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The biochemistry and physiology of long-chain dicarboxylic acid metabolism

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Abstract

Mitochondrial β-oxidation is the most prominent pathway for fatty acid oxidation but alternative oxidative metabolism exists. Fatty acid ω-oxidation is one of these pathways and forms dicarboxylic acids as products. These dicarboxylic acids are metabolized through peroxisomal β-oxidation representing an alternative pathway, which could potentially limit the toxic effects of fatty acid accumulation. Although dicarboxylic acid metabolism is highly active in liver and kidney, its role in physiology has not been explored in depth. In this review, we summarize the biochemical mechanism of the formation and degradation of dicarboxylic acids through ω- and β-oxidation, respectively. We will discuss the role of dicarboxylic acids in different (patho)physiological states with a particular focus on the role of the intermediates and products generated through the peroxisomal β-oxidation. This review is expected to increase the understanding of dicarboxylic acid metabolism and spark future research.

Introduction

Fatty acid oxidation (FAO) is a metabolic process that provides energy in times of starvation and metabolic stress and in the post-absorptive state. In these physiological situations, circulating lipid levels increase through the generation of free fatty acids via adipose tissue lipolysis and very-low-density lipoprotein particles through hepatic lipogenesis. Two organelles have the capacity to metabolize fatty acids through β-oxidation, the mitochondrion and the peroxisome. The primary pathway for long-chain FAO is mitochondrial [1], while longer-chain fatty acids (>C22) are selectively metabolized in the peroxisome [2]. In both organelles, FAO sequentially removes acetyl-CoA units from the acyl-CoA chain. Acetyl-CoA generated through mitochondrial FAO is a substrate for ketone body synthesis and fuels the tricarboxylic acid (TCA) cycle. Reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) are also formed through mitochondrial FAO and are used in oxidative phosphorylation to generate ATP

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directly. Ketone bodies serve as an auxiliary fuel to glucose, particularly in the brain. Thus, mitochondrial FAO is crucial in energy homeostasis. The peroxisomal function is crucial for the homeostasis of a variety of carboxylic acids, such as very long-chain fatty acids (VLCFA, >C22) and prevents toxicity due to their accumulation. The importance of mitochondrial and peroxisomal FAO in human physiology is highlighted by patients with inherited disorders caused by defects in these pathways. Patients with mitochondrial FAO defects display a clinical phenotype that may include fasting-induced hypoketotic hypoglycemia, cardiomyopathy, heartbeat disorders, sudden infant death, myopathy and rhabdomyolysis [1]. Defects in peroxisomal FAO also lead to multisystem disease and often involve the nervous system [2–4]. Neonatal screening for mitochondrial FAO disorders allows early intervention and has reduced the number of infants with neurologic damage or sudden death due to hypoglycemia. Unfortunately, some of the symptoms displayed by patients with FAO defects such as myopathy respond poorly to dietary interventions and other treatments [5]. Peroxisomal FAO disorders often lead to severe neurological disease with no curative treatment options available [4]. Therefore, there is a clear need to improve the understanding of the pathophysiology of FAO disorders to develop better therapies.

Mitochondrial β-oxidation is quantitatively the most important pathway for FAO, but alternative metabolic pathways are induced as an auxiliary mechanism when mitochondrial β-oxidation is overloaded or defective. One of these is peroxisomal β-oxidation [6]. While peroxisomes normally oxidize specific carboxylic acids such as VLCFAs, branched-chain fatty acids, bile acid precursors, and fatty dicarboxylic acids (DCAs), they can also contribute to the β-oxidation of medium and long-chain fatty acids that are typically handled by mitochondria [7,8]. Under specific physiological or pathological conditions such as fasting or a mitochondrial FAO defect, respectively, long-chain fatty acids undergo microsomal ω-oxidation, generating DCAs, which are chemically defined by two carboxyl groups, one at each side of the aliphatic chain (α- and ω-carbons). DCAs then undergo peroxisomal β-oxidation [9–14], which generates chain-shortened DCAs and acetyl-CoA units. DCA β-oxidation is particularly prominent when lipid flux is increased and when the mitochondrial FAO is impaired, which is demonstrated by the increased levels of DCAs in urine (dicarboxylic aciduria, **see** DCAs in human disease). Thus, fatty acid ω-oxidation and subsequent DCA β-oxidation may be an important metabolic pathway under lipid metabolic stress. However, compared with the β-oxidation of monocarboxylic acids, ω-oxidation is a poorly understood metabolic pathway. Isolated defects in DCA metabolism have not been described yet and detailed information on the physiological significance of DCA metabolism is not available. The biochemical mechanism of DCA β-oxidation has only been partially resolved. In this review, we will describe the current knowledge on DCAs, including their metabolism, the compartmentalization of DCA metabolism, the physiological functions of DCAs, and the association of DCAs with human disease. We will also identify gaps in our knowledge and define future directions for research.

DCA generation by ω-oxidation of monocarboxylic acids

Historical perspective

Fatty acid ω-oxidation was first described by Verkade et al. [15]. They administered triundecanoin, a triglyceride made of undecanoic acid (C11 fatty acid), to healthy subjects, including Verkade himself. Unexpectedly, they found a large amount of undecanedioic acid (C11-DCA) excreted in the urine [15]. This was the first observation of ω-oxidation *in vivo*, but the biochemical pathway was still unknown. In 1961, three independent groups started to elucidate the mechanism of fatty acid ω-oxidation *in vitro*. Wakabayashi and Shimazono demonstrated the formation of a hydroxylated intermediate in the ω-oxidation of sorbic acid (C6:2) to muconic acid (C6:2-DCA). This reaction was catalyzed by an enzyme that they called a “mixed-function oxidase”, which was present in the microsomal fraction of the guinea pig liver [16]. Robbins reported similar findings for the ω-oxidation of medium-chain fatty acids (C8-C12) by mammalian liver and kidney homogenates to their corresponding DCAs and described in more detail the biochemical reactions [17]. The first reaction is the microsomal conversion of the monocarboxylic acid to the ω-hydroxy acid and requires NADPH (nicotinamide adenine dinucleotide phosphate, reduced) and oxygen (Figure 1). Mitz and Heinrikson isolated an NAD^+-dependent alcohol dehydrogenase that catalyzed the second step, the ω-hydroxy acid conversion to an ω-oxo acid (Figure 1) [18]. In the last step, the ω-oxo acid is oxidized to the corresponding DCA in an NAD^+-dependent reaction catalyzed by an aldehyde dehydrogenase (Figure 1) [19]. Other cofactors required in these reactions are ATP and Mg^{2+}. Preiss and Bloch reported the first proof of ω-oxidation of physiological long-chain fatty acids in rat liver homogenates in 1964. While investigating the desaturation of stearate and palmitate to oleate and palmitoleate in rat liver preparations, they found that more polar metabolites were produced in smaller amounts. Among these polar metabolites, they identified ω-hydroxy acids, ω−1-hydroxy acids and DCA derivatives of these fatty acids [20]. Combined, these studies provide the basis for a more detailed description of the ω-oxidation pathway that came with the discovery of the role of cytochrome P450 (CYP) enzymes in the first step of ω-oxidation.

The first reaction in ω-oxidation is the microsomal NADPH-dependent hydroxylation of the monocarboxylic acid to an ω-hydroxy acid (Figure 1). In the early 1960s, it was known that this reaction could take place in microsomes, i.e. artificially fragmented membrane vesicles that originate from the endoplasmic reticulum [21], but the identity of the enzyme was unknown. Two independent groups identified CYP and NADPH-cytochrome p450 oxidoreductase as two enzymes involved in the hydroxylation of lauric acid and stearic acid [22,23]. Later, the third component of the first hydroxylation reaction of ω-oxidation, NADH-cytochrome b_5, was discovered [24].

CYP enzymes

CYP enzymes are well known for their role in hepatic drug metabolism and xenobiotic detoxification [25], but they also metabolize endogenous compounds. The CYP enzymes that catalyze the ω-oxidation of fatty acids to DCAs belong to the CYP4A and CYP4F subfamilies [26–28]. CYP4A11 and CYP4A22 enzymes in humans, and Cyp4a10, Cyp4a12a, Cyp4a12b and Cyp4a14 in mice, catalyze the 12-hydroxylation of lauric acid

to 12-ω-hydroxy lauric acid [28], the first step in the generation of dodecanedioic acid (C12-DCA, Figure 1). The initial studies suggested that ω-oxidation was restricted to saturated fatty acids of medium-chain length [19,29]. The affinity of the ω-hydroxylation enzymes for saturated monocarboxylic acids is variable depending on the chain length of the fatty acid. Mortensen et al. reported a higher affinity for capric acid (C10, K_m = 0.003 mM) and lauric acid (C12, K_m = 0.2 mM) than for caprylic acid (C8, K_m = 8.2 mM) or caproic acid (C6, K_m = 8.8 mM) in microsomes isolated from rat liver [30]. However, CYP enzymes hydroxylate many different fatty acids besides medium-chain saturated fatty acids, and the substrate preference is different for each CYP enzyme.

Most studies have investigated the ω-hydroxylation of lauric acid (C12) by CYP4A enzymes. Besides lauric acid, human CYP4A11 is active toward myristic acid (C14), palmitic acid (C16), stearic acid (C18), oleic acid (C18:1 omega-9), and arachidonic acid (C20:4 omega-6) [31,32]. CYP4A22 is another CYP4A enzyme that is active toward lauric and palmitic acid [33]. CYP4F enzymes also have ω-hydroxylase activity toward fatty acids, including CYP4F2, the major arachidonic acid ω-hydroxylase [34,35]. CYP4F2, together with CYP4FB3, are also active toward very long-chain fatty acids (C>22), a peroxisomal substrate [27,36,37]. Phytanic acid, another peroxisomal substrate, can also be metabolized by ω-oxidation, which became evident from studies in Refsum disease patients [38,39]. In this case, the CYP enzymes that catalyze phytanic acid ω-oxidation in humans are CYP4F3A, CYP4F3B, CYP4F2, and CYP4A11 [40–42].

Regulation of ω-oxidation

The formation of DCAs by ω-oxidation and their subsequent β-oxidation is regulated by similar pathways, as exemplified by the parallel induction of peroxisomal β-oxidation enzymes and CYP-mediated microsomal ω-oxidation by hypolipidemic drugs of the fibrate class [6,43–46]. In this review, we will focus on the regulation of enzymes of the CYP4A and CYP4F families, the major fatty acid ω-hydroxylases. The expression of the most relevant CYP enzymes for fatty acid ω-oxidation (CYP4A11, CYP4A22, CYP4F2, CYP4F3) is particularly high in liver and kidney and, in the case of CYP4F enzymes, also in the small intestine. These organs rely heavily on FAO for energy homeostasis, in line with the supportive role of ω-oxidation. In human liver, the expression of *CYP4A11* is significantly higher than *CYP4A22* [47]. CYP4A11 is also the major lauric ω-hydroxylase in human liver [26].

Specific physiological and pathophysiological conditions such as fasting, a high-fat diet, or diabetes, induce the expression of CYP4A enzymes. The induction of CYP4A enzymes by fibrates and these different stimuli is mediated by peroxisome proliferator-activated receptor α (PPARα) [48–52]. PPARα is a member of the nuclear receptor family and regulates the expression of genes involved in fatty acid homeostasis. PPARα binds as a heterodimer with the retinoid X receptor (RXR) to peroxisome proliferator response elements (PPREs) in the promoter region of target genes [53]. CYP4A induction by PPARα is coordinated with the transcriptional induction of mitochondrial and peroxisomal β-oxidation enzymes [54], in line with the role of ω-oxidation in the facilitation of FAO. Free fatty acids are activating ligands of PPARα [55], thereby creating an autoregulatory loop in fatty acid homeostasis

that regulates the activation of the microsomal, mitochondrial and peroxisomal fatty acid metabolizing pathways.

One interesting aspect of the effects of peroxisome proliferators and the induction of CYP4A enzymes by PPARα is the differential response between rodents and humans. Chronic exposure to peroxisome proliferators leads to hepatomegaly and hepatocellular carcinoma only in rodents and has been associated with a disproportionate increase in peroxisomal hydrogen peroxide production [45,56,57]. Induction of *CYP4A11* has been described after overexpression of PPARα and treatment with a peroxisome proliferator in HepG2 cells [47]. However, treatment with peroxisome proliferators only induces CYP4A enzymes robustly in mouse hepatocytes and not in human hepatocytes [58,59], and PPREs have been characterized in rodent CYP4A genes but not in human CYP4A genes [54,60]. These data suggest that PPARα may not mediate CYP4A induction in humans. The development of a mouse model containing a human *CYP4A11* transgene allowed the study of human *CYP4A11* regulation *in vivo* [61]. CYP4A11 transgenic mice exhibited an increase in hepatic CYP4A11 mRNA and protein levels after fasting or treatment with fibrates in a PPARα-dependent manner [61]. Thus, the importance of PPARα-mediated regulation of CYP4A enzymes in humans remains to be determined.

The regulation of the CYP4F enzyme family shows some differences compared to the CYP4A family. Unlike members of the CYP4A family, peroxisome proliferators inhibit *CYP4F2* gene promoter activity, whereas saturated fatty acids stimulate it in a PPARα-independent regulation [62]. Retinoic acids also regulate *CYP4F2* gene activity, with activating and repressive functions mediated by RXRα heterodimers and RARα, respectively [63]. CYP4F2 gene activity is also induced by statins, drugs used for the treatment of hypercholesterolemia, in a process that is mediated by the activation of the sterol regulatory element-binding protein 2 (SREBP2) [64]. This regulatory mechanism by statins may also be shared by CYP4A enzymes [65]. In line with the known role of CYP4F in the metabolism of eicosanoids that act as inflammatory mediators, such as leukotriene B4 [66], CYP4F genes are also regulated by inflammatory cytokines [67].

The basal and inducible expression of CYP4A enzymes shows a marked sexual dimorphism, mainly in rodents. In mice, *Cyp4a12* shows male-specific expression and a sex-specific differential response to fibrates, in which *Cyp4a12* levels only increase in female mice after fibrate treatment [68,69]. *Cyp4a12* expression is inducible by androgens in castrated males and females, especially in the kidney [70]. In the kidney, *Cyp4a14* shows a female-specific pattern of expression, whereas *Cyp4a10* expression is higher in females than in males varying with the strain of mice [69]. Moreover, *Cyp4a14* KO mice present with male-specific hypertension associated with increased plasma androgens and increased *Cyp4a12* expression in male KO kidneys [70]. In rats, *Cyp4a2* expression in liver and kidney is male-specific, whereas *Cyp4a1* and *Cyp4a3* are induced to a greater degree in the liver of male rats after clofibrate treatment [71]. Female rats are also less susceptible to fibrate-induced peroxisome proliferation than males, as judged by the degree of liver hepatomegaly and the induction of peroxisomal β-oxidation enzymes [72,73]. How this sexual dimorphism affects DCA metabolism is not known yet. However, our recent studies in mice with a deficiency of EHHADH, an important peroxisomal enzyme for DCA β-oxidation, have

identified sex-specific differences in the liver and kidneys [13,74] and sexual dimorphism of peroxisomal β-oxidation enzymes in mouse kidneys [74].

Besides their role in fatty acid catabolism, ω-oxidation catalyzed by CYP4A hydroxylases catalyzes the formation of arachidonic acid metabolites, which have been implicated in diverse biological functions, including the regulation of renal vasculature and ion channels. This aspect of ω-oxidation has been reviewed elsewhere [75] and is not part of this review's scope. In this review, we focus on the biological roles of DCAs. To answer this question, we need to know how DCAs are further metabolized after their formation by ω-oxidation. Pettersen found in 1972 that palmitic acid (C16), a long-chain monocarboxylic acid, was the precursor of adipic acid (C6-DCA) excreted in the urine of ketotic rats [76]. Thus, DCAs formed by ω-oxidation of long-chain fatty acids are shortened by β-oxidation. The *where* and *how* of DCA β-oxidation have been debated for years. In the next section, we provide the current state-of-the-art on the location and mechanisms of DCA β-oxidation.

DCA β-oxidation

Historical perspective

The early studies of Verkade et al. already suggested that DCAs undergo β-oxidation. When they administered the odd-chain triglyceride triundecanoin, besides large amounts of C11-DCA, they also found small amounts of azelaic acid (C9-DCA) and pimelic acid (C7-DCA) in the urine [77]. The same phenomenon was observed for even-chain triglycerides. Administration of tricaprin, composed of C10 chains, led to the excretion of smaller amounts of suberic acid (C8-DCA) and adipic acid in the urine [77]. These medium-chain DCAs adipic and suberic acids are almost absent in normal human urine so the authors concluded that, most likely, longer-chain DCAs undergo β-oxidation to generate chain-shortened DCAs.

DCA β-oxidation was confirmed by several groups using fatty acids with isotope-labeled carbons. Pettersen detected ^{14}C-labeled adipic acid in urine after administering ^{14}C-labeled palmitic acid (C16) to ketotic rats, proving that long-chain monocarboxylic acids are the precursors of medium-chain DCAs [76]. Passi et al. detected, in serum and urine, ^{3}H- or ^{14}C-labeled DCAs that were 2, 4, or 6 carbon atoms shorter than the injected [10,11-^{3}H]dodecanedioic acid or [1,9-^{14}C]azelaic acid, respectively [78]. Cerdan et al. detected ^{13}C-labeled chain-shortened DCAs derived from [1,2,11,12-$^{13}C_4$]dodecanedioic acid *in situ* in the liver of intact rats by nuclear magnetic resonance spectroscopy [79]. Thus, medium-chain DCAs are formed by ω-oxidation of long-chain fatty acids and subsequent β-oxidation. DCA β-oxidation can start from either carboxyl terminal group, a specific feature of dicarboxylic acids [80,81].

Mitochondrial DCA β-oxidation

The location of DCA β-oxidation has been a matter of debate for years. Different reports have shown that both mitochondria and peroxisomes participate in this process. [9,82–84]. In those first studies, DCA β-oxidation was thought to happen in peroxisomes and mitochondria, the latter being dependent on the carnitine shuttle [30,85]. However, even

though DCA β-oxidation was measured in mitochondrial fractions, it was more efficient in the peroxisomal fraction [85], suggesting that the peroxisome was the major organelle for DCA β-oxidation.

Long-chain FAO in the mitochondrion requires activation of the fatty acid into an acyl-CoA and subsequent transport through the carnitine shuttle [1]. Even though mitochondria can β-oxidize long-chain DC-carnitines such as hexadecanedioylcarnitine (C16-DC-carnitine) *in vitro* under certain conditions, the reaction is 10 times slower than with palmitoylcarnitine (C16-carnitine) [86,87]. Moreover, O_2 uptake with C16DC-carnitine is very low and transitory [86]. Vamecq and Draye observed that, in intact mitochondria from rat liver, dodecanedioic acid is β-oxidized only if the mitochondria had been previously permeabilized with digitonin [83]. Kølvraa and Gregersen reported the production of C4-DCA (succinic acid) in the mitochondria from exogenously added dodecanedioic acid and sebacic acid (C10-DCA) but not from suberic or adipic acid [9]. This could be explained because suberic and adipic acid cannot be transported across the mitochondrial membranes or due to the low affinity of the mitochondrial acyl-CoA dehydrogenases for the CoA esters of suberic and adipic acid. In contrast, exogenously added suberic and adipic acid were β-oxidized in the peroxisome due to the higher affinity of the peroxisomal acyl-CoA oxidase [9]. Moreover, the affinity of mitochondrial carnitine palmitoyltransferases toward DCA-CoA and DC-carnitines is much lower than for the corresponding monocarboxylic acids [10,86]. In a recent study, medium-chain acyl-CoA dehydrogenase (MCAD) was demonstrated to have activity with the CoA esters of dodecanedioic and adipic acid *in vitro* [88]. We recently provided evidence that DCAs are metabolized by mitochondrial fatty acid β-oxidation in mice when their peroxisomal import is not functional [14]. These data suggest that the mitochondrion contributes to DCA β-oxidation, but the extent of that contribution is relatively small. Mitochondrial β-oxidation of DCAs is restricted at the level of transport into the mitochondria and at the dehydrogenation step. It seems evident that mitochondrial DCA β-oxidation is less efficient than peroxisomal β-oxidation and unlikely to be of great physiological significance. However, mitochondria may play role when the peroxisomal machinery for DCA β-oxidation is defective.

Peroxisomal DCA β-oxidation

Lazarow and De Duve discovered that peroxisomes perform β-oxidation in the 70s [6]. They described a system that oxidizes monocarboxylic acids and is insensitive to cyanide, a poison that inhibits mitochondrial respiration [6]. Peroxisomal FAO follows a similar biochemical mechanism as mitochondrial FAO but with specific differences in the substrate specificity, the handling of reducing equivalents, the transport of fatty acids into the organelle, and the enzymes that participate in the process [2]. Most notably, the first step of peroxisomal β-oxidation is catalyzed by acyl-CoA oxidases (ACOX) that reduce oxygen to hydrogen peroxide illustrating the role of this organelle in peroxide metabolism [4,89]. Peroxisomal β-oxidation is induced by clofibrate and other hypolipidemic drugs that induce peroxisomal proliferation in the liver [90,91]. Besides DCAs, peroxisomal β-oxidation handles specific substrates such as VLCFA, bile acid precursors, and branched-chain fatty acids. Defects in peroxisome biogenesis or single peroxisomal enzymes illustrate

the essential role of peroxisomes in human physiology. For more details about diseases caused by peroxisomal defects, the reader is referred to excellent reviews on this topic [2–4].

Mortensen and coworkers discovered one of the clues on the subcellular location of DCA β-oxidation. They found that dodecanedioic acid β-oxidation in rat liver was enhanced by clofibrate, an inducer of peroxisomal β-oxidation [92]. Another interesting observation of Mortensen and his team was that cyanide, a mitochondrial respiratory chain inhibitor, not only did not inhibit DCA β-oxidation but enhanced it, both in control and clofibrate-treated rats [92]. Therefore, they pointed to the peroxisome as the place where DCA β-oxidation occurs. When Mortensen and coworkers incubated peroxisomal fractions with dodecanedioic and sebacic acid, they observed an increase in the concentration of chain-shortened adipic and suberic acid, alongside a decrease in dodecanedioic and sebacic acid amounts [30]. Moreover, hypolipidemic drugs concomitantly increased CYP450 enzyme activity, content, and peroxisomal β-oxidation [56,93,94]. Three studies confirmed that peroxisomes are the primary site for DCA β-oxidation. These studies used human fibroblasts from patients with established mitochondrial or peroxisomal FAO defects [11], hepatocytes from mice with peroxisomal gene knockouts [12], and HEK-293 cells in which selected genes encoding for proteins involved in mitochondrial or peroxisomal β-oxidation were knocked out through CRISPR/Cas9 genome editing [13].

Multiple enzymes accommodating the different substrates can catalyze each of the β-oxidation steps in peroxisomes and mitochondria. The pathway for DCA β-oxidation in peroxisomes is composed of ABCD3, ACOX1, the two bifunctional proteins EHHADH and HSD17B4, and the two thiolases SCPx and ACAA1 (Figure 2). Several lines of evidence support the essential role of ABCD3 (also known as PMP70) in long-chain DCA transport into the peroxisome. First, the expression of the human ABCD3 transporter but not that of human ABCD1 or ABCD2 restored C16-DCA (hexadecanedioic acid) β-oxidation in the β-oxidation-deficient *pxa1/pxa2Δ* yeast mutant [95]. Second, *ABCD3* KO HEK-293 cells are deficient in hexadecanedioic acid β-oxidation [13]. Finally, we recently reported the accumulation of long-chain DCAs in the liver of *Abcd3* KO mice [14], providing *in vivo* evidence for the role of ABCD3 in DCA β-oxidation.

Acyl-CoA oxidases catalyze the first step in peroxisomal β-oxidation. Data from ACOX1 deficient patient fibroblasts and *ACOX1* KO HEK-293 cells revealed a defect in hexadecanedioic acid β-oxidation suggesting that ACOX1 is essential for DCA β-oxidation [11,13]. The other two human acyl-CoA oxidases, ACOX2 and the recently identified ACOX3 [96], seem to play a less significant role in DCA β-oxidation. Although ACOX2 is active toward hexadecanedioyl-CoA in rat liver [97], there is no mention of DCA changes in three reports describing patients with ACOX2 deficiency [96,98,99] and in two patients with ACOX3 deficiency [100]. However, *in vivo* data supporting an essential role of ACOX1 in DCA β-oxidation are also lacking.

L- and D-bifunctional protein (EHHADH and HSD17B4, respectively) catalyze the second and third steps in peroxisomal β-oxidation. Several *in vitro* and *in vivo* studies suggest that EHHADH is more important than HSD17B4 in DCA β-oxidation. Fibroblasts from patients with DBP (HSD17B4) deficiency showed normal hexadecanedioic acid β-oxidation

activity [11]. Because no patients with EHHADH deficiency have been reported yet, the same authors studied the activities of human EHHADH and HSD17B4 enzymes with enoyl-C16:1DC-CoA in the *fox2Δ* yeast mutant. The *fox2* gene is the yeast ortholog of the human *HSD17B4* gene, and these mutants cannot metabolize enoyl-CoAs to the corresponding keto-acyl-CoAs. Both human bifunctional proteins formed 3-keto-C16DC-CoA, but EHHADH had a 3-times lower apparent K_m for C16:1DC-CoA than HSD17B4 [11]. We recently showed that EHHADH and HSD17B4 have overlapping functions in DCA β-oxidation in HEK-293 cells and that their contribution may depend mainly on their expression level [13]. The absence of patients with EHHADH deficiency makes animal models essential for studying EHHADH function. Dirkx et al. reported that tetradecanedioic acid (C14-DCA) β-oxidation was reduced in hepatocyte cultures from *Ehhadh* KO mice but not from *Hsd17b4* KO mice [12]. These studies provided the first evidence that EHHADH has a specific role in DCA metabolism.

EHHADH expression is exceptionally inducible, and the highest levels of expression are found in liver and kidney, the same organs with the highest rates of ω-oxidation and peroxisomal β-oxidation. Consistent with this, a mouse gene coexpression subnetwork using *Ehhadh* as the central node contained other relevant genes for fatty acid ω-oxidation and subsequent DCA β-oxidation, such as *Cyp4a10*, *Cyp4a14*, *Abcd3*, *Acaa1b*, and *Acot4*, among others, suggesting these genes function in a coordinated metabolic network [101]. EHHADH was also identified as a regulatory gene in a CYP450 regulatory transcriptional network in human liver [102]. *In vivo* studies have confirmed the prominent role of EHHADH in DCA β-oxidation. When EHHADH-deficient mice were fed with a coconut oil diet enriched in medium-chain triglycerides or with lauric acid, the generation of DCAs through ω-oxidation with apparently impaired β-oxidation caused severe hepatic toxicity [103]. We recently reported that EHHADH-deficient mice present with an accumulation of medium-chain 3-OH-DCAs and an increased suberic/adipic acid ratio in plasma, liver and urine, indicating an impaired medium-chain DCA β-oxidation [13].

Little is known regarding the last step of peroxisomal DCA β-oxidation. Two thiolases catalyze this last step, SCPx and ACAA1. ACAA1 belongs to the so-called classical peroxisomal β-oxidation pathway, together with ACOX1 and EHHADH [101]. This pathway is highly inducible by PPARα-dependent mechanisms. Indeed, *Acaa1b*, one of the two ACAA1 thiolases present in mice, was present in the gene coexpression subnetwork of mouse *Ehhadh*, suggesting its involvement in DCA β-oxidation [101]. However, patients with rhizomelic chondrodysplasia punctata type I, which lack the mature form of ACAA1, do not present a defect in long-chain DCA β-oxidation [11], arguing against an essential role for ACAA1 in DCA β-oxidation in humans. We recently reported considerable functional overlap between SCPx and ACAA1 in hexadecanedioic acid β-oxidation in HEK-293 cells, with a more important role for ACAA1 in the β-oxidation of medium-chain DCAs [13].

Similar to monocarboxylic acids, DCAs are activated to an acyl-CoA ester before β-oxidation via a dicarboxylyl-CoA synthetase that was identified in the microsomal fraction of rat liver [104–106] and was not able to activate adipic and suberic acid to the corresponding DC-CoA [104]. However, the molecular identity of the dicarboxylyl-CoA synthetase remains obscure. Other work identified a peroxisomal acyl-CoA synthetase,

although not specific for DCAs, on the cytoplasmic side of the peroxisome. The most likely candidates are the long-chain acyl-CoA synthetases ACSL1 and ACSL4 [107,108]. ACSL1 seems a good candidate since it is present in the *Ehhadh*-centered mouse gene coexpression subnetwork that contained other relevant genes for fatty acid ω-oxidation and subsequent DCA β-oxidation [101]. Interestingly, ACSL1 is present in different proteomics studies of hepatic peroxisomes [109,110], and some of these studies have identified an interaction between ACSL1 and ABCD3 [109,111], supporting the idea that ACSL1 activates DCAs to channel them into the peroxisomes. Another candidate is the long-chain acyl-CoA synthetase FATP2 (also known as ACSVL1, and encoded by the *SLC27A2* gene), which has been identified in the endoplasmic reticulum and in the peroxisome [112,113]. However, the role of these acyl-CoA synthetases in peroxisomal DCA metabolism has not been studied yet.

The β-oxidation rates of DCAs decrease as the chain length decreases. Different experiments have shown that suberic and, in particular, adipic acid, are metabolized at much lower rates than longer chain DCAs [10,114–117], which is likely one of the reasons for their appearance in urine. Other reasons for their appearance in urine may be related to their glomerular filtration and tubular reuptake. Fatty acid ω-oxidation and peroxisomal β-oxidation present a very similar specificity pattern, being less active with carbon chains ≤ 8 [118,119]. It has been debated whether fatty acid chain-shortening proceeds beyond C6 in the peroxisome [118,119]. The detection of 3-hydroxy-adipic acid and its derivative 3-hydroxyadipic acid 3,6-lactone in the urine of man and mice [13,120] suggests that peroxisomal DCA β-oxidation proceeds beyond adipic acid, thus generating the anaplerotic substrate succinic acid. We have discussed this possibility in detail in the next section.

The physiological role of DCAs

The physiological relevance of DCA metabolism and ω-oxidation are tightly connected, which is illustrated by the fact that ω-oxidation and urinary DCA excretion are increased under conditions in which the FAO flux is increased, such as fasting, diabetes, high-fat feeding, and after treatment with hypolipidemic drugs of the fibrate class [28,44,121–125]. Under these conditions, ω-oxidation is induced predominantly in metabolically demanding organs such as liver and kidney [19,20,126,127]. The general biological relevance of ω-oxidation has been reviewed by Miura [128]. Here, we will review the physiological roles of fatty DCAs in metabolism, focusing on the role of the intermediates and products generated by the peroxisomal β-oxidation of DCAs.

Energy homeostasis and generation of essential intermediary metabolites

The ω-oxidation of medium-, long-, and very long-chain fatty acids has received considerable interest because this pathway may represent an alternative way to reduce the accumulation of toxic fatty acids in conditions with increased lipid flux, such as fasting, diabetes, and a high-fat diet. However, the physiological role of fatty acid ω-oxidation remains poorly defined. One way to address this problem is to estimate the contribution of ω-oxidation and peroxisomal DCA β-oxidation to total FAO. A first study performed by Antony and Landau in 1968 reported that ω-oxidation contributed 4% to total stearate

(C18) oxidation in rat liver slices [129]. However, they did not consider β-oxidation from the original carboxyl group after ω-oxidation of the fatty acid. Thus, the actual contribution of ω-oxidation was not properly calculated. Other studies in liver of fasted or diabetic animals estimated a relative contribution of ω-oxidation that reached up to 20% of total FAO, depending on the length of the fatty acid used and the experimental conditions, being highest with lauric acid in conditions of increased lipid flux such as starvation and diabetes [81,130,131]. In other tissues with high rates of FAO, such as heart or skeletal muscle, the contribution of DCA β-oxidation to total FAO is very low, sometimes even undetectable [86,87,114,132], most likely as a result of the low level of ω-oxidation in these organs. These data suggest that the contribution of ω-oxidation and subsequent DCA β-oxidation to total FAO is relatively minor in normal conditions but becomes physiologically relevant in conditions of metabolic stress caused by an increased lipid flux, especially in liver and kidney.

Anaplerosis—Anaplerosis is the addition of intermediates to the TCA cycle, which compensates for pathways that consume TCA cycle intermediates (cataplerosis), such as gluconeogenesis or lipid synthesis [133]. DCA metabolism received attention because of its potential contribution to anaplerosis. This is in stark contrast to monocarboxylic fatty acids, which are not anaplerotic. It is generally accepted that peroxisomal β-oxidation of DCAs yields adipic and suberic acid as end-products that are lost in the urine. However, if DCA β-oxidation proceeds for one or two more cycles, respectively, the resulting product succinic acid can enter the TCA cycle [134]. However, the generation of succinic acid in peroxisome DCA β-oxidation has remained controversial. Peroxisomal succinic acid production has been detected after administering longer-chain DCAs even though the affinity of peroxisomal acyl-CoA oxidase towards adipic acid is much lower than for longer-chain DCAs [9,10,135,136]. Wada and Usami's study with ^{14}C-labeled DCAs found a gluconeogenic potential of DCAs that was even higher when the rats were starved or diabetic [81]. Mortensen's observations further corroborated this hypothesis by showing that medium-chain DCAs, such as adipic acid, also showed gluconeogenic potential [137,138]. Several studies using stable isotope-labeled DCAs were consistent with this hypothesis and detected labeled carbons in TCA intermediates, such as succinate and citrate, after administration of the labeled DCA, supporting the previous findings of the anaplerotic potential of DCAs [80,85,114]. Other studies did not find such a contribution [79,139]. More recently, Jin et al. used mass isotopomer analysis and demonstrated the anaplerotic potential of dodecanedioic acid [140]. We documented the excretion of 3-hydroxyadipic acid in the urine of fasted mice [13]. Together with the low but detectable labeling of TCA cycle intermediates in mouse liver slices after incubation with [U-^{13}C]-dodecanedioic acid [13], these data indicate that adipic acid can indeed undergo another round of peroxisomal β-oxidation. These findings, together with the discovery of ACOT4, a peroxisomal acyl-CoA thioesterase with a specific affinity for succinyl-CoA [141,142], provide substantial evidence to support the notion that peroxisomal DCA β-oxidation can generate succinate and thus provide substrates for anaplerosis. In addition, a recent study has suggested that dodecanedioic acid administration can refill the TCA cycle in fibroblasts from patients with very-long chain acyl-CoA dehydrogenase (VLCAD) deficiency [143].

The anaplerotic potential of DCAs and their potential ease of administration (medium-chain DCAs are water-soluble in contrast to long- and medium-chain triglycerides that have to be administered as emulsions) motivated several studies to investigate their use as nutritional compounds [144]. One study showed that odd- and even-chain DCAs were well tolerated and did not provoke side effects in volunteers [144]. Moreover, infusion of dodecanedioic acid in diabetic patients decreased plasma glucose significantly, suggesting that DCAs may be useful as fuel substrates for diabetes patients. However, the anaplerotic potential of DCA β-oxidation under different physiological and pathophysiological situations remains to be established.

Destination of peroxisomal acetyl-CoA units: Cholesterol and lipid metabolism—Peroxisomal DCA β-oxidation generates acetyl-CoA units, presumably released in the form of acetate or acetylcarnitine [145–147]. Administration of DCAs of different chain lengths increases the extramitochondrial pool of acetyl-CoA [115,135,145,148,149]. The most plausible source of this pool of acetyl-CoA is peroxisomal DCA β-oxidation. One of the suggested destinations of this pool of acetyl-CoA is the biosynthesis of cholesterol [149,150]. Recently we confirmed that disruption of peroxisomal DCA β-oxidation in *Ehhadh* KO and *Abcd3* KO mice induces alterations in hepatic cholesterol synthesis [13,14]. In other tissues, such as the heart, peroxisome-derived acetyl-CoA contributes substantially to the cardiac malonyl-CoA pool, thus participating in the regulation of fatty acid oxidation and lipogenesis [151,152]. Alternatively, acetyl-CoA is converted into acetylcarnitine by the peroxisomal carnitine acetyltransferase (CRAT), with subsequent transport to and metabolism in the mitochondrion [147,153]. Peroxisomal DCA β-oxidation may also participate in protein acetylation and succinylation, important regulatory mechanisms that control metabolic processes [154,155].

DCAs in human disease

Another fact that has attracted much attention to DCA metabolism is the detection of significant amounts of medium-chain or long-chain DCAs in urine under various pathological conditions. This is relevant because DCA levels are usually low in the urine of healthy subjects and animals [13,14,122,156]. Currently, dicarboxylic aciduria is a metabolic feature that is helpful for the diagnosis of several inborn errors of metabolism [156], particularly mitochondrial FAO defects. However, they have also been detected in cases of glycogen storage disorders and HMG-CoA lyase deficiency [156]. Urinary DCAs can also be present in acquired conditions such as Reye-like and Reyés syndrome, Jamaican vomiting disease [157], celiac disease [158], and diabetes [159], or even under non-pathological conditions, such as after medium-chain triglyceride (MCT) administration [160,161], or after prolonged fasting [76,122].

In Reyés syndrome patients, the urinary excretion of DCAs appeared to decrease with increasing chain length, with adipic and suberic acid the most abundant DCAs [162,163]. Strikingly, long-chain DCAs were the most abundant in the serum of comatose patients with Reye syndrome [162], suggesting that the chain length of the accumulated DCA may be associated with the severity of the disease. Accumulating medium-chain DCAs themselves can also be toxic and result in liver failure. This was demonstrated in *Ehhadh*

KO mice fed with lauric acid or a coconut oil diet (rich in lauric acid), which accumulate high levels of dodecanedioic acid [103]. In summary, dicarboxylic aciduria may hint at an underlying biochemical defect behind a clinical presentation, but cannot be used as the only biochemical parameter to diagnose an inherited metabolic disease. Moreover, the effects of DCA accumulation on human health are poorly understood. In this section, we review the role that DCA metabolism plays in different diseases.

DCA metabolism in mitochondrial FAO defects

Abnormal large amounts of medium-chain DCAs are detected in the urine of patients with a mitochondrial FAO defect. Dicarboxylic aciduria is a consistent biochemical feature in cases of systemic carnitine deficiency [164], CACT (carnitine acylcarnitine translocase) deficiency [165], CPT2 (carnitine palmitoyltransferase 2) deficiency [166], MADD (multiple acyl-CoA dehydrogenase deficiency) [167], VLCAD deficiency [168], MCAD deficiency [169], LCHAD or MTP (long-chain 3-hydroxyacyl-CoA dehydrogenase/mitochondrial trifunctional protein) deficiency [170] and some cases of CPT1A (carnitine palmitoyltransferase 1A) deficiency [171]. A recent report found increased levels of hexadecanedioic acid in dried blood spots from five severe VLCAD deficiency patients compared with nine mild cases [172]. The presence of urinary DCAs is also characteristic of mouse models of mitochondrial FAO defects [13,173–176]. The most likely biochemical explanation for the increased DCA excretion in many mitochondrial FAO disorders is an increased compensatory fatty acid ω-oxidation and subsequent peroxisomal DCA β-oxidation [92,177]. Another common feature in some mitochondrial FAO disorders is the excretion of unsaturated DCAs originating from oleic and linoleic acids [175,178–181].

Besides urinary DCAs, medium-chain dicarboxylylcarnitines (DC-carnitines) are present in mitochondrial FAO defects, such as severe cases of CPT2 deficiency [166], CACT deficiency [165] and the LCAD (long-chain acyl-CoA dehydrogenase) KO mouse model [13,182]. C6-C12 DC-carnitines have also been detected in the urine of one Reye syndrome case without an underlying metabolic disorder [183]. The metabolic explanation for the presence of these medium-chain DC-carnitines is still unknown. We hypothesize that these DC-carnitines are generated in the peroxisome given the high rate of DCA β-oxidation due to the mitochondrial defects. Indeed, peroxisomes possess the carnitine acyltransferases CRAT and CROT, which are theoretically capable of generating these metabolites [184]. Although CROT is the most likely origin of the DC-carnitines, this remains to be experimentally tested [147]. Another possibility we cannot exclude is that monocarboxylylcarnitines generated from the mitochondrial FAO defect undergo ω-oxidation in the microsomes to generate the DC-carnitines present in plasma and urine.

Impact of DCA metabolism on mitochondrial function

Mitochondria are not well equipped to metabolize DCAs. Thus, a substrate overload of DCAs may have negative consequences on the function of this organelle. For example, the DCA-rich serum of patients with Reye’s syndrome inhibits mitochondrial respiration and ATP synthesis in isolated mitochondria, acting as a mitochondrial uncoupler [185]. The amount of DCAs in the serum of Reye’s syndrome corresponded directly to the reduction in ATP synthesis. Moreover, substantial serum concentrations of DCAs may not be bound

to albumin, increasing the potential toxicity of these metabolites [186]. The antimicrobial action of azelaic acid (C9-DCA), which is used in treating acne and hyperpigmentary disorders [187], is in part mediated by an inhibition of mitochondrial respiration and some mitochondrial respiratory enzymes. This effect was observed in rat liver hepatocytes after adding dodecanedioic acid and other medium-chain DCAs [188]. Sebacic acid induced swelling of respiring mitochondria [189].

Recently, we reported that the mitochondrial FAO machinery metabolizes DCAs in *Abcd3* KO mice, a mouse model of defective peroxisomal DCA β-oxidation [14]. Alongside the detection of medium-chain DCAs and dicarboxylylcarnitines in plasma and liver of *Abcd3* KO mice, we also observed a decrease in the production of medium-chain dicarboxylylcarnitines in liver slices from *Abcd3* KO mice when treated with the CPT2 inhibitor L-aminocarnitine [14]. These results support that DCAs can be metabolized in mitochondria under exceptional circumstances. The mechanisms of DCA import into the mitochondria will need to be established to fully understand the effects of DCAs on mitochondrial function. Besides the low-efficient carnitine shuttle for DCA transport into the mitochondria, other mechanisms have been proposed [189]. Overall, the mechanisms of mitochondrial metabolism of medium- and long-chain DCAs remain largely unknown.

DCA metabolism in peroxisomal disorders

Urinary organic acid analysis is also helpful in the diagnostics of peroxisomal disorders. The central role of peroxisomes in DCA β-oxidation is illustrated by the presence of high levels of long-chain DCAs in urine and long-chain DC-carnitines in plasma (C16- to C22DC-carnitine) in peroxisome biogenesis disorders (PBD) patients [190–192]. We made similar observations in the liver of *Abcd3* KO mice [14]. Plasma long-chain DC-carnitines in patients with DBP (HSD17B4) deficiency are comparable to controls [190], indicating that DCA β-oxidation is functionally normal in DBP deficiency, and thus supporting a major role for EHHADH in DCA β-oxidation. Interestingly, long-chain DC-carnitines accumulate in patients with peroxisome biogenesis defects [190], who do not have β-oxidation enzymes in the peroxisomal matrix, suggesting that, under some conditions, CPT1 may handle long-chain DC-CoAs. This is supported by our observation of increased octadecenedioylcarnitine (C18:1-DC) in the liver of *Abcd3* KO mice [14].

Patients with PBD also excrete adipic acid, suberic acid, and sebacic acid in the urine [192–195]. The presence of these medium-chain DCAs (C6-C10) in the urine of patients who do not have functional peroxisomes suggests that DCA β-oxidation is not exclusively a peroxisomal process and is consistent with our recent findings in the *Abcd3* KO mice, which also accumulate medium-chain DCAs [14]. The pattern of DCA excretion in peroxisomal disorders differs from mitochondrial FAO defects, where adipic acid is the most abundant DCA in urine, followed by suberic acid and sebacic acid. In contrast, PBD patients present a relatively more prominent accumulation of suberic acid and sebacic acid than adipic acid [194], pointing to a block in the conversion of suberic and sebacic acid into adipic acid due to the lack of functional peroxisomes. Data in mouse models of peroxisomal dysfunction are consistent with this impairment in sebacic and suberic acid β-oxidation, as shown by the

increase in the suberic/adipic acid ratio in *Ehhadh* KO mice [13], and the increased levels of suberic and sebacic acid in the urine of *Abcd3* KO mice [14].

Patients with peroxisomal disorders also excrete odd-chain (C7-C15) DCAs [192,194]. The origin of these odd-chain DCAs has not been elucidated yet. They may arise from ω-oxidation of odd-chain fatty acids from dietary origin (such as dairy fat), or as a result of increased odd-chain fatty acid synthesis using propionyl-CoA as a primer, or from decarboxylation of 2-hydroxy fatty acids [192,196,197]. Other groups have proposed that medium-chain odd-numbered DCAs are generated by the peroxidation of polyunsaturated fatty acids [198], thus reflecting lipid peroxidation. However, the specific detection of odd-chain DCAs in peroxisomal disorders with chain lengths longer than 9 carbon atoms strongly suggests that these metabolites are generated in a mechanism similar to even-chain DCAs, i.e. through β-oxidation.

Patients with defects in α-oxidation of phytanic acid to pristanic acid (Refsum disease) excrete 3-methyladipic acid in the urine [38,39]. Moreover, the levels of urinary 3-methyladipic acid correlate with the plasma levels of phytanic acid [199], indicating that phytanic acid can undergo ω-oxidation. Therefore, ω-oxidation is an alternative pathway that enables the metabolism of a fraction of the accumulated phytanic acid. In fact, fatty acid ω-oxidation has been proposed as a rescue pathway in mitochondrial and peroxisomal fatty acid oxidation disorders, aiming to reduce the detrimental effects caused by the accumulation of fatty acids in these disorders [200].

DCA and diabetes

Available evidence supports that alterations in FAO are associated with insulin resistance and thus may contribute to the development of diabetes. Diabetic patients display high rates of FAO, reflected by elevated levels of acylcarnitines [201]. Inhibition of mitochondrial FAO induces the accumulation of ectopic lipids in the skeletal muscle, which is associated with an impairment in insulin signaling [201,202], and a compensatory increase in peroxisomal FAO [203,204]. Two recent reports in mice have shown that the expression of genes involved in fatty acid ω-oxidation and peroxisomal β-oxidation is increased in the liver and kidney of streptozocin-induced diabetic mice [205,206], but DCA levels in plasma or urine were not reported. Under these conditions, one would expect an induction of ω-oxidation. Indeed, diabetic rats and humans present high amounts of medium-chain DCAs in urine and DC-carnitines in plasma and a higher rate of DCA β-oxidation [123,138,159,207–209]. Excessive peroxisomal DCA metabolism would lead to an increased production of hydrogen peroxide, which is formed in the first reaction of peroxisomal β-oxidation [89]. It has been proposed that peroxisome-derived hydrogen peroxide is responsible for pancreatic β-cell lipotoxicity [210]. However, the role of DCAs in the pathophysiology of diabetes is not known yet.

DCAs have been proposed as an alternative nutritional substrate for type 2 diabetes patients based on the idea that their anaplerotic potential will improve glucose uptake in skeletal muscle and thus help prevent hyperglycemia. Animal and human studies have shown that administration of sebacic acid reduces postprandial glycemia in type 2 diabetes patients [211], and improves fasting glycemia and glucose tolerance in diabetic db/db mice [212].

Administration of dodecanedioic acid reduced muscle fatigue during exercise in diabetic patients [213]. Along similar lines, the risk of diabetes in a Chinese population was inversely associated with C10DC-carnitine and C12DC-carnitine plasma levels [214]. These findings suggest that DCAs improve energy utilization and metabolic flexibility. However, this possible beneficial effect of DCAs and the mechanisms behind them need to be better understood and deserve further studies.

Cardiovascular disease

Several studies have reported associations between DCA metabolism and cardiovascular disease. Shah et al. found an association between short- and long-chain DC-carnitines plasma levels and adverse cardiovascular events [215]. Elevated plasma tetradecanedioic and hexadecanedioic acid levels are associated with ischemic stroke [216]. Ruiz et al. found increased DC-carnitine levels (C6DC-, C16DC-, and C18DC-carnitine) together with increased circulating C26-carnitine (a metabolite that accumulates due to peroxisomal dysfunction) in two cohorts of patients with heart failure [217]. Furthermore, plasma levels of hexadecanedioic acid are associated with an increased risk of heart failure in two independent cohorts of patients [218], and also with high systolic and diastolic blood pressure levels [219]. These observations are supported by studies in rats indicating that hexadecanedioic acid increases blood pressure levels when added to the diet [219]. Abnormally elevated levels of long-chain DCAs (C14-, C16-, and C18-DCA) are also present in the lungs of patients with pulmonary arterial hypertension [220]. These data suggest that DCA metabolism plays a relevant role in cardiometabolic health. However, fatty acid ω-oxidation rates are low in the heart, suggesting that the generation of DCAs in cardiac tissue is less likely to be the direct cause of these cardiometabolic problems. Despite the low levels of fatty acid ω-oxidation in the heart, it has been shown that peroxisomal FAO can contribute to the acetyl moiety of cardiac malonyl-CoA [151], and that dodecanedioic acid β-oxidation contributes to the acetyl moiety of cardiac malonyl-CoA [152]. These results indicate that DCA β-oxidation may play a role in the cardiovascular system.

The mechanism behind the association between DCA metabolism and blood pressure changes is not clear. 20-HETE (20-hydroxyeicosatetraenoic acid), a major player in blood pressure regulation [221], is formed by ω-oxidation of arachidonic acid by CYP450 enzymes, mainly CYP4F2 and CYP4A11 [35]. A loss-of-function SNP in *CYP4A11* is associated with hypertension [222], and mice deficient in *Cyp4a10* and *Cyp4a14* (*CYP4A11* orthologs) are more prone to develop hypertension [70,223]. Given the known association between hexadecanedioic acid and blood pressure [218,219], we speculate that peroxisomal DCA β-oxidation may play a role in the control of blood pressure. The underlying mechanisms have not been elucidated yet, but the availability of animal models defective in DCA β-oxidation provides an excellent opportunity to investigate this association further.

Conclusion

Fatty acid ω-oxidation, defined as the biological oxidation of fatty acids at the ω-carbon, is a well-established pathway in animals and plants. The mechanism of ω-oxidation of long-chain monocarboxylic acids has been elucidated in detail. However, the biological

relevance of DCA metabolism remains largely unknown. In particular, there is a lack of understanding of the physiological consequences of alterations in this metabolic pathway. Future studies should aim to fill this knowledge gap. Animal models that are deficient in one of the components of this pathway, such as the *Abcd3, Ehhadh, Cyp4a10* and *Cyp4a14* KO mice, are a great resource for investigating this aspect of DCA metabolism. A better understanding of fatty DCA metabolism may help the development of therapies for inborn errors of metabolism and other metabolic diseases in which DCAs may play a role.

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Abbreviations:

CPT	carnitine palmitoyltransferase
CYP	cytochrome P450
DCA	dicarboxylic acid
FAD	flavin adenine dinucleotide
FAO	fatty acid oxidation
MCAD	medium-chain acyl-CoA dehydrogenase
MCT	medium-chain triglyceride
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
PBD	peroxisome biogenesis disorder
TCA	tricarboxylic acid
VLCAD	very long-chain acyl-CoA dehydrogenase
VLCFA	very long-chain fatty acids

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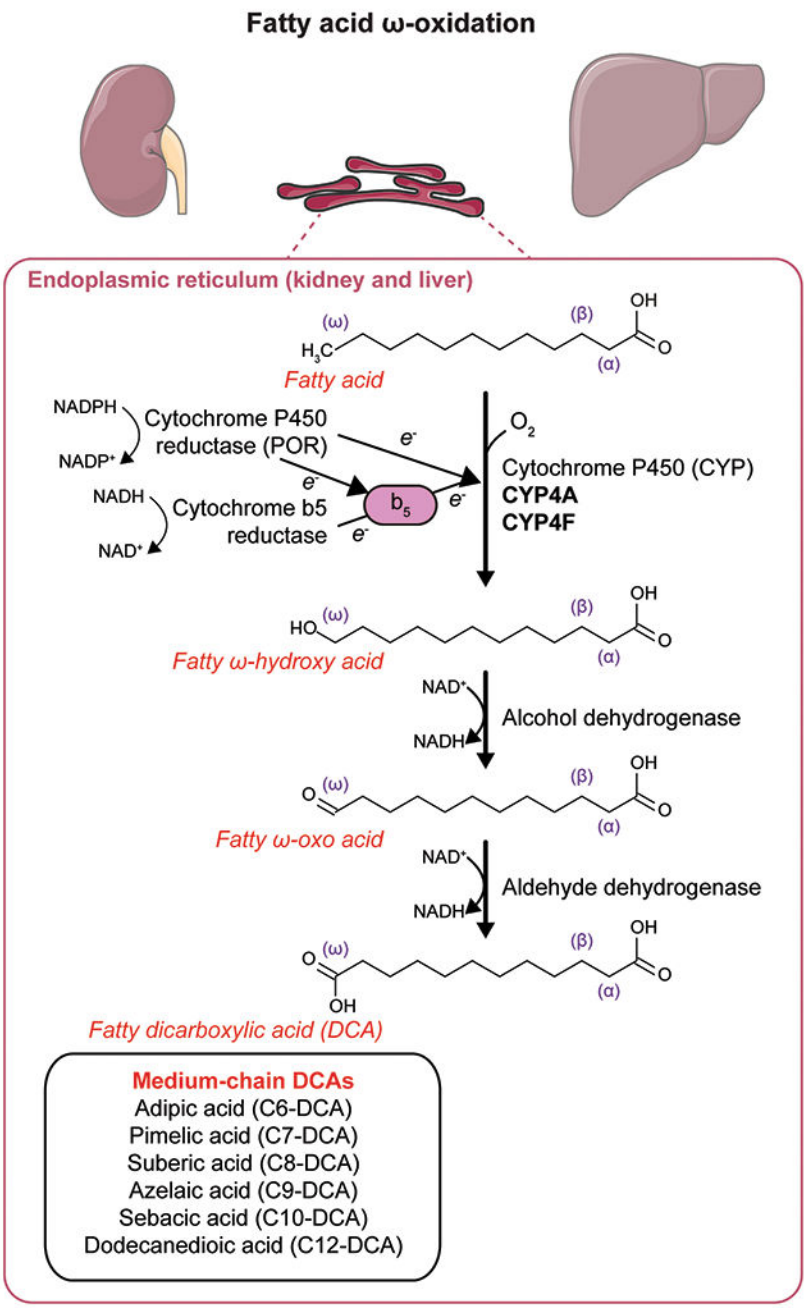

Figure 1.

Enzymatic steps of fatty acid ω-oxidation using the example of lauric acid (C12) ω-oxidation to dodecanedioic acid (C12-DCA). This figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

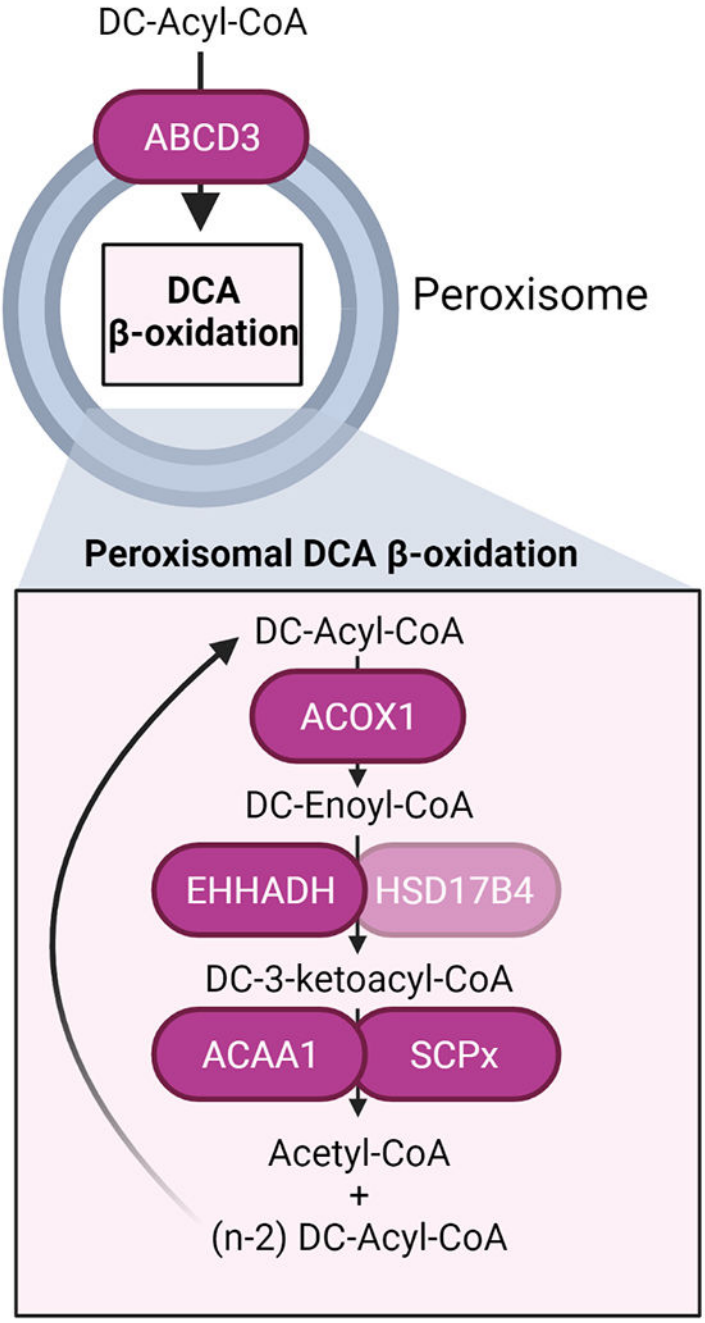

Figure 2.
Schema of the peroxisomal steps in DCA β-oxidation with the name of the enzymes involved in each step. Created with BioRender.com