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Advances in the Understanding and Treatment of Mitochondrial Fatty Acid Oxidation Disorders

Eric S Goetzman, PhD [Associate Professor of Pediatrics]

University of Pittsburgh

Abstract

Purpose of review—This review focuses on advances made in the past three years with regards to understanding the mitochondrial fatty acid oxidation (FAO) pathway, the pathophysiological ramifications of genetic lesions in FAO enzymes, and emerging therapies for FAO disorders.

Recent findings—FAO has now been recognized to play a key energetic role in pulmonary surfactant synthesis, T-cell differentiation and memory, and the response of the proximal tubule to kidney injury. Patients with FAO disorders may face defects in these cellular systems as they age. Aspirin, statins, and nutritional supplements modulate the rate of FAO under normal conditions and could be risk factors for triggering symptoms in patients with FAO disorders. Patients have been identified with mutations in the *ACAD9* and *ECHS1* genes, which may represent new FAO disorders. New interventions for long-chain FAODs are in clinical trials. Finally, post-translational modifications that regulate fatty acid oxidation protein activities have been characterized that represent important new therapeutic targets.

Summary—Recent research has led to a deeper understanding of FAO. New therapeutic avenues are being pursued that may ultimately cause a paradigm shift for patient care.

Keywords

mitochondrial fatty acid oxidation; FAO; FAO disorders; clinical genetics; FAO enzymes

Introduction

Mitochondrial fatty acid oxidation (FAO) is the pathway by which fatty acids are broken down for energy. While the pathway can metabolize fatty acids up to 22 carbons in length, the bulk of the fatty acids fluxed through the mitochondria *in vivo* are 16 or 18 carbons long. This reflects the most abundant fatty acids in the diet as well as in adipose stores [1]. FAO breaks fatty acids into two carbon units (acetyl-CoA) which are subsequently oxidized to completion by the TCA cycle. This process involves only four sequential biochemical reactions (Fig 1, inset top left). However, there are multiple enzymes at each step with

Address correspondence to: Eric Goetzman, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Avenue, Pittsburgh, PA 15224, Eric.goetzman@chp.edu, 412-692-7952.

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different chain length specificities. Additional enzymes are required to activate fatty acids to acyl-CoAs (acyl-CoA synthetases), to transport them across the mitochondrial membrane (carnitine palmitoyltransferases), to deal with double bonds that block chain shortening (dienoyl-CoA reductase and enoyl-CoA isomerase), and to accept the electrons produced by the first step (electron transferring flavoprotein, electron transferring flavoprotein dehydrogenase)(Fig 1). All told, there are at least 21 genes encoding the enzymatic machinery required for transporting fatty acids across the mitochondrial membrane and chain-shortening them to completion. This does not include recently described putative new members of the pathway, to be discussed below. To date, human patients have been described with pathogenic mutations in 15 of the enzymes in Figure 1. Some of these diseases are exceedingly rare (i.e., medium-chain 3-ketothiolase deficiency) while others are as common as 1/10,000 births in certain populations (medium-chain acyl-CoA dehydrogenase deficiency). When summed together, the FAO disorders (FAOD) are the most common inborn error of metabolism. The incidence, diagnosis, and clinical characteristics of these disorders have been extensively reviewed elsewhere [2-5]. The scope of the current review is to summarize recent (2014 to present) advances in our understanding of the pathogenesis of these disorders, newly described diseases related to FAO, and new therapies under development.

Advances in understanding pathophysiology

While FAODs have been studied for over 40 years, we still have a lot to learn about their natural history and about the pathophysiology. As our general understanding of biology and metabolism has deepened so has our understanding of how FAO contributes to human health. The sections below cover several recent areas of discovery in this regard.

FAO defects in “non-traditional” organs may contribute to disease pathology

Historically, the organs studied in the context of FAOD are those that become most severely compromised in the patient and cause mortality: heart and liver. Close behind heart and liver are organs that are severely affected but are not life-threatening—skeletal muscle and, in the case of trifunctional protein (TFP) deficiencies, the retina [6]. Why eye symptoms are seen in TFP patients but not in other long-chain FAOD remains unknown. In addition to these organs the recent literature has uncovered key roles for FAO in organs not traditionally examined in FAOD. Organs/cell types now known to conduct high rates of FAO are listed in Table 1. In the age of newborn screening and better management of FAODs, patients are living longer and it therefore becomes pertinent to understand how functional defects in these organs may contribute to quality of life issues and in some cases, mortality. A related factor with as-yet-unknown ramifications is aging; it has only been 44 years since the first report of a genetic FAOD [7], and many FAODs have come to light much more recently than that, meaning that little is known about the natural history of these disorders and what pathologies may emerge in an aging population with genetic lesions of FAO.

Lung—The lung is a complicated organ that contains over 40 different cell types. While much of the physical work of the lung is outsourced to the surrounding muscles and diaphragm, there is one minority cell type in the lung that is rich in mitochondria and has

intense bioenergetic demands: the alveolar type II pneumocyte (ATII cell), which produces pulmonary surfactant. It was recently shown that ATII cells conduct high rates of FAO [8]. Mice deficient in the FAO enzyme long-chain acyl-CoA dehydrogenase (LCAD) show altered breathing mechanics. The amount of pulmonary surfactant is reduced in these animals as is the composition of the acyl chains on surfactant phospholipids [9, 8]. Human ATII cells also express high levels of LCAD [8]. While no LCAD-deficient patients have yet been described, it is tempting to speculate that they might present with respiratory distress or other lung symptomology, given the very restricted nature of LCAD expression in the human [10]. Patients with known FAO lesions in mitochondrial trifunctional protein (TFP) or carnitine palmitoyltransferase-2 (CPT2) may also be at risk for lung disease, which has not been systematically evaluated. Anecdotally, this possibility is supported by case reports documenting respiratory distress in TFP and CPT2-deficient neonates [11, 12].

Kidney—As with the ATII cells in the lung, the proximal tubules of the kidney conduct highly energy-intensive processes such as ion transport. Proximal tubule cells are packed with mitochondria and express FAO proteins abundantly [13, 14]. There have been some reports of renal cysts in CPT2 deficient patients and acute kidney injury in a very long-chain acyl-CoA dehydrogenase (VLCAD) patient [15-17]. Autopsy of MCAD patients has revealed fatty infiltration of the kidney [18]. Importantly, Kang et al recently linked dysfunctional FAO to renal fibrosis, which is typically seen in chronic end-stage kidney disease [19]. These findings could mean that FAOD patients are at risk for chronic kidney diseases as they age.

Immune System—The connection between FAOD and the immune system is two-fold. First, whenever FAO is blocked, acylcarnitines accumulate (discussed in following section). Acylcarnitines have been shown *in vitro* to activate a central regulator of the inflammatory response known as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [20]. Accumulation of acylcarnitines thus drives secretion of pro-inflammatory cytokines [21, 22]. The second connection lies in the recently documented reliance of certain T-cell populations upon FAO to meet their bioenergetics demands including tissue-resident memory T-cells and regulatory T-cells [23, 24]. The immune system must walk a fine line between protecting the host from pathogens and self-damaging inflammation. Part of the system of checks and balances inherent in the immune system are regulatory T-cells, which temper exaggerated immune responses and help to maintain self-tolerance. Recent studies have shown that effector T-cells and regulatory T-cells have very different metabolic phenotypes, with effector T-cells favoring glycolysis and regulatory T-cells favoring FAO [25, 26]. Etomoxir, an irreversible inhibitor of FAO that targets carnitine palmitoyltransferase-1 (CPT1), prevents differentiation of naïve CD4+ T-cells into regulatory t-cells [26]. It is thus tempting to speculate that FAOD patients, with compromised FAO in all cell types including T-cells, would have an impaired ability to generate regulatory T-cells. Indeed our laboratory has shown that naïve T-cells isolated from VLCAD knockout mouse spleens show a reduced capacity to differentiate into regulatory T-cells *in vitro* (unpublished observation). A paucity of regulatory T-cells, combined with possible proinflammatory effects of acylcarnitines, could conceivably result in chronic low-

grade inflammation in FAOD patients, or an exaggerated immune response to pathogens. Further research is required to explore these possibilities in human patients.

Pathological effects of lipid intermediates

FAOD involve a bottleneck in the pathway resulting in an accumulation of the acyl-CoA substrate for the dysfunctional/missing enzyme. The accumulating acyl-CoA species may interchange with the carnitine or glycine conjugate of that species, which, being more soluble and able to cross membranes, appear in the blood and urine. This has been recognized for decades and forms the basis of newborn screening programs for FAOD [27, 28]. For any given disorder a characteristic pattern of metabolites will accumulate in the affected tissues and blood with regards to chain length, degree of unsaturation, etc. For example, a patient deficient in VLCAD will accumulate a mixture of long-chain free fatty acids, long-chain acyl-CoAs, and long-chain acylcarnitines while an MCAD patient accumulates medium-chain lipid species and TFP patients accumulate long-chain hydroxylated species. Only recently have the biological effects of these lipid species been investigated in any detail. Free fatty acids appear to be particularly toxic to mitochondria. Free long-chain hydroxylated species, which may accumulate in TFP patients, inhibit the respiratory chain and induce opening of the permeability transition pore [29]. Similar toxicities were seen for medium-chain free fatty acids that may be accumulating in MCAD-deficient patients [30]. Hoffmann et al established similar effects of unsaturated fatty acids in cultured cardiomyocytes [31]. These findings echo a robust literature showing the toxic effects of the prototypical fatty acid palmitate in a variety of experimental models [32-36].

More surprising than the toxicity of free fatty acids are recent reports demonstrating pathological consequences of acylcarnitine accumulation, which was previously thought to be benign. In fact, patients with FAOD are typically supplemented with carnitine under the assumption that driving substrate accumulation away from free fatty acids/CoAs toward acylcarnitines would be beneficial [37]. While the effects of acylcarnitines in human patients remains unknown and difficult to study, *in vitro* experiments and animal models have indicated that acylcarnitines are associated with a plethora of negative effects. For example, in a mouse model of cardiac ischemia-reperfusion injury, accumulating acylcarnitines inhibited the respiratory chain and drove ROS production and oxidative stress [38]. Infusing the heart with palmitoylcarnitine prior to arterial occlusion significantly exacerbated the size of the resulting infarct. In cultured mouse myotubules, acylcarnitines induce insulin resistance, cell membrane permeability, and promote apoptosis [39, 21, 40, 41]. Acylcarnitines can activate NF- κ B and promote a pro-inflammatory response [22, 20]. In cultured intestinal epithelial cells, acylcarnitines disrupt tight junctions between cells [42]. Acylcarnitines secreted from Schwann cells cause axonal degeneration and may play a role in peripheral neuropathies [43]. Finally, acylcarnitines secreted by type II pneumocytes into the extracellular space can incorporate into pulmonary surfactant and inhibit its surface tension-lowering properties [9, 44]. This acylcarnitine secretion can be further stimulated by infection and lung injury [9]. Together, these literature indicate that acylcarnitines are not simply benign biomarkers but may contribute directly to the pathophysiology of FAOD.

Interactions between FAO lesions and the environment

A corollary to the greatly improved survival being seen in FAOD patients since the advent of newborn screening is that living longer means increased exposure to environmental factors that could exacerbate their metabolic disease. Environmental toxins, pharmaceuticals taken for non-FAOD related conditions, anesthetics, viral infections, and nutritional supplements may all alter the FAO pathway with negative and potentially life-threatening consequences for the FAOD patient. Modulating FAO either up or down could be problematic for the FAOD patient. On the one hand, decreasing FAO further in a patient with an existing defect could push them to the brink of decompensation, while on the other hand driving FAO in the face of a genetic block can result in accumulation of toxic intermediates.

Aspirin—Perhaps the most prominent example of an environmental factor exacerbating an FAOD is that of aspirin ingestion and “Reye-like” syndrome. Prior to newborn screening, and prior to the near-complete discontinuation of aspirin therapy in children due to successful public health campaigns, aspirin ingestion was frequently found to trigger metabolic decompensation and even death in young children, typically in the context of a viral fever or other common illness [45]. Many of these children were later found to suffer from FAODs, most prominently MCAD deficiency [46]. Our laboratory has recently reported that, at least in cultured cells, aspirin inhibits peroxisomal FAO while driving mitochondrial FAO [47]. Based on our findings we postulate that aspirin overwhelms the defective MCAD enzyme by enhancing long-chain FAO flux, while at the same time inhibiting the peroxisomal pathway which could otherwise act as a safety valve to prevent lipotoxicity.

Infection—In the past few years it has come to light that viral infections can alter FAO flux, either up or down depending upon the virus and the context [48-52]. Further, a CPT2 polymorphism has been identified in Asian populations that causes influenza-associated encephalopathy, which is frequently fatal [53, 54]. The polymorphism creates a thermolabile CPT2 variant that functions well under normal conditions but becomes dysfunctional during fever.

Supplements—Over-the-counter supplements are particularly dangerous due to the lack of regulation over their use combined with limited scientific data regarding their biological effects. Creatine, taken to boost athletic performance, has been found to drive FAO and, similar to aspirin, could stress the mitochondria of those with FAO lesions [55, 56]. Mildronate is another supplement thought to boost performance [57]. This compound made headlines in 2016 when professional tennis player Maria Sharapova was suspended from the sport after testing positive for mildronate. In eastern Europe mildronate is commonly used as an anti-ischemic therapy [58-60]. Mildronate's main biologic effect is to inhibit carnitine synthesis in the liver, and it also competitively inhibits carnitine uptake via the transporter OCTN2 [61, 62]. The result is a severe restriction of FAO due to limited carnitine availability for the carnitine shuttle. In LCAD knockout mouse lung mildronate was beneficial due to eliminating acylcarnitine secretion to the extracellular space [9], but in the context of human FAOD patients this supplement would be damaging.

Statins—As FAOD patients age and develop medical conditions requiring pharmacotherapy, they may unknowingly suffer unexpected side effects due to their underlying genetic disease. Statins, one of the most widely prescribed drug classes in the world, were recently shown to increase hepatic FAO flux [63]. Again, as with aspirin, driving fatty acids into mitochondria bearing an FAO defect is likely to exacerbate disease.

New disorders and enzymes

Acyl-CoA dehydrogenase-9 (ACAD9) deficiency

There are four well-described acyl-CoA dehydrogenases that catalyze the first step of mitochondrial FAO: short-chain acyl-CoA dehydrogenase (SCAD), MCAD, LCAD, and VLCAD. The names “LCAD” and “VLCAD” are somewhat of a misnomer since it has now become clear that these two enzymes have largely overlapping substrate specificities in the range of C₁₄ to C₂₀ acyl-CoAs [10]. It was therefore a surprise that ACAD9, first reported by a Chinese group while data mining the human genome in the early 2000s [64], also utilizes these same substrates, although it tends to favor unsaturated acyl-CoA [65]. Human ACAD9 can clearly dehydrogenate acyl-CoA substrates *in vitro* and pass electrons to electron transferring flavoprotein (ETF), the same electron acceptor used by all acyl-CoA dehydrogenases, but whether it does so *in vivo* has become the subject of controversy. He et al reported the first ACAD9-deficient patients in 2007 [66]. However, while these patients had no detectable ACAD9 antigen, the disease-causing mutations were not identified and therefore a direct causal link between ACAD9 and an FAOD could not be definitively established. No additional ACAD9 patients were reported until 2010 when Nouws et al used whole exome sequencing to identify ACAD9 mutations as a cause of electron transport chain (ETC) Complex I deficiency [67]. Since that time, ACAD9 mutations have become recognized as a major cause of Complex I deficiency and ACAD9 is now established as an assembly factor for Complex I [68-71]. ACAD9 interacts strongly with another known assembly factor called “evolutionarily conserved signaling intermediate in toll pathway,” or ECSIT for short [67], but how exactly these proteins facilitate Complex I assembly is unknown. The symptoms of ACAD9 deficiency resemble those of other ETC deficiencies rather than FAODs. It is presently not known whether ACAD9 maintains dual functions, i.e., operating in both Complex I assembly and the FAO pathway. Schiff et al have presented evidence to support the dual function hypothesis [69]. They proposed that in cell types where ACAD9 is abundantly expressed it contributes to both Complex I assembly and FAO; in support of this, when ACAD9 was deleted from HEK293 cells, which normally express high levels of ACAD9, both complex I and the rate of FAO were significantly decreased. Further, when several patient mutations were evaluated for their effects on ACAD9 dehydrogenase activity *in vitro*, it was found that mutations that most severely affected the dehydrogenase activity were associated with more severe symptoms in the patient. Further clarification of the biochemical roles of ACAD9 is needed in order to understand the nature of ACAD9 deficiency and whether or not it involves an FAOD component.

ECHS1 Deficiency

Short-chain enoyl-CoA hydratase (ECHS1), previously known as crotonase, catalyzes the second biochemical reaction in FAO for short-chain substrates (Fig 1). It is also active in the

degradation pathways for branched-chain amino acids isoleucine and valine. Toxic intermediates from the valine pathway were elevated in the original ECHS1 patients described by Peters et al [72], while intermediates from short-chain FAO and the isoleucine pathway were normal. Peters et al postulated that the valine intermediates methacrylyl-CoA and acryloyl-CoA, which are very reactive and cause downstream damage to the pyruvate dehydrogenase complex (PDC) and the electron transport chain (ETC), are primarily responsible for the severe brain disease observed in ECHS1 deficiency. The original two patients succumbed at ages 4 and 8 months. Haack et al [73] identified 10 patients using whole exome sequencing and found the phenotype to be highly variable. While four had died between ages 1 month and 7 years, the remaining six were ages 2, 3, 5, 8, 16, and 31 years at time of publication. Regardless, all 10 suffered severe debilitating “Leigh syndrome-like” symptoms including encephalopathy, developmental delay, and impaired psychomotor function. Most suffered hearing loss, optic atrophy, and epileptic seizures. While the hallmark symptoms of FAOD were absent, such as elevated blood acylcarnitines or hypoketotic hypoglycemia, when ECHS1-deficient cells were challenged with palmitate loading short-chain acylcarnitines did accumulate and the overall rate mitochondrial respiration on palmitate was significantly lower. Thus, as with ACAD9 above, ECHS1 deficiency is an FAOD in which the predominant disease is not directly related to FAO. ECHS1 deficiency closely resembles 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency, a disorder of valine metabolism, and the consensus of the cases described thus far is that ECHS1 pathology is almost entirely driven by disruption of valine catabolism.

“Orphan” FAO enzymes without associated human disorders

There are currently at least four FAO enzymes awaiting corresponding human patients. These are 2,4-dienoyl-CoA reductase (DECR), enoyl-CoA isomerase (ECI), LCAD, and the heart/muscle-specific isoform of CPT1 (CPT1b). Deficiencies of these genes may be lethal, or alternatively, the disease phenotypes may not resemble other FAOD and therefore have not been identified due to ascertainment bias. With the advent of whole exome sequencing we may identify relevant diseases for these genes in the near future, as was done with ACAD9. In addition to these four, there are two other genes which may encode FAO enzymes based on homology to the acyl-CoA dehydrogenase family. These are known as ACAD10 and ACAD11. Very little is known about either gene other than that they contain an acyl-CoA dehydrogenase domain by homology [74]. Polymorphisms in ACAD10 have been linked to obesity/diabetes among the Pima Indians and a similar phenotype was reported in ACAD10 knockout mice [75, 76]. Very recently ACAD10 was shown to play an instrumental role in the metabolic benefits of metformin in *c. elegans* [77]. Understanding the role of these enzymes in human biology is one of the next frontiers in FAO research.

Advances in therapy

Current therapies for FAODs focus on removing lipids from the diet or replacing them with lipids that bypass the genetic block [2]. For example, patients with long-chain defects will avoid the fats typically found in our Western diet which are primarily long-chain fatty acids 16 to 18 carbons in length, replacing this with a specially produced medium-chain (C8) triglyceride formulation (MCT) which is efficiently chain-shortened by the medium-chain

and short-chain FAO enzymes to produce energy. For MCAD deficiency, however, there is no such dietary formulation to bypass the block. These patients focus on avoidance of fat and avoidance of fasting. In addition to these dietary regimens, many FAOD patients are given carnitine supplementation to prevent secondary carnitine deficiency due to acylcarnitine secretion. While a few studies have demonstrated positive effects of MCT [78-80] there is most certainly a need for better therapeutic options. Unfortunately, a drug known as bezafibrate that showed promise in cell culture studies [81] was recently assessed in clinical trials and found to have no efficacy in patients with long-chain FAOD [82, 83]. However, there are other new drugs on the horizon. These are summarized below.

Triheptanoin

Triheptanoin is a triglyceride oil like MCT, except with C7 fatty acids instead of C8. Triheptanoin was conceived by Dr. Charles Roe et al in 2002 who postulated that it would provide a benefit over C8 by yielding a C3 unit after two rounds of beta-oxidation [84]. C3 can feed into the TCA cycle at the level of succinyl-CoA, thereby replenishing the intermediates of the TCA cycle which can become depleted in FAOD. Triheptanoin has gone through a Phase 2 trial, the result of which were recently published. In this study, 29 patients who had ongoing symptoms despite their current treatment regimen were given triheptanoin for 6 months. Patients reported an improved quality of life and many demonstrated improved exercise performance and tolerance [85, 86]. A larger open-label study is now underway to further explore the benefits of triheptanoin over current MCT therapy.

Ravicipiti

Glycerol phenylbutyrate, known as Ravicipiti, is currently FDA-approved to treat urea cycle disorders. Ravicipiti has been shown to bind as a substrate to the MCAD enzyme [87, 88] and has been postulated to have benefits in stabilizing the most common disease variant of MCAD, caused by the K304E amino acid substitution. A Phase I clinical trial for Ravicipiti in MCAD patients homozygous for K304E was recently concluded at the University of Pittsburgh with results forthcoming.

Future therapies

Gene therapy remain the gold standard that all genetic diseases strive to achieve. New technologies in gene therapy such as stabilized therapeutic mRNAs and gene-editing methods such as CRISPR offer the promise of modifying disease genes or replacing them, temporarily if not permanently. Progress in gene therapy has been reviewed elsewhere and will not be covered here [89, 90]. In the interim, while the inborn errors community awaits the clinical availability of gene replacement therapies, other strategies continue to be pursued. Current therapy, treatments in clinical trials, and attempted therapies cover three basic strategies: 1) nutritional bypass of the defect to provide energy (MCT, triheptanoin), 2) stabilize an unstable enzyme variant to increase activity (Ravicipiti), and 3) increase expression of semi-active enzyme variants (bezafibrate). In addition to these, a fourth strategy has been proposed, which is to boost enzymatic activity via the modulation of post-translational modifications (PTMs). Advancements have been made in our understanding of how FAO enzymes are regulated and it has become apparent that PTMs such as lysine acetylation and succinylation play a role [91-94]. Virtually all the FAO enzymes are

modified with multiple PTMs. TFP is one of the most highly modified proteins in the mitochondria, with 173 unique modifications reported on *phosphosite.org* which tracks the PTM literature and large datasets deposited online. These modifications include lysine acetylation, succinylation, glutarylation, and ubiquitylation, many of which occur on the same lysine residues suggesting they may compete with each other. Other modifications on TFP include phosphorylation, nitrosylation, and methylation. The function of these PTMs on TFP remains to be determined. For VLCAD, more progress has been made. VLCAD has reversible lysine acetylation and succinylation sites that change enzymatic activity, and perhaps more importantly, determine its ability to bind to cardiolipin on the inner mitochondrial membrane [91]. Thus, PTMs have the potential to influence localization of VLCAD inside the mitochondria which may then in turn have effects on flux through the proposed long-chain FAO complex on the inner mitochondrial membrane [95]. Finally, regulatory effects on VLCAD have been observed for cysteine nitrosylation [96]. While acetylation/succinylation inhibit VLCAD activity and membrane localization, cysteine nitrosylation impressively activates VLCAD. In mice, nitrosylation of cysteine 238 was shown to increase the catalytic efficiency of VLCAD by 29-fold [96]. Because this modification is transient and reversible, it is open to being therapeutically exploited to increase the activity of partially active VLCAD variants in human patients. In a proof-of-principle study, Tonopoulous et al treated VLCAD patient fibroblasts bearing missense mutations that cause low residual enzymatic activity with a compound that induces cysteine nitrosylation [97]. In all four patient cell lines the compound increased VLCAD activity by 7-10 fold without changing the abundance of the protein. This boost in VLCAD activity completely normalized flux of radiolabeled palmitate through the FAO pathway.

Conclusion

In the 44 years since the first inborn error of FAO was described in the literature tremendous advancements have been made in our understanding of the enzymology and architecture of the FAO pathway and how it intersects with the electron transport chain and other metabolic pathways. Mass spectrometry technology has allowed for early identification of FAOD patients, at least in economically developed nations. After decades of little advancement with regards to therapeutics, the first clinical trials for FAODs have been conducted and new interventions are on the horizon. Additionally, “drilling down” to the finer details of how FAO enzymes are regulated has revealed promising new therapeutic avenues such as cysteine nitrosylation. Together, these advancements are greatly lowering the risk for mortality and increasing the quality of life of FAOD patients.

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Figure 1.

Mitochondrial beta-oxidation of a typical fatty acid (palmitate). Step 1 is catalyzed by a family of flavine adenine dinucleotide (FAD)-containing enzymes that includes short-chain, medium-chain, long-chain, and very long-chain acyl-CoA-dehydrogenases (SCAD, MCAD, LCAD, VLCAD) and acyl-CoA dehydrogenase-9 (ACAD9) whose role in FAO is still uncertain. The acyl-CoA dehydrogenases pass electrons to electron transferring flavoprotein (ETF) which in turn passes them to ETF dehydrogenase (ETFDH). All enzymes in yellow are flavoproteins. Step 2 is hydration across the double bond by enoyl-CoA hydratases (rendered in gray), and Step 3 is carried out by hydroxyacyl-CoA dehydrogenases (rendered in green) which reduce NAD^+ to NADH. The final step is catalyzed by ketoacyl-CoA thiolases (beige) which cleave two carbons off the acyl-chain, utilizing free coenzyme-A (CoA) as a cofactor to do so. Note that mitochondrial trifunctional protein (TFP) catalyzes Steps 2-4 for long-chain substrates. Long-chain FAO takes place on the inner mitochondrial membrane while the medium and short-chain enzymes are in the matrix. The ultimate fate of all NADH and FADH_2 produced by FAO is re-oxidation by the respiratory chain (blue).

Bottom inset: unsaturated fatty acids pose a special problem which is dealt with by auxiliary enzymes 2,4 dienoyl-CoA reductase (DECR) and enoyl-CoA isomerase (ECI) which can reduce and move the problematic double bonds, respectively. Finally, shown in orange are the enzymes of the carnitine shuttle which carry long-chain fatty acids into the mitochondria. Carnitine palmitoyltransferase-1 (CPT1) is the "gatekeeper" of FAO. In the case of FAO disorders where acyl-CoA species accumulate intra-mitochondrially, CPT2 runs in reverse and the diagnostic hallmark acylcarnitines are secreted.

Table 1

Tissues and cell types that rely upon FAO for energy.

Tissue/Cell Type	Role of FAO	Reference
Brown adipose	Thermogenesis in newborns	[98]
Heart	>80% of basal energy demand	[99]
Liver	Provide acetyl-CoA for ketogenesis and ATP to support gluconeogenesis	[100]
Skeletal Muscle	ATP to support exercise	[101]
Type II pneumocytes	ATP to support surfactant synthesis	[8]
T-cells	Drives differentiation of regulatory T-cells	[25]
Renal proximal tubules	ATP to support solute reabsorption and ion transport	[15, 102]