450 hydroxylases catalyzing the formation of omega-hydroxy-C16:0-CoA followed by the formation of the dicarboxylic acid via one of two different mechanisms involving CYP4A and/or CYP4F or an alternative route including a alcohol and aldehyde dehydrogenase. This is followed by activation of the dicarboxylic acid via an ER-bound acyl-CoA synthetase followed by transfer of the CoA-ester to the peroxisomal membrane, transport across the peroxisomal membrane by means of ABCD3 and beta-oxidation in the peroxisome. The products of dicarboxylic acid beta-oxidation in peroxisomes include acetyl-CoA and probably different medium-chain dicarboxylyl-CoAs whose identify has not been established definitively, followed by their transport out of the peroxisome most likely in their free-acid form.

Figure 6. Formation of docosahexaenoic acid (C22:6 n-3) and its subsequent incorporation into complex lipids. Docosahexaenoic acid is produced from C16:3 (n-3)-CoA which undergoes chain elongation and desaturation at the endoplasmic reticulum membrane to produce C24:6 (n-3)-CoA which is then transported to the peroxisomal membrane after which import of C24:6 (n-3)-CoA occurs possibly via ABCD2. C24:6 (n-3)-CoA then undergoes one cycle of beta-oxidation after which the C22:6 (n-3)-CoA is converted into the free acid followed by its export from the peroxisome back to the endoplasmic reticulum for incorporation into complex lipids.

Figure 7. Formation of the primary bile acids cholic acid and chenodeoxycholic acid from cholesterol and the complicated interplay between different subcellular organelles including the endoplasmic reticulum, mitochondria, cytosol and peroxisomes. Bile acids start their life as cholesterol which is converted into di- and trihydroxycholestanoic acid via the concerted action of a large number of different enzymes, localized in the endoplasmic reticulum, mitochondrion and cytosol. Both DHCA and THCA are activated at the site of the endoplasmic reticulum membrane and are then transferred to the peroxisomal membrane where uptake into the peroxisome takes place via ABCD3. After one round of beta-oxidation chenodeoxycholoyl-CoA and choloyl-CoA are produced which are then converted into the corresponding taurine and/or glycine esters via the enzyme bile acid-CoA: aminoacid transferase (BAAT) which is exclusively localized in peroxisomes. After export of tauro/glycocholate and tauro/glycochenodeoxycholate the primary bile acids are extruded into bile mediated by the bile canalicular transporter ABCB11, also known as bile salt export protein (BSEP).

Figure 8. Glyoxylate homeostasis in human hepatocytes. Glyoxylate is either produced in the cytosol or inside mitochondria from 4-hydroxyproline. In mitochondria glyoxylate is converted into glycolate via the enzyme glyoxylate reductase (GR/HPR) followed by export of glycolate to the cytosol and subsequent uptake into peroxisomes,probably via one of the peroxisomal porines including PXMP2. Once inside the peroxisome glycolate undergoes oxidation to glyoxylate via the enzyme glycolate oxidase (HAO1) after which glyoxylate is converted into glycine via the enzyme alanine glyoxylate aminotransferase (AGXT). The glycine is then shuttled back to the mitochondrion where glycine is cleaved via the glycine cleavage system to CO2 and H2O.

Figure 9. Phytanic acid metabolism in human cells and the complicated interplay between peroxisomes and mitochondria. Phytanic acid first undergoes activation via one of several different acyl-CoA synthetases to produce phytanoyl-CoA which then enters the peroxisome via ABCD3 (PMP70). The first step involved in phytanic acid alpha-oxidation is catalyzed by the enzyme phytanoyl-CoA 2-hydroxylase which generates 2-hydroxyphytanoyl-CoA from phytanoyl-CoA. This enzyme reaction requires 2-oxoglutarate which is derived from the mitochondrion in the citric acid (Krebs) cycle (see Figure). The product of the phytanoyl-CoA hydroxylase reaction is succinate which is then shuttled back to the mitochondrion to enter the citric acid cycle and produce 2-oxoglutarate which can then undergo another cycle of phytanoyl-CoA 2-hydroxylase mediated conversion of phytanoyl-CoA into 2-hydroxyphytanoyl-CoA. Furthermore, the NADH produced in the pristanal dehydrogenase step in peroxisomes is ultimately reoxidized by the oxidative phosphorylation system in mitochondria as mediated by the malate/aspartate- or glycerol-3-phosphate/dihydroxyacetone phosphate-shuttle (see text for more details).

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