Mechanistic Bases of Neurotoxicity Provoked by Fatty Acids Accumulating in MCAD and LCHAD Deficiencies

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Abstract
Fatty acid oxidation defects (FAODs) are inherited metabolic disorders caused by deficiency of specific enzyme activities or transport proteins involved in the mitochondrial catabolism of fatty acids. Medium-chain fatty acyl-CoA dehydrogenase (MCAD) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiencies are relatively common FAOD biochemically characterized by tissue accumulation of medium-chain fatty acids and long-chain 3-hydroxy fatty acids and their carnitine derivatives, respectively. Patients with MCAD deficiency usually have episodic encephalopathic crises and liver biochemical alterations especially during crises of metabolic decompensation, whereas patients with LCHAD deficiency present severe hepatopathy, cardiomyopathy, and acute and/or progressive encephalopathy. Although neurological symptoms are common features, the underlying mechanisms responsible for the brain damage in these disorders are still under debate. In this context, energy deficiency due to defective fatty acid catabolism and hypoglycemia/hypoketonemia has been postulated to contribute to the pathophysiology of MCAD and LCHAD deficiencies. However, since energetic substrate supplementation is not able to reverse or prevent symptomatology in some patients, it is presumed that other pathogenetic mechanisms are implicated. Since worsening of clinical symptoms during crises is accompanied by significant increases in the concentrations of the accumulating fatty acids, it is conceivable that these compounds may be potentially neurotoxic. We will briefly summarize the current knowledge obtained from patients with these disorders, as well as from animal studies demonstrating deleterious effects of the major fatty acids accumulating in MCAD and LCHAD deficiencies, indicating that disruption of mitochondrial energy, redox, and calcium homeostasis is involved in the pathophysiology of the cerebral damage in these diseases. It is presumed that these findings based on the mechanistic toxic effects of fatty acids may offer new therapeutic perspectives for patients affected by these disorders.

Keywords
fatty acid oxidation defects, MCAD deficiency, LCHAD deficiency, medium-chain fatty acids, long-chain 3-hydroxy fatty acids, mitochondrial dysfunction, oxidative stress

Introduction
Medium-chain fatty acyl-CoA dehydrogenase (MCAD) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiencies are among the most common fatty acid oxidation defects (FAODs) that are caused by deficient activities of MCAD and LCHAD, respectively. The biochemical hallmark of these disorders is tissue accumulation of medium-chain fatty acids (MCFAs) and long-chain 3-hydroxy fatty acids (LCHFAs) and their carnitine derivatives.

Medium-Chain Acyl-CoA Dehydrogenase Deficiency
Medium-chain acyl-CoA dehydrogenase (E.C. 1.3.99.3) deficiency (OMIM # 201450) is the most common FAOD with a
prevalence of around 1:10 000 newborns. It is caused by a defect in MCAD, leading to the accumulation of octanoate, decanoate, cis-4-decenoate, and their respective carnitine derivatives. Lactic acidosis has been found in the affected patients, especially during episodes of metabolic decompensation. Clinical presentation usually occurs during fasting or metabolic stress and is characterized by lethargy, seizures, and coma. Progressive encephalopathy with brain abnormalities is commonly found in many untreated patients. Muscle weakness and rhabdomyolysis during acute episodes can also be observed. Death may occur in 25% of patients with MCAD deficiency undiagnosed in the first presentation. In this context, diagnosis is usually performed by the detection of increased octanoylcarnitine concentrations in blood and medium-chain dicarboxylic acids and glycine derivatives in urine.

An important measure for MCAD deficiency treatment is to reverse catabolism and sustain anabolism by administrating high amount of carbohydrates and to prescribe frequent meals and fasting avoidance to prevent episodes of decompensation. On the other hand, L-carnitine sometimes coupled to riboflavin supplementation may be helpful in MCAD deficiency, as well as for very long-chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiencies, although this is still controversial. L-Carnitine administration may increase urinary excretion of accumulating toxic metabolites by its property of binding to these compounds and correct carnitine deficiency, whereas riboflavin was shown to activate octanoyl-CoA dehydrogenase in lymphocytes from patients with MCAD deficiency, as well as the respiratory chain. On the other hand, newborn screening of MCAD deficiency has dramatically improved the clinical course of the disease, significantly preventing mortality and morbidity.

Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

Long-chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211) deficiency (OMIM # 609016) is FAOD with an approximate prevalence of 1:50 000 newborns. Laboratory and clinical features include hypoglycemia, metabolic acidosis, hyperlactacidemia, hyperammonemia, skeletal myopathy, hypotonia, cardiomyopathy, and hepatopathy. Mortality is mainly caused by cardiac decompensation and liver failure and may be as high as 80%. Milder cases surviving into adolescence and adulthood usually present hypotonia, seizures, mental retardation, hypoglycemia, cardiomyopathy, peripheral neuropathy, and retinopathy. Metabolic crises are characterized by encephalopathy with seizures, hypoketotic hypoglycemia, vomiting, and dehydration precipitated by infections. Diagnosis of LCHAD deficiency is performed by identification of high urinary excretion of dicarboxylic acids with a hydroxyl group and their carnitine derivatives in blood.

Therapy is based on the prevention of fasting and acute infections, as well as a high carbohydrate consumption at frequent intervals associated with medium-chain triglyceride supplementation. The use of L-carnitine as adjuvant therapy is also controversial and may even aggravate the clinical condition possibly by generating toxic long-chain carnitine derivatives. It is emphasized that patients affected by LCHAD deficiency are difficult to treat since many of them present severe clinical manifestations despite the use of an appropriate diet and medications.

### Table 1. Potential Pathogenetic Mechanisms Underlying Brain Damage in Fatty Acid Oxidation Disorders (FAOD).

<table>
<thead>
<tr>
<th>Pathogenetic Mechanisms</th>
<th>Possible Causes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy deficiency</td>
<td>Mitochondrial dysfunction</td>
<td>21,24</td>
</tr>
<tr>
<td></td>
<td>Hypoketotic hypoglycemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss or deficiency of carnitine and CoA</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>β-Oxidation blockage</td>
<td>22,23</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain inhibition</td>
<td></td>
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<tr>
<td></td>
<td>Misfolded protein</td>
<td></td>
</tr>
<tr>
<td>Hyperammonemia</td>
<td>Reduction of hepatic ATP availability from fatty acid oxidation</td>
<td>21,25</td>
</tr>
<tr>
<td>Lipotoxicity</td>
<td>Toxicity of fatty acids and carnitine derivatives altering cellular functions</td>
<td>21</td>
</tr>
</tbody>
</table>

### Potential Mechanisms of Neurotoxicity in FAOD

Although the pathophysiology of FAOD is still poorly known, brain dysfunction has been attributed to inadequate energy supply due to hypoketotic hypoglycemia, as well as by the toxic effects of high ammonia levels that occur in some patients with these disorders. However, other pathomechanisms seem to be implicated in their pathogenesis. In this context, observations of disturbed redox homeostasis in tissues from patients with MCAD deficiency and more recently neurotoxic properties of the accumulating fatty acids and/or carnitine derivatives may represent contributory factors to FAOD pathophysiology. In the present review, we focus on the role of disrupted mitochondrial homeostasis and oxidative stress observed in humans with MCAD and LCHAD deficiencies. We also provide animal experimental data demonstrating that disruption of mitochondrial functions is caused by the fatty acids found at high concentrations in tissues of the affected patients. This is consistent with the observations showing that symptomatology worsening is associated with substantial increase in the concentrations of the potentially toxic fatty acids, particularly during catabolic crises. Table 1 displays the proposed major pathomechanisms of brain damage in FAOD.

It should be stressed that neural cells express β-oxidation enzyme activities at low levels, especially the thiolase activity that is nearly absent in this tissue, explaining the inability of brain to oxidize fatty acid to sustain ATP generation. However, the presence of β-oxidation pathway enables neural
cells to potentially produce and accumulate fatty acids in patients affected by MCAD and LCHAD deficiencies. On the other hand, although brain lipid composition is high, central nervous system is capable of synthesizing only a few nonessential fatty acids, so that both essential and some nonessential fatty acids must enter into the brain from the blood to be incorporated into complex lipids. Short-chain and medium-chain fatty acids with less than 12 carbons can be efficiently transported from the systemic circulation to the brain by passive diffusion through the blood–brain barrier (BBB), whereas long-chain fatty acids are less soluble and must be transported in the nonionized form linked to fatty acid transport proteins to cross the BBB. The protein-mediated transport is carried out by specific protein transporters expressed on the cell membrane, including the fatty acid transport proteins 1 to 6, the fatty acid translocase/CD36, and the plasma membrane fatty acid–binding protein.

### Disruption of Mitochondrial Homeostasis in MCAD and LCHAD Deficiencies

Mitochondria are essential for cellular homeostasis and survival, since they play critical roles in energy (ATP) production and intracellular transfer, as well as in the regulation of redox and calcium homeostasis, fatty acid oxidation, and apoptosis.

Oxidative phosphorylation (OXPHOS) is the major source of cellular ATP and reactive oxygen species (ROS) formation. Reactive oxygen species have essential functions in cellular signaling mainly by regulating the expression/activity of many genes and enzymes. However, when at high concentrations, ROS become toxic to the cell causing oxidative damage to mitochondrial proteins, lipids, and DNA that may lead to a cascade of apoptosis or necrosis.

Oxidative damage and elevated intracellular calcium concentrations cause mitochondrial stress and collapse of internal membrane potential in a process called mitochondrial permeability transition that also leads to cell death.

Brain is highly dependent on OXPHOS for energy production and therefore is highly susceptible to alterations in mitochondrial function. When OXPHOS is compromised, ATP synthesis is decreased and free radical production increased, potentially leading to cell damage. Thus, it is expected that disordered mitochondrial functions is associated with encephalopathy.

Available human studies point to mitochondrial dysfunction as an important mechanism in the pathogenesis of brain damage in patients affected by MCAD and LCHAD deficiencies. This is consistent with the observations of hyperlactic acidemia, decreased activity of respiratory chain complexes, oxidative stress biomarkers, mitochondrial morphological abnormalities, and rhabdomyolysis that have been demonstrated in patients affected by these diseases.

However, the exact underlying mechanisms of mitochondrial deregulation are still unclear in these disorders.

### Fatty Acid Toxicity

Table 2 shows potential mechanisms of mitochondrial disruption. Long-chain fatty acids that are routinely present in plasma of normal individuals were shown to have cytotoxic effects at high concentrations, impair ATP generation, uncouple OXPHOS, inhibit the respiratory chain, depolarize mitochondria by their protonophoric properties, induce oxidative stress, and have permeability transition.

Medium-chain fatty acid effects on mitochondrial functions are less studied, but may similarly affect mitochondrial respiration and disturb the translocation of ADP in mitochondria. Therefore, it is feasible that the fatty acids that accumulate in tissues of patients with MCAD and LCHAD deficiencies (Table 3) may similarly induce cellular toxicity. This presumption is supported by mounting evidence of deleterious effects on mitochondrial functions attributed to these compounds. We present below evidence that lipotoxicity caused especially by the principal fatty acids accumulating in MCAD and LCHAD deficiencies may contribute decisively to disrupt mitochondrial homeostasis in brain tissue.

### Table 2. Toxicity of Fatty Acids Commonly Found in High-Concentration Human Plasma.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Mitochondrial Homeostasis Disruption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid, stearic acid</td>
<td>Inhibition of oxidative phosphorylation in fibroblasts</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Uncoupler of oxidative phosphorylation in liver</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Induction of permeability transition in liver</td>
<td>48,49</td>
</tr>
<tr>
<td>Oleic acid, palmitoleic acid</td>
<td>Reduction of Na(^+), K(^+)—ATPase activity in purified enzyme</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Decrease of Ca(^{2+})—ATPase activity in human red cell membranes</td>
<td>45</td>
</tr>
</tbody>
</table>

### Table 3. Major Fatty Acids Accumulating in Medium-Chain Acyl-CoA Dehydrogenase (MCAD) and Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) Deficiencies.

<table>
<thead>
<tr>
<th>FAOD</th>
<th>Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAD deficiency</td>
<td>Octanoic acid, decanoic acid, cis-4-decenoic acid, 3-Hydroxydodecanoic acid, 3-hydroxytetradecanoic acid, 3-hydroxypalmitic acid</td>
</tr>
<tr>
<td>LCHAD deficiency</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FAOD, fatty acid oxidation defect.

### Evidence That Mitochondrial Dysfunction Is Caused by the Major Fatty Acids Accumulating in MCAD and LCHAD Deficiencies

It can be seen in Table 4 that the MCFAs accumulating in MCAD deficiency deregulate various crucial mitochondrial functions in the brain. It has been demonstrated that MCFA, particularly decanoic and cis-4-decenoic acids, behave as uncouplers of OXPHOS and metabolic inhibitors, as well as inducers of mitochondrial permeability transition and oxidative stress. These effects may be associated at least partly
with the blockage of the respiratory chain provoked by MCFA that may stimulate superoxide and other ROS production and with the protonophoric action of these fatty acids due to the transbilayer movement of undissociated (linked to protons) fatty acids through the mitochondrial inner membrane toward the mitochondrial matrix.

Mitochondrial dysfunction has also been suggested as an important pathomechanism involved in the neurological symptoms that affect individuals with LCHAD deficiency since there are clear human evidences of mitochondrial damage in these patients. Furthermore, the major hydroxylated fatty acids accumulating in LCHAD deficiency deregulate crucial mitochondrial functions such as maintenance of membrane potential, NAD(P)H redox status, and calcium retention capacity (Table 5). They also uncouple the OXPHOS, induce mitochondrial permeability transition pore opening, and provoke metabolic inhibition in forebrain of adolescent rats, besides inducing oxidative stress.

These data allied to previous observations demonstrating that long-chain 3-hydroxyacyl-CoA derivatives inhibiting ATP production in human fibroblasts support the hypothesis that LCHFAs disrupt energy and redox mitochondrial homeostasis, probably representing a relevant underlying mechanism in the pathophysiology of the cerebral alterations observed in LCHAD deficiency.

Taken together, the available data strongly indicate that the major fatty acids accumulating in MCAD and LCHAD deficiencies probably contribute to the pathogenesis and perhaps symptomatology of affected patients, by disrupting mitochondrial homeostasis, especially during catabolic situations in which their concentrations significantly increase in blood and other tissues due to accelerated lipolysis. Figure 1 depicts the potential mechanisms involved in FAOD pathophysiology, emphasizing the important role of lipotoxicity provoked by the accumulating metabolites inducing deregulation of mitochondrial homeostasis.

### Concluding Remarks

Growing evidence obtained from human and animal studies revealed that disturbance of mitochondrial functions associated with oxidative stress at least partly caused by accumulating fatty acids is involved in the pathophysiology of MCAD and LCHAD deficiencies. It is presumed that this pathomechanism possibly contribute to the chronic and neurologic symptoms seen in these defects. Moreover, although it is difficult to evaluate the relative contribution of the toxic fatty acids in the neuropathology of these diseases, it is conceivable that there may be a synergistic action between the toxicity of these

### Table 4. Disruption of Brain Mitochondrial Homeostasis Provoked by the Major Medium-Chain Fatty Acids Accumulating in Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency.

<table>
<thead>
<tr>
<th>Accumulating Fatty Acids</th>
<th>Mitochondrial Homeostasis Disruption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanoic acid; decanoic acid; cis-4-decenolic acid</td>
<td>Uncoupling of oxidative phosphorylation; Metabolic inhibition</td>
<td>51,54-55</td>
</tr>
<tr>
<td></td>
<td>Decrease of NAD(P)H content</td>
<td>54-55</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain and creatine kinase activities inhibition</td>
<td>54,56-58</td>
</tr>
<tr>
<td></td>
<td>Induction of oxidative stress</td>
<td>52,53</td>
</tr>
<tr>
<td></td>
<td>Decrease of Ca$^{2+}$ retention capacity</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Induction of permeability transition</td>
<td>56</td>
</tr>
</tbody>
</table>

### Table 5. Disruption of Brain Mitochondrial Homeostasis Provoked by the Major Long-Chain 3-Hydroxy Fatty Acids Accumulating in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) Deficiency.

<table>
<thead>
<tr>
<th>Accumulating Fatty Acids</th>
<th>Mitochondrial Homeostasis Disruption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxydodecanoic acid</td>
<td>Uncoupling of oxidative phosphorylation</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Induction of oxidative stress</td>
<td>63</td>
</tr>
<tr>
<td>3-Hydroxytetradecanoic acid; 3-hydroxypalmitic acid</td>
<td>Uncoupling of oxidative phosphorylation</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Induction of oxidative stress</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Decrease of NAD(P)H content</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Decrease of Ca$^{2+}$ retention capacity</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>ATP production impairment</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Induction of permeability transition</td>
<td>62</td>
</tr>
</tbody>
</table>

![Figure 1. Lipotoxicity as an important pathomechanism involved in the brain damage of medium-chain acyl-CoA dehydrogenase (MCAD) and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiencies.](image)
metabolites, hyperammonemia, and hypoketotic hypoglycemia (energy deficit) leading to brain damage. It is expected that the development of new drugs targeting the mitochondrion, initially in animal models and thereafter as adjuvant therapeutic approaches for the patients, may become an important focus in the future. In this context, bezafibrate, which activates the peroxisome proliferator-activated receptor that induces transcription of various enzymes involved in mitochondrial fatty acid oxidation, was shown to normalize impaired β-oxidation in skin fibroblast from patients with various FAOD, as well as improve the clinical symptoms of late-onset type glutaric acidemia type II. Similar effects were attributed to the antioxidant and anti-inflammatory natural compound resveratrol with positive effects on mitochondrial energy metabolism improving mitochondrial fatty acid oxidation capacities in fibroblasts from patients with VLCAD and carnitine palmitoyltransferase II deficiency. Thus, these compounds may represent potential novel candidates for the treatment of these diseases by a dual mechanism, improving fatty acid oxidation and counteracting oxidative stress.

**Declaration of Conflicting Interests**

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