NIH Public Access
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J Inherit Metab Dis. Author manuscript; available in PMC 2011 October 1.

Published in final edited form as:
J Inherit Metab Dis. 2010 October ; 33(5): 539–546. doi:10.1007/s10545-010-9121-7.

MITOCHONDRIAL FATTY ACID OXIDATION DISORDERS: PATHOPHYSIOLOGICAL STUDIES IN MOUSE MODELS

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Summary

Mouse models have been designed for a number of fatty acid oxidation defects. Studies in these mouse models have demonstrated that different pathogenetic mechanisms play a role in the pathophysiology of defects of fatty acid oxidation.

Supplementation with L-carnitine does not prevent low tissue carnitine levels and induces acylcarnitine production having potentially toxic effects, as presented in very long-chain acyl-CoA dehydrogenase (VLCAD) – deficient mice.

Energy deficiency appears to be an important mechanism in the development of cardiomyopathy and skeletal myopathy in fatty acid oxidation defects and is also the underlying mechanism of cold intolerance.

Hypoglycemia as one major clinical sign in all fatty acid oxidation defects occurs due to a reduced hepatic glucose output and an enhanced peripheral glucose uptake rather than to transcriptional changes that are also observed simultaneously as presented in medium-chain acyl-CoA dehydrogenase (MCAD) -deficient mice.

There are reports that impaired fatty acid oxidation also plays a role in intrauterine life. The embryonic loss demonstrated for some enzyme defects in the mouse supports this hypothesis, however the exact mechanisms are unknown. This observation correlates to maternal HELLP-syndrome as observed in pregnancies carrying a long-chain 3-hydroxyacyl-Co-A dehydrogenase (LCHAD)- deficient fetus.

Synergistic heterozygosity has been shown in isolated patients and in mouse models to be associated with clinical phenotypes common to fatty acid oxidation disorders. Synergistic mutations may also modulate severity of the clinical phenotype and explain in part clinical heterogeneity of fatty acid oxidation defects.

In summary, knowledge about the different pathogenetic mechanisms and the resulting pathophysiology allows the development of specific new therapies.

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The authors, Ute Spiekerkoetter and Philip A. Wood, act as guarantor and take full responsibility for the material in this article.

The authors confirm that they have no competing interests for declaration.

The animal studies presented here were performed with the approval of the respective Institutional Animal Care and Use Committees.

There were no new animal studies done by the authors for this paper.

No personal information about any patient is included and no ethics approval was necessary.

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Keywords

carnitine; acylcarnitines; energy; fatty liver; glucose metabolism; synergistic heterozygosity

Fatty acid oxidation defects comprise enzyme deficiencies of long-chain fatty acid oxidation and medium chain fatty acid oxidation. A number of mouse models for the different enzyme deficiencies have been designed in order to study the different aspects of disease. Importantly, many of the different mouse models present with similar, stress-induced clinical phenotypes as observed in humans and are excellent models for pathophysiological studies relevant to human patients. The available mouse models of defects in mitochondrial β-oxidation of fatty acids are displayed in Table 1.

In humans, long-chain fatty acid oxidation defects present with heterogeneous clinical phenotypes affecting mainly three different organ systems: the heart, the liver and skeletal muscles (Wanders et al 1999). Whereas some patients present with the full spectrum of disease and a multisystemic disease, others only exhibit hypoglycemia during illness or episodes of rhabdomyolysis due to vigorous exercise. In general, clinical symptoms are mainly induced by catabolism when energy from fatty acid oxidation is needed. Since implementation of newborn screening a large number of patients remained asymptomatic with preventive measures during the observation period of 5 to 10 years (Spiekerkoetter et al 2009).

In patients with medium-chain acyl-CoA dehydrogenase (MCAD) – deficiency (MCADD), the common defect of medium-chain fatty acid oxidation, only hepatic symptoms are observed such as fatty liver and impaired glucose metabolism due to prolonged fasting or catabolism during illness.

Factors determining the heterogeneous clinical course of disease and the pathophysiology have been studied in gene-targeted mouse models of long-chain and medium-chain fatty acid oxidation defects (Schuler and Wood 2002; Spiekerkoetter et al 2005; van Vlies et al 2005; Cox et al 2009; Kurtz et al 1998).

Mouse models and correlation of clinical phenotype in murine and human disease

Mouse models with carnitine palmitoyltransferase-1a (liver isoform) (OMIM 600528) and 1b (muscle isoform) (OMIM 601987) deficiencies have been developed and characterized. Both are homozygous lethal in early gestation (Nyman et al 2005; Ji et al 2008). In humans, only CPT 1a-deficiency occurs and presents, as MCAD-deficiency, solely with the hepatic phenotype characterized by hypoketotic hypoglycemia during catabolism.

Very long-chain acyl-CoA dehydrogenase (VLCAD) – deficiency (VLCADD) (OMIM 609575) is the commonest of the human long-chain fatty acid oxidation defects. There are two independently developed and characterized VLCAD-deficient mouse models (Cox et al 2001; Exil et al 2003). Both VLCAD-deficient mouse models are characterized by no definite clinical phenotype at rest but present with similar stress-induced phenotypes as humans (Spiekerkoetter et al 2005; Cox et al 2001). Therefore, they are good models to study the stress-induced changes occurring in this group of disorders. In the mouse, physical exercise results in impaired running speed and overall impaired running distance (Spiekerkoetter et al 2005; Spiekerkoetter et al 2004). Fasting and cold exposure result in severe hypoglycemia, hypothermia and lethargy and is lethal in one third of VLCAD deficient mice (Spiekerkoetter et al 2004). The full picture of cardiomyopathy is not induced in VLCAD-deficient mice by short-term exercise, however metabolic changes occur in cardiac tissue.

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The β-oxidation cycle contains enzymes with overlapping substrate specificity that oxidize fatty acids with the same chain length. Long-chain acyl-CoA dehydrogenase (LCAD) (OMIM 609576) is highly expressed in mouse tissues covering a great part of palmitoyl-CoA oxidation, as does VLCAD in humans (Schuler and Wood 2002). In contrast to the mouse, LCAD is not as highly expressed in human tissues. For LCAD-deficiency, a knock-out mouse model has been designed (Kurtz et al 1998; Cox et al 2001) presenting with non-progressing cardiac hypertrophy, that is not exercise- or stress-induced, in the first weeks of life, however, cardiac function remains normal (Schuler and Wood 2002; Cox et al 2009). The LCAD-deficient mouse is a good model to study cardiac morphological changes in long-chain fatty acid oxidation disorders and closely mimics cardiac changes in human VLCADD.

For medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (MCADD) (OMIM 607008), also an MCAD-deficient mouse model has been designed (Tolwani et al 2005) presenting with fatty liver and profound cold intolerance at 4 degrees C with prior fasting. There was significant neonatal mortality of MCAD-deficient pups (Tolwani et al 2005) demonstrating similarities to patterns of clinical episodes and mortality in MCADD patients prior to the newborn screening era. Since the MCAD-deficient mouse reproduces important aspects of human MCADD and mainly presents with hepatic clinical disease, it is a valuable model to study solely hepatic pathogenetic mechanisms.

The mouse model of TFP-deficiency (TFPD) (HADHB OMIM 143450, HADHA OMIM 600890) with deficiency of the TFP-α-subunit is lethal such as severe TFP-deficiency in humans (Ibdah et al 2001; Spiekerkoetter et al 2004). In humans, in addition, a milder phenotype of TFP-deficiency exists presenting with myopathy and irreversible peripheral neuropathy with later onset.

Pathophysiology

Importantly, most symptoms occurring in human patients with long-chain fatty acid oxidation defects are completely reversible with therapeutic measures and sufficient energy supply (Spiekerkoetter et al 2009). Cardiomyopathy and pericardial effusion completely reverse within a few weeks (Brown-Harrison et al 1996; Spiekerkoetter et al 2003). Skeletal myopathy can be prevented with medium-chain triglyceride (MCT) supplementation prior to exercise (Gillingham et al 2006; Spiekerkoetter 2007). Some long-term complications occurring in disorders of the mitochondrial trifunctional protein (TFP)-deficiency including human long-chain 3-hydroxacyl-CoA dehydrogenase (LCHAD) – deficiency (OMIM 600890), however, are not reversible with treatment such as peripheral neuropathy and retinopathy suggesting other pathogenetic mechanisms than energy deficiency. Different mechanisms underlying the pathophysiology have been demonstrated in the respective mouse models (Schuler and Wood 2002; Spiekerkoetter et al 2005; Cox et al 2009). This paper discusses these mechanisms and related pathophysiology along with the available evidence in the development of different clinical phenotypes. The mechanisms addressed here are:

- Carnitine depletion
- Accumulation of toxic metabolites
- Energy deficiency
- Disturbed hepatic carbohydrate management
- Impairment of intrauterine fatty acid oxidation
- Synergistic heterozygosity

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Carnitine depletion

VLCAD-deficient mice, as humans with VLCADD, present with lower concentrations of free carnitine in blood than healthy controls (Spiekerkoetter et al 2004). Whereas, 1-hour exercise training on the treadmill does not result in any changes as compared to baseline free carnitine concentrations, after fasting and cold exposure for 8 hours blood free carnitine concentrations are significantly lower in knockout but also in wild-type mice as compared to resting conditions (Spiekerkoetter et al 2004).

Occurrence of intermittently low free carnitine in blood has led to the hypothesis that carnitine depletion develops and is important in disease pathogenesis. This situation has been characterized as “secondary carnitine-deficiency” in fatty acid oxidation defects. With accumulating acyl-CoA esters in defects of fatty acid oxidation, free carnitine is needed for their conversion into acylcarnitines and their shuttle out of the mitochondria in order to provide sufficient free CoA for other metabolic reactions. The hypothesis of depleted free CoA and depleted tissue carnitine has resulted in therapeutic L-carnitine supplementation in defects of fatty acid oxidation (Winter 2003).

Recent studies in the mouse model of VLCADD, however, have shown for the first time, that blood and tissue free carnitine concentrations do not correlate (Spiekerkoetter et al 2005). In VLCAD knock-out mice free carnitine concentrations significantly decreased in skeletal muscle after 1 hour exercise with this effect not being visible in blood (Spiekerkoetter et al 2005). At the same time free carnitine concentrations in liver significantly increased, strongly suggesting induced endogenous carnitine biosynthesis. Skeletal muscle lacks the final enzyme of the carnitine biosynthesis pathway, and therefore, is not feasible to replenish low carnitine concentrations itself (Vaz and Wanders 2002). Importantly, 24 hours after 1-hour exercise training low free carnitine in skeletal muscle is again replenished and reaches initial baseline concentrations (Primassin et al 2008). The same is observed in heart from VLCAD-deficient mice. In addition, further studies with L-carnitine supplementation (200 mg/kg) demonstrate that exercise-induced reduction of free carnitine in skeletal muscle cannot be prevented with L-carnitine supplementation. It is again endogenous carnitine biosynthesis that is active to replenish low carnitine tissue concentrations, even with concurrent L-carnitine supplementation.

In contrast to reduced free carnitine in muscle after exercise, fasting and cold exposure do not result in reduced carnitine concentrations in tissues. However, carnitine biosynthesis is also induced in the liver. The fasting stress mainly affects the liver, therefore, a reduced free carnitine would be expected most likely in the liver. The low free carnitine concentrations in blood after fasting and cold exposure are misleading and point to “carnitine-deficiency”, however, they do not correlate with any carnitine tissue concentration measured simultaneously (Spiekerkoetter et al 2005).

In conclusion, studies in the VLCAD-deficient mouse model suggest, that endogenous carnitine biosynthesis plays the most important role in carnitine homeostasis and replenishes low tissue carnitine concentrations. In contrast, despite continuous oral carnitine supplementation constantly high enough carnitine tissue levels cannot be provided as presented for skeletal muscle after physical exercise. In addition, blood free carnitine concentrations do not reflect the tissue levels and are insufficient markers for carnitine biosynthesis. These data suggest 1) no permanent carnitine-deficiency in tissues is responsible for the development of clinical symptoms in fatty acid oxidation defects and 2) a lack of effectiveness of L-carnitine supplementation to prevent low tissue carnitine concentrations and to replenish them.

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Accumulation of toxic metabolites

In humans with long-chain fatty acid oxidation defects, long-chain acylcarnitines accumulate during metabolic derangement. This observation is also used for diagnostic purposes including newborn screening. However, it is also known, that acylcarnitines can be completely normal during anabolic conditions (Schymik et al 2006). The mouse model of VLCADD, especially after fasting and cold exposure, presents with elevated plasma long-chain acylcarnitines; however, there is also a significant rise in long-chain acylcarnitines in wild-type mice under these conditions (Spiekerkoetter et al 2004). These effects are well confirmed by measurements of liver acylcarnitines (Spiekerkoetter et al 2005). In contrast, physical exercise of 1 hour in mice does not result in an increase in acycarnitines in blood as compared to resting conditions (Spiekerkoetter et al 2004). However, concurrent acylcarnitine measurements in skeletal muscle after physical exercise reveal a significant increase in acylcarnitine production suggesting no correlation between muscle and blood concentrations. Importantly, after a regeneration period of 24 hours, these long-chain acylcarnitines decrease again to baseline levels (Primassin et al 2008). This has not only been observed in skeletal muscle but also in cardiac muscle.

In VLCAD-deficient mice, carnitine supplementation significantly induces long-chain acylcarnitine production in skeletal muscle. This rise in tissue long-chain acylcarnitines is even higher when mice are additionally subjected to physical exercise. These results are threatening since toxic and arrhythmogenic effects are attributed to long-chain acylcarnitines (Bonnet et al 1999). It is under debate whether unphysiologically high concentrations of long-chain acyl-CoA esters are also toxic. Hep G2 cells have been incubated with palmitoyl-carnitine and palmitoyl-CoA at different concentrations and cell viability is affected by palmitoyl-carnitine at much lower concentrations as compared to palmitoyl-CoA, suggesting a higher toxic potential of long-chain acylcarnitines (Primassin et al 2008). This also proved that acylcarnitine accumulation and acyl-CoA accumulation directly correlate and are in balance as demonstrated by ter Veld et al (ter Veld et al 2009). However, acylcarnitines are in general measurable in higher concentrations than acyl-CoA ester (ter Veld et al 2009). With carnitine supplementation, the balance of both metabolites would shift to increased production of acylcarnitines.

In summary, in the mouse model of VLCADD, there is tissue-specific acylcarnitine production according to the stressor applied with highly elevated acylcarnitine production in skeletal muscle after exercise. Threatening is the fact that L-carnitine supplementation significantly induces acylcarnitine production, especially after exercise (without replenishing free carnitine in skeletal muscle). In vitro studies suggest that long-chain acylcarnitines are more toxic than the corresponding long-chain acyl-CoA ester, however, this has to be further studied in vivo. Toxic effects of accumulating metabolites seem to play an important role in disease pathogenesis since the tissue-specific accumulation due to different stressors directly correlates with the clinical symptoms namely skeletal myopathy after exercise and hepatopathy after fasting.

Energy deficiency

Energy deficiency due to impaired long-chain fatty acid oxidation especially affects high-energetic organs such as the heart or skeletal muscle. Disease episodes occur in situations of increased energy demand such as exercise or illnesses when energy from carbohydrates has been used up and fatty acid oxidation is activated to provide energy from fat. Exil and coworkers have shown in different tissues from VLCAD-deficient mice that mitochondrial proliferation appears to be compensating for impaired β-oxidation and energy deficiency (Exil et al 2003). In fact, under resting conditions VLCAD-deficient mice, as occurs in human patients, do not present with skeletal myopathy. The same occurs to the heart at rest with no echocardiographic

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signs of cardiomyopathy, however the same group demonstrated abnormal mitochondrial bioenergetics and heart rate dysfunction (Exil et al 2006). Additionally, cardiac function of VLCAD-deficient mice assessed by MRI revealed a significantly reduced stroke volume as compared to wild-type mice suggesting impaired cardiac function without changes in cardiac morphology (ter Veld et al 2008). Longer-term exercise over a period of 2 weeks, however, also induced morphological cardiac changes (ter Veld et al 2008).

There are reports from clinical practice in different long-chain fatty acid oxidation defects demonstrating prevention of muscle weakness and pain and also episodes of rhabdomyolysis with medium-chain triglyceride (MCT) supplementation right prior to exercise providing sufficient energy during increased energy demand (Spiekerkoetter 2007; Gillingham et al 2006). The effects have also been studied in the mouse model of VLCADD and could be directly confirmed with a much less accumulation of long-chain acylcarnitines in skeletal muscle after exercise after an MCT bolus prior to exercise suggesting sufficient energy production from MCT fat.

Cold intolerance

There are at least three crucial components of nonshivering thermogenesis as demonstrated in gene targeted mouse models. These include an intact β-adrenergic signaling system, a mitochondrial FAO pathway, and expression of mitochondrial uncoupling protein-1 (UCP-1). The first example of cold intolerance demonstrated in mouse models of FAO deficiencies was described by Guerra et al. (Guerra et al 1998). Fatal cold intolerance in the well characterized SCAD-deficient mouse model (Wood et al 1989; Amendt et al 1992; Hinsdale et al 1993; Armstrong et al 1993) and in the LCAD- deficient mouse model (Kurtz et al, 1998, Cox et al., 2001) was reported to occur due to the inability to adequately accomplish non-shivering thermogenesis when placed in a laboratory cold room at 4°C (Guerra, et al. 1998). Previously, it had been shown that mice deficient in UCP-1 were also cold intolerant (Enerback et al 1997). Although they had an intact adrenergic system and a complete fatty acid oxidation (FAO) pathway, they were unable to uncouple the generated energy for ATP production and turn it into heat. In contrast, the mice with FAO deficiencies are unable to generate the energy required to uncouple and produce heat. Additionally, in the case of the SCAD-deficient mice significantly lower expression of UCP-1 was demonstrated; whereas, the UCP-2 expression was significantly higher (Guerra et al 1998). While FAO is crucial for non-shivering thermogenesis, acyl-CoA dehydrogenase gene expression in brown fat is not influenced by cold exposure and FAO genes are not regulated by PPAR-α as they are in liver (Goetzman et al 2005).

Disturbed hepatic carbohydrate management

In contrast to long-chain fatty acid oxidation defects, MCADD generally presents with a hepatic phenotype in human patients. Cardiomyopathy and myopathy do not occur. However, the hepatic disease signs such as hypoglycemia, hepatic dysfunction and hepatomegaly due to steatosis completely resemble those found in human VLCADD or other long-chain fatty acid oxidation defects. Also in MCADD, symptoms are mainly induced by catabolism e.g. during prolonged fasting or illnesses. Therefore, in mice with MCADD, the hepatic carbohydrate management has been studied during fasting (Tolwani et al 2005) and during a lipopolysaccharide (LPS)-induced acute phase response (APR) (Herrema et al 2008). During the acute phase response, both, MCAD-deficient and wild-type mice became hypoglycemic, however, after a 24-hour fasting period only, no hypoglycemia was observed. Flux studies have shown, that the de novo synthesis of glucose-6-phosphate (G6P) was not impaired on fasting, however, was significantly decreased (−20%) during the acute phase reaction. In addition, newly formed G6P was preferentially directed towards glycogen under both conditions.

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Interestingly, the expression of different transcription factors, such as hepatic peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α), peroxisome proliferator-activated receptor alpha (PPARα) and pyruvate dehydrogenase kinase 4 (PDK4), which all regulate fatty acid and glucose oxidation, however, suggest altered control of hepatic glucose metabolism during fasting and during the acute phase reaction (Herrema et al 2008). PPARα is known to be a major regulator of fatty acid oxidation, regulating the expression of FAO enzymes and fatty acid transporters. PGC-1α is a major energy regulating transcriptional co-activator upstream of PPARα and has wide influence on most energy producing pathways depending on its binding partners such as PPARα. PDK4 is the most important kinase that phosphorylates and therefore inhibits the pyruvate dehydrogenase complex in times of reduced glucose utilization.

In summary, transcriptional changes of factors regulating fatty acid and glucose oxidation occur in MCAD-deficient mice after different stressors and suggest an altered control of hepatic glucose metabolism. In addition, hepatic glucose output is reduced in MCAD-deficient mice during an acute phase response. Overall, enhanced peripheral glucose uptake due to increased glucose consumption during energy deficiency appears to be the major pathogenetic mechanism responsible for the development of hypoglycemia in the study presented (Herrema et al 2008).

Impairment of intrauterine fatty acid oxidation

It is proposed that mitochondrial fatty acid oxidation does not play an important role in intrauterine life, and it has been demonstrated that fatty acid oxidation genes are significantly upregulated after birth (Oey et al 2006). However, there are reports in humans of severe intrauterine cardiomyopathy that is lethal after birth (Spiekerkoetter et al 2008). As one reason for fetal disease and maternal HELLP (hemolysis, elevated liver enzymes, low platelets) – syndrome, deficient fatty acid oxidation in the placenta has been proposed (Rakheja et al 2002). There is also a significant embryonic loss in LCAD- (Kurtz et al 1998), CPT-1a- (Nyman et al 2005) and CPT-1b- (Ji et al 2008) deficient mice. LCAD-deficiency produces significant gestational losses of both the homozygous and heterozygous mutant offspring (Kurtz et al 1998); however, around 50–60% of mice with LCAD-deficiency survive to birth and live into adulthood. Homozygous LCAD-deficient embryos appear to die around day 4 to 5 of gestation when the embryo is transforming from a morula into a blastocyst (Berger and Wood 2004), a process that requires considerable energy. Furthermore, it was not possible to rescue LCAD-deficient embryos in culture with medium or short-chain fatty acid supplementation (Berger and Wood 2004).

In contrast to the reduced gestational survival of homozygous LCAD mice, there has never been a live born homozygous CPT-1a (Nyman et al 2005) or CPT-1b mouse (Ji et al 2008). These two enzyme deficiencies appear to be much more severe in homozygous mice and apparently kill the embryo sometime before day 10 of gestation (Nyman et al 2005; Ji et al 2008).

In conclusion, these data suggest that intrauterine mitochondrial fatty acid oxidation is of relevance and that energy production from glucose oxidation is not always sufficient in utero. However, the exact mechanisms for the embryonic loss remain unsolved, e.g. whether energy deficiency is the main problem or whether additional toxic effects of accumulating metabolites also play a role.

Synergistic heterozygosity

Vockley and coworkers (Vockley et al 2000) hypothesized that patients found with typical clinical/pathologic features of an inborn error of FAO, yet had no clear enzyme deficiency or

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metabolite pattern indicating a specific homozygous deficiency, could be double heterozygous for two different enzyme deficiencies resulting in a clinical disease episode. They reported tantalizing data from a few patients with clearly two partial deficiencies and clinical disease symptoms. They called this genetic hypothesis “synergistic heterozygosity.” That is, individuals with two different heterozygous enzyme deficiencies in the same metabolic pathway (mitochondrial fatty acid oxidation) will be at risk for acute metabolic disease. This might explain some patients with a disease phenotype without an unequivocal diagnostic enzyme deficiency/metabolite pattern.

This concept was further investigated in some mouse models of FAO deficiencies. As reported previously (Schuler et al 2002, Schuler et al 2005), synergistic heterozygosity was demonstrated in various heterozygous combinations of VLCAD+/−//LCAD+/−//SCAD+/− mice. In general, double heterozygous combinations of VLCAD+/−//LCAD+/−, or LCAD+/−//SCAD+/−, and the triple combination VLCAD+/−//LCAD+/−/SCAD+/− all produced fatal cold intolerance in ~33% of mice. In contrast, wild-type and single heterozygous control groups showed 100% normal cold tolerance. Furthermore, the accompanying biochemical characteristics such as acylcarnitine and fatty acid profiles appeared modestly abnormal. Overall, as far as serum acylcarnitine profiles were concerned, LCAD+/−//SCAD+/− and VLCAD+/−//LCAD+/− mice had patterns similar to both LCAD−/− and VLCAD−/− mice. VLCAD+/−//LCAD+/−//SCAD+/− mice had a pattern most similar to wild-type. There was no indication such as butyrylcarnitine indicating SCAD+/− deficiency (Wood et al 1989). Regarding free fatty acid profiles in serum, VLCAD+/−//LCAD+/− mice had patterns similar to VLCAD−/− (i.e., no $C_{14:1}$), whereas LCAD+/−//SCAD+/− and VLCAD+/−//LCAD+/−//SCAD+/− mice had patterns similar to wild-type. Thus, double/triple heterozygosity in the mouse models can produce acute disease under metabolic challenge with ambiguous biochemical profiles. Furthermore, the disease phenotype is not as severe as that found in homozygous enzyme deficiencies involving the same enzymes.

These initial studies require further investigations with special respect to genotype/phenotype relationships. Critical areas of investigation include whether and how changes in metabolite pools affect the normal rate limiting steps from being properly regulated ones to ones that occur by default because of the 50% functional deficiency without the proper normal regulation.

In summary, synergistic heterozygosity may explain why some patients with a disease phenotype do not present with an unequivocal diagnostic enzyme deficiency/metabolite pattern. This mechanism may also be of importance in severe clinical manifestations of otherwise milder deficiencies such as VLCADD due to an additional mutation in one of the other fatty acid oxidation enzymes.

Conclusions

As reviewed here the mouse models representing a range of enzyme deficiencies in the FAO pathway have been instrumental in our further understanding of the pathophysiology of these disorders in human patients. These studies point the way to new therapies or preventive measures for this group of potentially fatal disorders affecting children.

Acknowledgments

Part of the work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG, SP1125/1-1; SFB 575; SFB 612).

Other parts of the work were supported by NIH Grant Number RO1-RR02599 and T-32-RR00493 to PAW from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.

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Abbreviations

APR	acute phase response
CPT-1a	carnitine palmitoyl-CoA transferase-1a (liver isoform)
CPT-1b	carnitine palmitoyl-CoA transferase-1b (muscle isoform)
DAG	diacylglycerol
FAO	fatty acid oxidation
G-6-P	glucose-6 phosphate
LCAD	long-chain acyl-CoA dehydrogenase
LCT	long-chain triglycerides
MCAD	medium-chain acyl-CoA dehydrogenase
MCADD	medium-chain acyl-CoA dehydrogenase deficiency
MCT	medium-chain triglycerides
PPAR-α	peroxisomal proliferator activated receptor-alpha
PGC1-α	PPAR-γ co-activator-1 alpha
PDK4	pyruvate dehydrogenase kinase four
SCAD	short-chain acyl-CoA dehydrogenase
TFP-α	mitochondrial trifunctional protein-α
TFP-β	mitochondrial trifunctional protein-β
TFPD	trifunctional protein deficiency
VLCAD	very long-chain acyl-CoA dehydrogenase
VLCADD	very long-chain acyl-CoA dehydrogenase deficiency

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Table 1

Mouse models of defects in mitochondrial β-oxidation of fatty acids

Enzyme deficiency	Mouse phenotype	References
Carnitine palmitoyl-CoA transferase-1a (liver isoform)	Homozygous lethal in early gestation, heterozygous mice have normal phenotype	Nyman et. al., 2005 ([8])
Carnitine palmitoyl-CoA transferase-1b (muscle isoform)	Homozygous lethal in early gestation, heterozygous mice have normal phenotype	Ji et al., 2008 ([9])
Very long-chain acyl-CoA dehydrogenase deficiency: two independent mouse models	Cardiac phenotype: VLCAD −/− displays milder version of LCAD−/− phenotype; Hepatic and myopathic phenotypes after stress	Cox et al., 2001 and 2009 ([10], [6]) Exil et al., 2003 ([11]) Spiekerkoetter et al., 2004 and 2005 ([12], [4])
Long-chain acyl-CoA dehydrogenase deficiency	Sudden death, gestational loss, fatty change of liver and heart, cold intolerance,	Kurtz et al., 1998 ([7]), Guerra, et al., 1998 ([13])
Medium-chain acyl-CoA dehydrogenase deficiency	neonatal deaths, fasting intolerance, cold intolerance	Tolwani, et al., 2005 ([14])
Mitochondrial trifunctional protein deficiency (α-subunit deficiency)	Neonatal hypoglycemia, fatty change of liver, necrosis and degeneration of cardiac and diaphragmatic myocytes, lethal	Ibdah et al. 2001 ([15])
Mitochondrial trifunctional protein (β-subunit deficiency)	Viable, no further characterization	Personal communication Arnold Strauss, Cincinnati, USA
Medium-/short-chain 3-hydroxyacyl-CoA dehydrogenase	Fasting and cold intolerance with development of fatty liver and kidney	Personal communication Arnold Strauss, Cincinnati, USA