Postepy Hig Med Dosw. (online), 2009; 63: 266-277
e-ISSN 1732-2693

www.phmd.pl
Review

**Received:** 2009.01.29
**Accepted:** 2009.04.14
**Published:** 2009.06.08

# An Overview of β-Oxidation Disorders

## Przegląd zaburzeń β-oksydacji kwasów tłuszczowych

**Dariusz Moczulski[1], Iwona Majak[2], Dariusz Mamczur[1]**

[1] Uniwersytet Medyczny w Łodzi; Katedra Medycyny Wewnętrznej
[2] Politechnika Łódzka, Wydział Biotechnologii i Nauk o Żywności, Instytut Podstaw Chemii Żywności

## Summary

Fatty acids (FAs) are components of cell membrane, enzymes, and hormones and are one of the most important energy sources for many organisms. There are several types of fatty-acid oxidative degradation processes in the cell, namely alpha-, beta-, and omega-oxidation, which take place in specialized cellular structures: mitochondria and peroxisomes. The best-known pathway is β-oxidation taking place in the matrix of mitochondria. It is responsible for the degradation of straight-chain FAs. The pathway of β-oxidation of fatty acids is comprised of at least 25 enzymes and specific transport proteins. Deficiencies in 18 of them have been demonstrated to cause disease in humans. These diseases show a wide variety of symptoms, which can be expressed at random, one at a time, or in sets, characteristic of the individual rather than the metabolic character of the disease. Disorders of β-oxidation are believed to cause about 1–3% of unexplained sudden infant deaths (SIDS). Acute fatty liver of pregnancy (AFLP) and the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome), which have significant neonatal and maternal morbidity and mortality, have also been associated with β-oxidation deficiency in fetuses. This review summarizes recent observations on disorders associated with fatty-acid oxidation: deficiencies of β-oxidation enzymes, namely VLCAD, TFP and LCHAD, MCAD, MCKAT, M/SCHAD, and SCAD, and deficiencies of the enzymes TCP I, CT, and CPT II of the carnitine cycle.

**Key words:** **Mitochondrial Fat Oxidation • Fatty Acids • Organic Aciduria • SIDS • AFLP • HELLP**

## Streszczenie

Kwasy tłuszczowe (FA) są składnikiem błon komórkowych, enzymów, hormonów oraz jednym z najważniejszych źródeł energii dla wielu organizmów. W komórce odbywa się wiele procesów oksydacyjnej degradacji kwasów tłuszczowych: alfa-, beta- oraz omega-oksydacja, które zachodzą w wyspecjalizowanych organellach komórkowych – mitochondriach i peroksysomach. Najlepiej poznanym szlakiem jest β-oksydacja zachodząca w matriks mitochondrium. Odpowiada ona za degradację prostych łańcuchów kwasów tłuszczowych. Szlak β-oksydacji kwasów tłuszczowych składa się z co najmniej 25 enzymów i wyspecjalizowanych białek transportowych. Stwierdzono, że deficyty 18 z nich powodują choroby u ludzi. Zaburzenia te przedstawiają dużą różnorodność symptomów, które mogą się objawiać pojedynczo lub w zestawach objawów, charakterystycznie raczej dla indywidualnych przypadków niż metabolicznego charakteru zaburzenia. Uważa się, że zaburzenia β-oksydacji powodują 1–3% przypadków zespołu nagłej śmierci niemowląt (SIDS). Ostre stłuszczenie wątroby ciężarnych oraz zespół niedokrwistości hemolitycznej, podwyższonych enzymów wątrobowych i małopłytkowości, przedstawiające znaczącą zapadalność i śmiertelność wśród noworodków oraz matek również są łączone z zaburzeniem β-oksydacji u płodu.

Poniższy przegląd podsumowuje aktualne obserwacje na temat zaburzeń związanych z oksydacją kwasów tłuszczowych: niedoborów aktywności enzymów β-oksydacji, tj. VLCAD, TFP oraz LCHAD, MCAD, MCKAT, M/SCHAD, SCAD, a także niedoborów aktywności enzymów cyklu karnitynowego: CPT I, CT i CPT II.

**Słowa kluczowe:** **oksydacja kwasów tłuszczowych • kwasy tłuszczowe • kwasica organiczna • SIDS • AFLP • HELLP**

**Full-text PDF:** http://www.phmd.pl/fulltxt.php?ICID=887784

**Word count:** 5841
**Tables:** 4
**Figures:** 1
**References:** 85

**Author's address:** Iwona Majak, mgr inż., Politechnika Łódzka, Wydział Biotechnologii i Nauk o Żywności, Instytut Podstaw Chemii Żywności; ul. Stefanowskiego 4/10, 90-924 Łódź; e-emal: ihime.m@gmail.com

**Abbreviations:** **AFLP** – acute fatty liver of pregnancy; **CACT** – carnitine/acylcarnitine translocase; **CAT** – carnitine transporter; **CPT I** – carnitine palmitoyltransferase I (liver); **CPT II** – carnitine palmitoyltransferase II; **ETF/ETFDH** – electron-transport flavoprotein/electron-transport flavoprotein dehydrogenase; **FATP** – fatty acid transport protein; **HELLP** – hypertension, elevated liver enzymes, and low platelets; **MCAD** – medium-chain acyl-CoA dehydrogenase; **MCKAT** – medium-chain 3-oxoacyl-CoA thiolase; **MCT** – medium-chain triglyceride; **MTP** – mitochondrial trifunctional protein (including long-chain enoyl-CoA hydratase, long-chain 3-hydroxyacyl-CoA dehydrogenase, and long-chain 3-oxoacyl-CoA thiolase); **SCAD** – short-chain acyl-CoA dehydrogenase; **SCHAD** – short-chain 3-hydroxyacyl-CoA dehydrogenase; **SCKAT** – short-chain 3-oxoacyl-CoA thiolase; **SIDS** – sudden infant death syndrome; **VLCAD** – very-long-chain acyl-CoA dehydrogenase.

## Fatty acids and the pathways of their degradation

Fatty acids (FAs) are components of cell membrane, enzymes, and hormones and are one of the most important energy sources for many organisms. The primary sources of fatty acids are triglycerides, esters of glycerol, and three identical or different fatty acids which are the main components of animal fats and plant oils. Chemically, fatty acids are a group of compounds containing a long aliphatic chain and a carboxylic group; in other words, they are carboxylic acids containing at least eight carbon atoms. The carbon chain can be either saturated or mono- or polyunsaturated as well as straight or branched. The majority of natural fatty acids contains an even number of carbons because they are synthesized from acetyl-CoA. The digested triglycerides are hydrolyzed into monoglycerides and free fatty acids by lipases in order to pass through the intestinal wall. After this they are re-formed and released into the lymph and blood stream in chylomicrons or liposomes, lipoproteins in which proteins have charged groups directed outwards, which makes them soluble in salt solutions such as blood, or as free fatty acids. Furthermore, they can be stored in adipose tissue or hydrolyzed and oxidized by tissues that require energy. The transport of free fatty acids through the cell membrane and cytoplasm is accomplished by specific transporter molecules with the aid of fatty acid-binding proteins [29,33,71,72].

There are several types of fatty-acid oxidative degradation processes in the cell, namely alpha-, beta-, and omega-oxidation, which take place in specialized cellular structures: the mitochondria and peroxisomes. The best-known pathway is β-oxidation taking place in the matrix of mitochondria. It is responsible for the degradation of straight-chain FAs. Long-chain fatty acids require activation before they enter the matrix, which is achieved by the formation of a thioester bond between the acid and CoA. The reaction is catalyzed by acyl-CoA synthase on the outer mitochondrial membrane with consumption of energy from ATP. The released pyrophosphate is immediately hydrolyzed by pyrophosphatase, which drives the reaction forward. Furthermore, very-long-chain and long-chain FAs are actively transported into the mitochondrium by a set of enzymes, i.e. carnitine palmitoyl transferase I located on the inner side of the outer mitochondrial membrane and acylcarnitine translocase and carnitine palmitoyl transferase II present on the inner side of the inner membrane. The first of these converts acyl-CoA into acylcarnitine, which requires a molecule of carnitine. Acylcarnitine translocase transports the acylcarnitine through the inner mitochondrial membrane and, finally, restores acyl-CoA in the matrix. The liberated carnitine is exchanged for another incoming molecule of acylcarnitine. Medium- and short-chain fatty acids are thought to enter the mitochondrial matrix directly [41].

β-oxidation represents a spiral pathway that can be divided into four repetitive enzymatic reactions resulting in the cleavage of two-carbon-unit acetyl-CoA. In the first step, acyl-CoA is dehydrogenated by a member of the FAD-dependent acyl-CoA dehydrogenases family, which shows high specificity to substrate chain length. Secondly, the obtained 2-enoyl-CoA is hydrated to 3-hydroxyacyl-CoA, which undergoes NAD-dependent 2,3-dehydrogenation to produce 2-ketoacyl-CoA. Finally, a specific thiolase cleaves the thioester bond, releasing acetyl-CoA and acyl-CoA, which is two carbons shorter and can enter another turn of the spiral. The process is repeated until the whole molecule has been reduced to acetyl-CoA molecules. One turn of the β-oxidation spiral yields 14 ATP molecules [6].

Fatty acids with an odd number of carbons are degraded in the same way, but the product of the last turn is propionyl-CoA, which is converted into succinyl-CoA and enters the tricarboxylic acid cycle. The long-chain acyl-CoA dehydrogenase (VLCAD) and the trifunctional protein complex (TFP), expressing the enzymatic activities of long-chain enoyl-CoA hydratase, L-3-hydroxyacyl-CoA dehydrogenase (LCHAD),

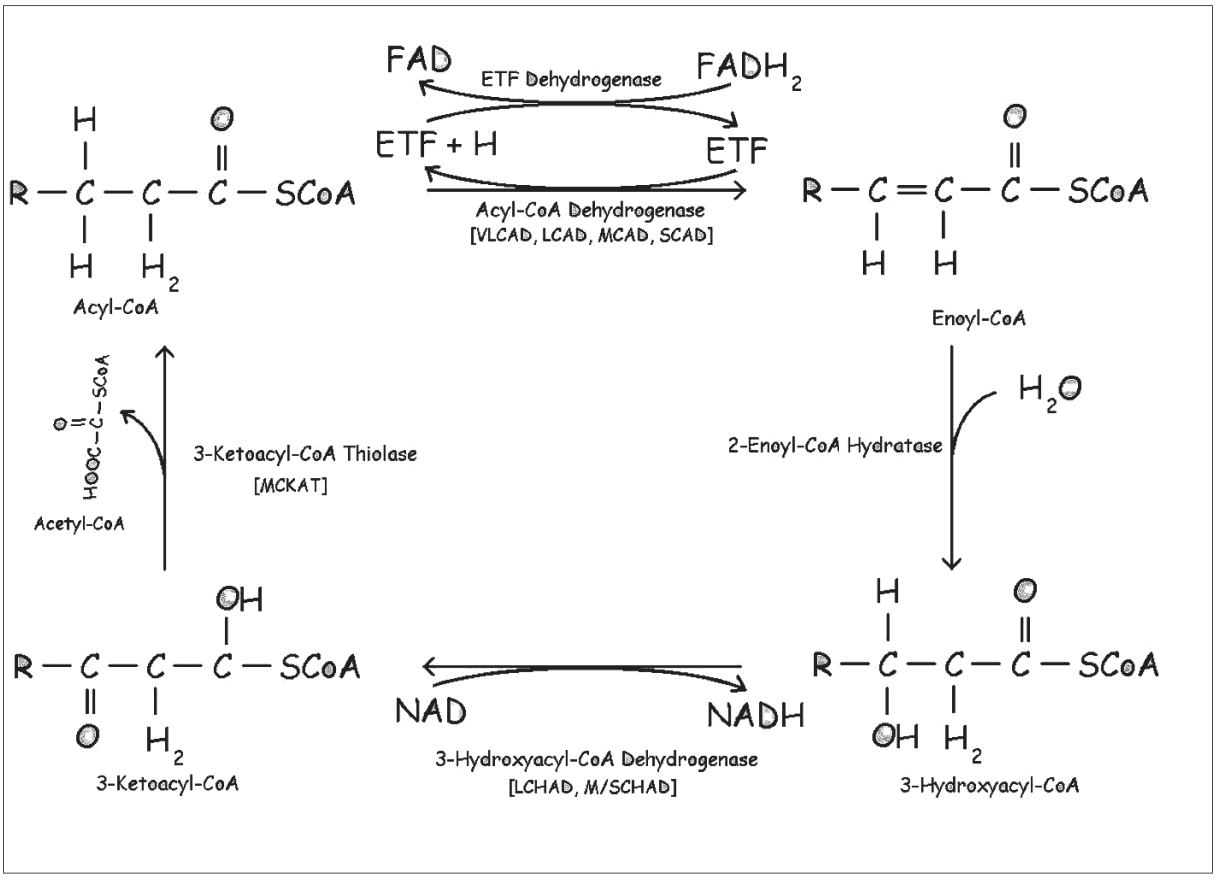


Figure 1. Mitochondrial β-oxidation spiral [28,72]

and thiolase, are located on the inner mitochondrial membrane, while medium-chain acyl-CoA dehydrogenase (MCAD), short-chain acyl-CoA dehydrogenase (SCAD), short-chain enoyl-CoA hydratase, short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD), and short-chain thiolase are present in the mitochondrial matrix [17,18,43,58,71,72].

Unsaturated fatty acids undergo the same reactions until the cis- configuration double-bond, usually present in these compounds, prevents the formation of a trans-configuration molecule which is a substrate for acyl-CoA dehydrogenase and enoyl-CoA hydratase. Odd-numbered double-bonds are handled by cis-δ3-enoyl-CoA isomerase and even-numbered bonds by 2,4-dienoyl-CoA reductase, which creates an odd-numbered double-bond subsequently adjusted by the isomerase [59].

The variation of β-oxidation taking place in peroxisomes is specific only to very-long-chain and long-chain fatty acids. Although the mitochondrial and peroxisomal enzymes have different genetic origins, the mechanisms of reaction in mitochondria and peroxisomes are similar except for the second step after activation in which peroxisomes utilize acyl-CoA oxidase, which transfers the electrons to oxygen, yielding hydrogen peroxide instead of ATP, further converted to water and oxygen by catalase. Another attempt is required for methyl-branched fatty-acid degradation, which is achieved by peroxisomal α-oxidation in which they are shortened by one carbon. Phytanic acid is the only established physiological substrate of α-oxidation in humans. First its CoA derivative is synthesized by a specific synthetase with the use of one ATP molecule and then it undergoes hydroxylation on á-carbon. Further steps are thiamine pyrophosphate-dependent cleavage and aldehyde dehydrogenation, which lead to the formation of $CO_2$ and formyl-CoA. The last known type of FA oxidation is ω-oxidation. It is a three-step process resulting in the formation of dicarboxylic acids. First, monocarboxylic fatty acid undergoes ω-hydroxylation catalyzed by mono-oxygenase associated with the endoplasmic reticulum. The resulting ω-hydroxymonocarboxylic acid is oxidized by cytosolic long-chain alcohol and aldehyde dehydrogenases to ω-ketomonocarboxylic acid and, finally, dicarboxylic acid. Dicarboxylic acids can be activated by a microsomal dicarboxylyl-CoA synthetase and undergo peroxisomal β-oxidation from both ends [25,77].

## General characteristics of β-oxidation disorders

The pathway of β-oxidation of fatty acids is comprised of at least 25 enzymes and specific transport proteins. Deficiencies in 18 of them have been demonstrated to cause disease in humans. All the defects identified so far seem to be inherited in an autosomal recessive fashion, although obligate heterozygotes have been found to have intermediate levels of enzyme activity [72]. These diseases show a wide variety of symptoms, which can be expressed at random, one at a time, or in sets, characteristic of the individual rather than the metabolic character of the disease. What is more, the signs of the disorders, including biochemical

Table 1. Enzymes involved in mitochondrial β-oxidation [72]

| Enzyme | Proven clinical disorder |
|---|---|
| *Fatty-acid transport and activation* | |
| Fatty-acid transporter(s) (plasma membrane) | Yes |
| Acyl-CoA synthetase(s) | No |
| *Carnitine cycle* | |
| Plasma membrane carnitine transporter | Yes |
| CPT I (liver) | Yes |
| CPT I (muscle) | No |
| Carnitine/acylcarnitine translocase | Yes |
| CPT II | Yes |
| *Mitochondrial β-oxidation spiral* | |
| VLCAD (membrane) | Yes |
| LCAD (matrix) | No |
| MCAD | Yes |
| SCAD | Yes |
| Electron-transferring flavoprotein | |
| ETF: ubiqiunone oxidoreductase | |
| Trifunctional protein | Yes (all activities) |
| Long-chain 2-enoyl-CoA hydratase | |
| Long-chain 3-hydroxyacyl-CoA dehydrogenase | Yes (isolated) |
| Crotonase (short-chain 2-enoyl-CoA hydratase | No |
| M/SCHAD | Yes |
| Short-chain 3-ketoacyl-CoA thiolase | Yes |
| Medium-chain 3-ketoacyl-CoA thiolase | Yes |
| *Enzymes of β-oxidation of unsaturated fats* | |
| Long-chain Δ3, Δ2-enoyl-CoA isomerase | No |
| Short-chain Δ3, Δ2-enoyl-CoA isomerase | No |
| 2,4-dienoyl-CoA reductase | Possible |

abnormalities, are not persistent and may not be evident when the patient is between episodes of metabolic crisis. Generally, the symptoms can be associated with energetic deficiency in fatty-acid-oxidation-dependent (FAO-dependent) tissues, such as the heart, liver, and muscle, and also with the secretive and toxic properties of the accumulating intermediates. They may be triggered by prolonged fasting, exercise, infection, exposure to cold, or fat -rich diet [6,17,18,19,42].

Energetic deficiency of the heart may cause cardiomyopathy and cardiac arrhythmia. The accumulation of fatty acids in the liver and muscles will result in fatty liver and hepatomegaly, muscle weakness, or hypotonia. β-oxidation disorders are often misdiagnosed as Reye's syndrome. Patients may also show recurrent episodes of rhabdomyolysis and myoglobinuria as well as peripheral neuropathy. Progressive retinopathy can be observed mostly in long-chain FA oxidation disorders [46].

ω-oxidation disorders present a paroxysmal character. Usually the first observed sign of the incoming metabolic crisis is a change in behavior, unusual irritability or lethargy, sometimes accompanied by vomiting or poor appetite. It is often associated with hypoketotic or mildly ketotic hypoglycemia, probably caused by hepatic glycogen deficiency and impaired gluconeogenesis, which can lead to coma or sudden death. However, low blood sugar levels may not appear until the metabolic crisis is well developed and are therefore not a definite indicator of the state [51,58]. The second symptom is a quick build-up of metabolic acidosis with or without hyperammonemia. Hyperammonemia is a result of secondary urea cycle dysfunction due to decreased availability of N-acetylglutamate and acetyl-CoA. Because of the blockage of β-oxidation, the accumulating fatty acids are processed by β-oxidation, which yields the characteristic dicarboxylic acids detectable in these disorders. As recurrent myoglobinuria can damage the kidneys, increased levels of serum creatine kinase and hyperuricemia may be observed. Fatty-acid oxidation disorders, except for CPT II deficiency, cause a secondary carnitine (L-3-hydroxy-4-N,N,N-trimethylaminobutyrate) deficiency because excess fatty acids are removed in the form of acylcarnitines by the elimination of carnitine-fatty acid esters from the blood through the kidneys [62].

β-oxidation disorders are believed to cause about 1–3% of unexplained sudden infant deaths (SIDS). A postmortem diagnosis is difficult because some disorders of β-oxidation cause minimal or no tissue changes. Diagnosis may be accomplished by the study of organic and fatty acids in urine and blood, histological and biochemical analysis of the liver, acylcarnitine profiling in bile and dried blood spots, and a study of fatty-acid oxidation in cultured fibroblasts. Proper diagnosis can help protect present and future siblings of SIDS victims [5,6,65,73].

Acute fatty liver of pregnancy (AFLP) and the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome), which carry significant neonatal and maternal morbidity and mortality, have also been associated with β-oxidation deficiency in fetuses, mainly LCHAD, although cases affected with CPT I, MCAD, and SCAD have also been reported [45,65]. The exact mechanism of development of these severe diseases of the third trimester of pregnancy is unclear. Pregnant women who are obligate heterozygotes have reduced activity of enzymes of fatty-acid β-oxidation. Conditions of increased energy requirement, as in pregnancy, result in increased triglyceride breakdown, which cannot be further processed efficiently. The accumulating FAs from disabled maternal and fetal β-oxidation may act as maternal hepatic toxin [6,36,60,68].

## Carnitine cycle

The carnitine palmitoyltransferase system is made up of two separate proteins, carnitine palmitoyltransferase 1 (CPT1),

which is tightly bound to the outer mitochondrial membrane, and carnitine palmitoyltransferase 2 (CPT2), which is loosely associated with the inner membrane, and it is aided by carnitine acylcarnitine translocase (CACT). It is responsible for the transport of long-chain fatty acids through the mitochondrial membrane. CPT1 catalyzes the formation of long-chain acylcarnitine from acyl-CoA and L-carnitine. CPT2 restores the acyl-CoA and releases free carnitine into the matrix. Carnitine acylcarnitine translocase exchanges acylcarnitine from the cytosol for free carnitine from the mitochondrial matrix. Although the carnitine cycle is not literally a step in mitochondrial β-oxidation, these cycles are strictly bound and it is generally agreed that the total flux of FAs in β-oxidation is regulated mainly by CPT1 [65]. Deficiency of CPT in a human was first described in 1973, when the CPTs were believed to be a single protein [22].

**CPT I deficiency**

Carnitine palmitoyltransferase I exists in three tissue-specific isoforms. The liver isoform (L-CPT1 or CPT1A) is present in the liver, lymphocytes, and fibroblasts, the muscle isoform (M-CPT1 or CPT1B) mainly in skeletal muscle, and the recently described brain isoform (CPT1C) has been found exclusively in the brain [10].

L-CPT1 is a single polypeptide, 773 amino acids long and from a 4.7 kb transcript, containing both inhibitor-binding and catalytic sites. Its gene is located on chromosome 11q13. 1-q13.5 [9]. The mutations causing L-CPT1 deficiency are mainly private mutations distributed throughout the entire sequence of the gene. Twenty-one of them are point mutations, five of which are nonsense mutations, one splice mutation on intron 15, and missense mutations, among which can be distinguished mutations affecting the active site functional determinant directly and indirectly by affecting the structural determinant [10]. The onset of L-CPT1 deficiency usually becomes apparent between a few days and 18 months of life in the form of a paroxysmal Reye-like attack with hypoketotic hypoglycemia or hepatomegaly with or without acute liver failure and subsequent hypoglycemic attacks and elevated creatine kinase level, liver enzymes, and ammonia in plasma. Organic aciduria may not be prominent in this disorder, but distal renal tubular acidosis is usually found during acute metabolic episodes [71,72]. Mild dicarboxylic aciduria without the presence of ketone bodies may be occasionally found in urine. Most often there is no heart involvement, but in several cases slight cardiomegaly, heart-beat disorders, or myocardial steatosis were reported. Neurologic deficit is common, but it is probably rather a result of the initial insult than inborn. The most characteristic feature of this disorder, present exclusively in CPT1 deficiency among all the β-oxidation disorders, is elevated plasma carnitine level [9].

M-CPT1 exhibits 63% amino-acid identity with L-CPT1 and has a smaller transcript size (3 kb). Its gene is located on chromosome 22q13.31-q13.32. To date there have been no cases of either M-CPT1 or CPT1C deficiency reported. There might be several explanations:
1) deficiency of these enzymes is lethal in early embryonic life,
2) the disease has not been identified in the pool of undefined inherited disorders,
3) it is scarcely possible that such a deficiency would not lead to noticeable health problems because of the important role these enzymes play in the heart and brain.

**CPT II deficiency**

The CPT2 gene is located on chromosome 1p32. The predicted product of its 3-kb transcript is 658 amino acids long. In CPT2 deficiency, the long-chain acylcarnitines are translocated across the inner mitochondrial membrane, but they are not efficiently recovered to acyl-CoA. Although CPT2 is present in one isoform in all tissues, its deficiency presents in three phenotypes of different severity: an adult-onset, an infantile, and a neonatal form. This may be explained by the level of residual activity of CPT2 sufficient to maintain β-oxidation flux varies among tissues. The phenotypic presentation can be highly variable, even in one family [9]. Of the over 40 characterized mutations distributed throughout the entire coding sequence, three genotypic subsets have been distinguished:
1) 17 "mild" mutations associated with the adult-onset phenotype, including a mutation exchanging serine for leucine at position 113 (S113L) on exon 3 and several mutations located on exons 4 and 5;
2) 8 mutations associated with the infantile form of disease located on exons 4 and 5;
3) 6 mutations determined in compound heterozygotes, whose phenotype severity depends on the presence of the mutation from subset one ("mild") or subset two ("severe").

Mutations of the first subset cause adult-onset disease or the "mild" phenotype in homozygous or compound heterozygous persons, while mutations of the second subset cause the "severe" disease phenotype. In compound heterozygotes with mutations of both sets, the mild form is prevalent. In the third subset the severity depends on the presence of the mutation of subset one, which will cause the "mild" phenotype, or subset two, resulting in "severe" phenotype. One mutation exchanging arginine for cysteine at position 631 (R631C) has been found to cause both the mild and severe phenotypes in homozygous persons, which suggests the existence of additional factors determining the severity of the disease [10, 82].

The adult form of CPT2 deficiency is generally restricted to a muscular symptomatology. First symptoms usually appear between 6 and 20 years of age, with recurrent attacks of myalgia, muscle stiffness or weakness, and myoglobinuria, most often induced by exercise but sometimes also by emotional stress, viral infections, fever, exposure to cold, fasting, or a fatty diet [42]. These attacks can last from a couple of hours to several weeks and are severe enough to lead to acute renal failure and respiratory insufficiency if respiratory muscles are involved. Patients are well between attacks. Serum creatine kinase and transaminases levels can be elevated 20- to 400-fold during the metabolic crisis. Fasting ketogenesis is delayed or decreased, but there is usually no hypoglycemia. Total serum carnitine level is low or normal, with a significantly elevated fraction of acylcarnitines. About 20% of patients had elevated triglyceride levels in serum and a comparable proportion of patients had lipid secretion in muscle accompanied by other structural anomalies, i.e. atrophy and necrosis of type I

muscle fibers. Dicarboxylic aciduria is usually undetectable [9,21,54,55,71,72].

The onset of the infantile form is usually between 6 and 24 months with recurrent liver failure, transient hepatomegaly, or hypoketotic hypoglycemia leading to coma or seizures, caused by fasting or febrile illness, although in several cases the precipitating factors are unknown. About half of the cases show heart involvement, i.e. dilated or hypertrophic cardiomyopathy or arrhythmia and conduction disorders, which may recede spontaneously. Laboratory findings reveal metabolic acidosis and hyperammonemia during attacks and almost constant low total and free carnitine, high long-chain acylcarnitines in plasma, which may point to the possible existence of a system which transports acylcarnitines out of mitochondria, and hepatic steatosis. The infantile form is a frequent cause of sudden death due to paroxysmal heart beat and Reye's syndrome [26].

The last and most severe form probably has a prenatal onset because fetal dysmorphic features can be detected ultrasonographically or soon after birth. Patients suffer from cystic renal dysplasia and neuronal migration defects, seizures, hepatomegaly, cardiomegaly, and rhythm and conduction disorders, often accompanied by metabolic acidosis and hyperammonemia. All affected children die shortly after birth, from a few hours to four days, due to respiratory distress and hypoglycemia [10,54,55,58].

**Carnitine/acylcarnitine translocase deficiency**

Carnitine-acylcarnitine transferase is a transporter protein located in the mitochondrial membrane which catalyses the exchange between carnitine from the mitochondrial matrix and different acylcarnitine species. The gene encoding CATC, 16.5 kb long, is located on chromosome 3p21.31. It comprises 9 exons and 8 introns. The mRNA transcript is 1250 bp long with an open reading frame of 903 bp. The translation product's amino-acid sequence reveals three homologous structural domains, each of about 100 amino acids, containing two membrane-spanning alpha-helices joined by hydrophobic regions, characteristic of the mitochondrial carrier-protein family. Nine mutations causing CATC deficiency have been identified, among them three point deletions (one resulting in incorrect splicing), two missense and two nonsense mutations, one point insertion, and one splice-site mutation. Two other incorrect splices resulting in exon 3 and 7 deletion were noted, but the mutations remain unidentified. Although CATC deficiency presents a broad spectrum of phenotypes, no strict division of phenotypes has been established so far [61,62].

CATC deficiency presents with severe hypoketotic hypoglycemia, severe secondary carnitine deficiency, mild hyperammonemia, ventricular arrhythmia, progressive muscle weakness, and episodes of coma triggered by intercurrent illness or fasting [53]. Patients are profoundly weak for 2–3 weeks after the metabolic crisis. Organic aciduria is usually not prominent. The acylcarnitine profile of patients affected with CATC deficiency is identical to that of a CPT2-deficient person; therefore the enzymatic defect has to be confirmed by direct determination of CATC activity in mitochondria [62,72].

## β-OXIDATION DISORDERS

During the last 25 years at least 22 different inborn defects in mitochondrial fatty-acid oxidation enzymes and transport proteins involved in the oxidation of fatty acids have been described. Due to the extensiveness of this group of disorders, this review will consider only defects of enzymes of the main pathway of saturated fatty-acid mitochondrial β-oxidation, namely very-long-chain acyl-CoA dehydrogenase, mitochondrial trifunctional protein, medium-chain acyl-CoA dehydrogenase, medium-chain 3-ketoacyl-CoA thiolase (also referred to as short-chain 3-oxoacyl-CoA thiolase), 3-hydroxyacyl-CoA dehydrogenase, and short-chain acyl-CoA dehydrogenase. Short-chain 3-ketoacyl-CoA thiolase (or mitochondrial acetoacetyl-CoA thiolase, T2) is an enzyme of isoleucine and ketone body metabolism and is therefore not recognized as a part of the β-oxidation pathway. Of the many clinical problems encountered in these disorders, hypotonia, myopathy (often with lipid storage), peripheral neuropathy, recurrent rhabdomyolysis, and hypoglycemia are frequent.

**VLCAD deficiency**

Very-long-chain acyl-CoA dehydrogenase (VLCAD) is a homodimeric enzyme located on the inner mitochondrial membrane with substrate specificity for long-chain FAs (>16 carbons) using electron-transfer flavoprotein (ETF) as an electron acceptor [27]. It is a rate-limiting enzyme of β-oxidation flux. The human VLCAD gene was located on chromosome 17 between bands p11.2 and p11.13105. It is about 5.4 kb long and contains 20 exons. The activity of VLCAD overlaps with that of LCAD. The existence of LCAD deficiency remains questionable because of the very low activity of this enzyme in fibroblasts and heart and its insignificant role in energy production. All patients suspected of LCAD deficiency were re-diagnosed as VLCAD deficient [12].

The first VLCAD-deficient patient was characterized in 1993 by C. Vianey et al. [70]. Further findings proved the existence of three phenotypes: severe or cardiomyopathic (VLCAD-C), mild or hypoglycemic (VLCAD-H). and late-onset or myopatic (VLCAD-M). The first is characterized by an early onset of symptoms, mainly hypertrophic cardiomyopathy, hepatomegaly, and a high incidence of death in the course of the first metabolic crisis, while the second presents mainly with hypoketotic hypoglycemia and hepatic symptoms. The third phenotype comprises adults who do not show cardiac and hepatic symptoms but who suffer from muscle weakness. The mutations causing VLCAD deficiency consist of different missense, nonsense, and splice mutations. The most severe phenotype is often caused by null mutations. However, no pattern or most frequent mutation allowing for direct genetic diagnosis has been found. What is more, the residual enzyme activity does not correlate with phenotype. Nevertheless, the incubation of VLCAD-deficient fibroblasts with palmitate revealed two patterns of accumulating long-chain acylcarnitines, suggesting different chain-length specificities for the two phenotypes in VLCAD deficiency [31]. Similar patterns were obtained in tests with unsaturated fatty acids, which led to the conclusion that VLCAD also contributes to the 2,3-dehydrogenation of cis-5- and trans-5-acyl-CoA [59].

Table 2. The discovery of certain mitochondrial β-oxidation disorders and recent updates [31]

| | Year disease discovered | Typical organ involvement | Recent updates |
|---|---|---|---|
| *Transporters* | | | |
| FATP (plasma membrane) | 1998, Odaib [49] | Liver | Rinaldo 2002 [57] |
| CAT (plasma membrane) | 1975, Karpati [40] | Liver, heart, muscle | Wang 2001 [76] |
| CACT (mitochondrial membrane) | 1992, Stanley [67] | Heart, liver, muscle | Hsu 2001 [35] |
| *Enzymes (mitochondrial membrane)* | | | |
| CPT I (liver) | 1981, Bougneres [11] | Liver | Bonnefont 1999 [9] |
| CPT II | 1973, DiMauro [22] | Heart, liver, muscle | Bonnefont 1999 [9] |
| ETF/ETFDH | 1976, Przyrembel [56] | Heart, liver, muscle | Frerman 2001 [27]<br>Olsen 2003 [52] |
| VLCAD | 1993, Bertrand [8] | Heart, liver, muscle | Andresen 1999 [4] |
| MTP | | | |
| LCHAD | 1989, Wanders [73] | Liver, heart, muscle | Hintz 2002 [34] |
| LCKAT | 1992, Hintz [34] | Liver, heart, muscle | Hintz 2002 [34] |
| *Enzymes (mitochondrial matrix)* | | | |
| MCAD | 1976, Gregersen [32] | Liver | Andresen 2001 [3] |
| SCHAD | 1991, Tein [69] | Liver | Clayton 2001 [15] |
| SCKAT | 1997, Kamijo [38] | Liver, muscle | – |
| SCAD | 1987, Amendt [1] | Muscle, brain | Corydon 2001 [16] |

CACT – carnitine/acylcarnitine translocase; CAT – carnitine transporter; CPT I – carnitine palmitoyltransferase I (liver); CPT II – carnitine palmitoyltransferase II; ETF/ETFDH – electron-transport flavoprotein/electron-transport flavoprotein dehydrogenase; FATP – fatty-acid transport protein; MCAD – medium-chain acyl-CoA dehydrogenase; MTP – mitochondrial trifunctional protein (including long-chain enoyl-CoA hydratase, long-chain 3-hydroxyacyl-CoA dehydrogenase, and long-chain 3-oxoacyl-CoA thiolase); SCAD – short-chain acyl-CoA dehydrogenase; SCHAD – short-chain 3-hydroxyacyl-CoA dehydrogenase; SCKAT – short-chain 3-oxoacyl-CoA thiolase; VLCAD – very-long-chain acyl-CoA dehydrogenase.

**TFP and LCHAD deficiency**

Mitochondrial trifunctional protein (TFP) is an enzymatic complex that catalyzes the last three steps of long-chain fatty-acid oxidation. It comprises four α-subunits catalyzing the hydration of long-chain enoyl-CoA and the dehydrogenation of long-chain L-3-hydroxyacyl-CoA (LCHAD) and four β-subunits harboring long-chain 3-ketoacyl-CoA thiolase (LKAT) activity. Their genes are located separately in the same region of chromosome 2p23 [39].

Mutations can affect either the α- or β-subunits. Null mutations are more frequent in the α-subunit, while missense mutations predominate in the β-subunit. The most common disability of TFP is an isolated LCHAD deficiency. It is often caused by a mutation exchanging glutamic acid to glutamine at position 474 (E474Q) in the catalytic site of the α-subunit, with occurrence in 60% of abnormal alleles. Mutation in each of the subunits can destabilize the whole complex and speed up degradation, which results in general TFP deficiency. Mutations in both subunits, regardless of the type of deficiency, yield three heterogeneous phenotypes: a lethal cardiomyopathic form, an infant-onset hepatic form, and a late-onset neuromyopathic form. The lethal phenotype is characterized by severe dilated cardiomyopathy, lactic acidosis and Reye-like syndrome, hypoketotic hypoglycemia, and neonatal death. The hepatic phenotype's onset is in the first months of life, presenting with episodes of hypoketotic hypoglycemia, lactic acidemia, and lethargy. The late-onset neuromyopathic form is characterized by progressive peripheral neuropathy and episodic myoglobinuria. Most patients suffer from chronic nonspecific symptoms before the metabolic crises, such as failure to thrive, cholestatic liver, hypotonia, and recurrent muscle cramps, which may be caused by the accumulation of long-chain acyl-CoA esters. Progressive retinis pigmentosa is a long-term consequence of both isolated LCHAD and TFP deficiencies [20,34,37,39,44,66,73].

**MCAD deficiency**

Medium-chain acyl-CoA dehydrogenase is the first enzyme in the chronological sequence of β-oxidation enzymes that is not bound to the mitochondrial membrane, but suspended in the matrix. The human MCAD gene is 44 kb long and encodes a 421-amino-acid precursor protein which is imported into the mitochondria, where it undergoes folding assisted by the chaperones Hsp70 and Hsp60 and assem-

bly to the mature MCAD homotetramer [2]. Medium-chain acyl-CoA dehydrogenase deficiency was first described in 1976 [32]. It is the most common defect of β-oxidation in humans, with a frequency in the population estimated at 1/15,000 [3]. The most common mutation causing MCAD deficiency is the transition of adenine to guanine at position 985 (985A–>G), resulting in a missense mutation which subsequently exchanges lysine for glutamic acid at position 304 (K304E) in the protein. It is present in 80% of affected homozygous people and on one of the alleles in a further 18%, leaving 2% for rare sequence variations on both alleles. The exchanged amino acid is located at the site responsible both for folding the monomer and assembling the tetramer; thus it disrupts the process of folding and alters the stability of the protein [30,31]. Another reported mutation substitutes Y for H at position 42 (Y42H) [48].

In spite of the genetic uniformity, the phenotypic presentation of MCAD deficiency is quite heterogeneous and may be modulated to a variable extent by the available number of chaperonins [50]. It usually includes hypoketotic hypoglycemia with lethargy, which may eventually develop into coma. Disease presentation may occur at any time of life. During episodes of acute metabolic decompensation, patients show urinary excretion of C6-C10 dicarboxylic acids. Cardiac symptoms characteristic of longer-chain oxidation disorders caused by energetic deficiency give in for the consequences of the hepatotoxic properties of acylcarnitines. Although MCAD deficiency is regarded as one of the mildest β-oxidation disorders, 20-25% of patients die suddenly at the first presentation of the disease [2].

**MCKAT deficiency**

To date, a single patient with medium-chain 3-ketoacyl-CoA thiolase (also referred to as short-chain 3-oxoacyl-CoA thiolase) activity reduced by 60% compared with controls has been reported by Kamijo et al. [38]. A male neonate presented with vomiting, dehydration, metabolic acidosis, liver dysfunction, terminal rhabdomyolysis, and myoglobinuria at two days of age. Analysis of organic acids in urine revealed increased concentrations of lactic acid, 3-hydroxybutyric acid, and saturated and unsaturated C6-C16 dicarboxylic acids. The patient died at thirteen days of age [30,31,38].

**M/SCHAD deficiency**

Mitochondrial 3-hydroxyacyl-CoA dehydrogenase (HAD) catalyzes the oxidation of straight-chain 3-hydroxyacyl-CoA, preferably of medium chain length but also of short chain substrates. This enzyme has a broad chain-length specificity and is sometimes referred to as M/SCHAD. It is a homodimeric protein with a subunit molecular mass of 33 kDa. Its activity is partially overlapped by that of short-chain hydroxyacyl-CoA dehydrogenase (SCHAD), also known as type 10 17β-hydroxysteroid dehydrogenase (HSD10), which belongs to the short-chain hydrogenase/reductase superfamily and primarily acts on steroids, cholic acids, and short-chain methyl-branched acyl-CoAs. Both enzymes reside in the mitochondrial matrix. The dehydrogenation of 3-hydroxyacyl-CoAs is catalyzed mainly by HAD.

The human disease referred to as SCHAD deficiency is actually due to HAD activity impaired by missense mutations of its gene at chromosome 4q22-26. The 45-kb-long gene contains nine exons. The two known disease-causing mutations result in the exchange of proline for leucine at position 258 (P258L) or aspartic acid for glutamic acid at position 45 (D45E) in the protein [83]. Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency patients presented with fasting-induced vomiting, ketosis, and low blood glucose, with possible optional hepatoencephalopathy, myopathy, and cardiomyopathy. The condition was characterized by elevated blood concentration of intermediate corresponding to the substrate chain length – butyrylcarnitine. A rare but distinctive feature of SCHAD deficiency is hyperinsulinism of unexplained mechanism, possibly due to the information-transducive role of short-chain acylcarnitines or acyl-CoAs. In such patients the plasma acylcarnitine profile fits in the lower normal range with an elevated, but still within the normal range, fraction of 3-hydroxybutyrylcarnitine, because insulin suppresses lipolysis and ketogenesis [23,24,47].

**SCAD deficiency**

Short-chain acyl-CoA dehydrogenase deficiency in nearly all cases is caused by missense sequence variations in the SCAD gene, with the exception of one in-frame deletion of three nucleotides, resulting in production of protein with some residual activity. About half of the missense variations are cytidine to thymine substitutions at CpG dinucleotides. Inactivating missense variations are rare in homozygous or compound heterozygous form, but frequent in compound heterozygous form together with one of two common susceptibility missense variations, namely the transition of guanine to adenine at position 625 (625G–>A) and cytosine to thymine at position 511 (511C–>T). The scarcity of mutations abolishing all enzyme activity could be explained by the negative selection of cells bearing such mutations. Moreover, a significant number of SCAD-deficient patients harbor susceptibility genes in homozygous or compound heterozygous form, although their phenotype is indistinguishable from patients carrying the rare inactivating sequence variations. The susceptibility gene variant in the homozygous state is not likely to result in SCAD deficiency. Although such susceptibility variations are present in 14% of the general population, it is assumed that only a minority of individuals is at risk of developing disease, which reveals the complexity of the conditions required for the development of clinically relevant disease. However, in the compound heterozygous state with a more severe mutation it is almost certain to produce a SCAD deficiency. It was demonstrated that the folding of the SCAD protein is highly dependent on the Hsp60/10 chaperonin system and this dependency is increased in the case of the susceptibility protein variant. As long as folding efficiency is sustained at a high level, the wild-type and variant proteins express the same activity at physiological temperatures. Nevertheless, the biogenesis of the two variant proteins is delayed and compromised and the protein stability is decreased at higher temperatures, which leads to the conclusion that high temperature, as in fever, and high fatty-acid oxidation activity may result in manifestation of SCAD deficiency.

Since SCAD deficiency blocks only the last cycle of β-oxidation, it results in the production of nearly normal

Table 3. Characteristic clinical findings in β-oxidation disorders [72]

| Clinical findings in defects of mitochondrial β-oxidation | |
|---|---|
| Suggestive symptoms | Reye's syndrome (especially recurrent) |
| | Hypotonia and/or myopathy |
| | Peripheral neuropathy |
| | Altered levels of consciousness |
| | Sudden unexplained death |
| Suggestive findings | Hypoketotic or ketotic hypoglycemia |
| | Cardiomyopathy |
| | Cardiac arrhythmia |
| | Unexplained metabolic acidosis with |
| | or without hyperammonemia |
| | AFLP/HELLP* |
| | Recurrent rhabdomyolysis |
| | Dicarboxylic aciduria |
| | Carnitine deficiency |
| | Recurrent/fulminant liver failure |

* AFLP – acute fatty liver of pregnancy; HELLP –hypertension, elevated liver enzymes, and low platelets.

amounts of acetylo-CoAs and the accumulation of butyric acid. Thus only a few patients presented with cardiac symptoms or hypoglycemia, which are associated with energy deficiency. What is more, butyric acid and its derivatives are not toxic to heart or liver, but they promote cell differentiation and inhibit the cell cycle, inducing apoptosis, which explains the predominance of neuromuscular symptoms.

## Diagnostics and treatment of defects of fatty-acid β-oxidation

Patients presenting with Reye's syndrome, hypotonia, myopathy, peripheral neuropathy, cardiomyopathy, cardiac arrhytmia, altered levels of consciousness, or even sudden unexplained death (Table 3) should be suspected of β-oxidation disorders. Initial examination could reveal hypoglycemia, mild lactic acidosis with or without hyperammonemia, rhabdomyolysis, myoglobin in blood and urine, dicarboxylic or 3-hydroxydicarboxylic aciduria, recurrent liver failure, elevated plasma creatine kinase levels, and alteration of carnitine levels. The diagnosis of defects of mitochondrial fatty-acid metabolism is difficult since most of the biochemical findings may disappear when the patient is not in an acute crisis. A fasting test with or without a subsequent challenge test with medium- or long-chain triglycerides may induce the excretion of a diagnostic metabolite, but this is dissuaded due to its high risk, even in a hospital setting when performed by someone experienced. Such tests are abandoned to the advantage of specific and highly sensitive techniques, including mass spectrometry, which has been developed for the detection of minute quantities of intermediates which are often undetectable by

Table 4. Characteristic laboratory testing and treatment in β-oxidation disorders [72]

| Initial laboratory evaluation | Blood |
|---|---|
| | Glucose |
| | Free fatty acids |
| | pH |
| | Ammonia |
| | Carnitine |
| | Creatnie kinase |
| | Liver function tests |
| | Lactate/pyruvate |
| | Electrolytes |
| Specialized metabolic testing | Urine organic acids/acylglycines |
| | Plasma acylcarnitine profile |
| | Free fatty acids profile |
| | Fibroblast oxidation studies |
| | Fibroblast enzyme analysis |
| | Specific enzyme analysis |
| | Challenge testing |
| Possible therapies | Low fat diet (25% calories from fat) |
| | MCT oil supplement (not for MCAD deficiency) |
| | High calorific intake (from carbohydrates) with illness or stress |
| | Night time nasogastric drip feedings |
| | Corn starch at bedtime |
| | Carnitine only for transporter defect |

MCT – medium chain triglyceride; MCAD – medium chain acyl-CoA dehydrogenase.

routine organic acid analysis, as well as blood carnitine level, blood acylcarnitine profile, and urine organic acid analysis. Elevated carnitine levels clearly indicate CPT I deficiency, while decreased levels point to other β-oxidation disorders. The blood acylcarnitine profile is the most specific and direct diagnostic approach [50,64]. Specific enzyme analysis can be performed *in vitro* for many of these defects on cultured fibroblasts, liver, or skeletal muscle, and since it is performed on cells under standard conditions, the diagnosis is always apparent. Dicarboxylic acids and moderate amounts of 3-hydroxydicarboxylic acids are products of omega-oxidation in peroxisomes. Their presence does not identify the specific enzyme defect, but clearly indicates a disabled mitochondrial oxidation. Direct DNA mutational analysis is available for MCAD and LCHAD deficiencies [13,14,58,72].

Medical treatment comes down to conservative therapy, which should limit endogenous lipolysis, enhance gluconeogenesis, ensure the availability of CoA in the mitochondrion, and provide an appropriate energy source alternative to fat. The efforts are concentrated on arresting lipolysis. To prevent patients from becoming dependent on β-oxidation, the basis of therapy is avoidance of fasting. Fasting and even minor infections switch the metabolism to increased endogenous lipolysis, which causes an excessive accumulation of toxic intermediates. Therefore, FAO-deficient patients require fast recognition of infections, intense treatment, and intravenous glucose support (10 mg/kg/min). It is advised to introduce this support as fast as possible, without waiting for laboratory results, since the accumulation of toxic metabolites can be extremely rapid. It must also be stated that the glucose blood level is not an indicator of state and intravenous glucose is administered even when its levels are normal in order to restrict lipolysis. What is more, dietary fat intake is restricted to less than 25% of total calories at all times and calorific intake from carbohydrates is increased. In case of feeding problems, which are frequent in this group of disorders, nasogastric or intravenous feeding is introduced. In some patients, orally administered corn starch (1–2 g/kg/dose) has been used as an alternative glucose source.

In case of defects affecting the metabolism of long-chain fatty acids, significant effects were achieved by supplementation of MCT oil (containing medium-chain fatty acids), which pass around a metabolic block. The same oil is extremely toxic to MCAD-deficient patients [58,71,72].

Although supplementation of L-carnitine in carnitine-transporter defect (primary carnitine deficiency) is undoubtedly beneficial, carnitine supplementation in other disorders of β-oxidation causing a secondary carnitine deficiency is at least controversial. Carnitine may help to dispose of toxic metabolites and improve exercise tolerance, but long-chain acylcarnitines are toxic and arrhythmogenic and other acylcarnitines species are suspected of playing a regulatory role in the cell [14,60].

## Conclusions

Inborn errors of mitochondrial β-oxidation form a large and diverse group of diseases. It is generally thought that these disorders may account for as much as 3–5% of sudden unexpected death in infants and children. The sudden onset of acute illness in an apparently normal child is life-threatening in the absence of proper diagnosis. What is more, an affected fetus significantly increases the risk of potentially life-threatening AFLP (acute fatty liver of pregnancy) or HELLP syndrome in the mother. Therefore, fast and accurate diagnosis is crucial. The diagnostics of these disorders requires a high level of suspicion because of the considerable variation in severity and the impermanence and nonspecificity of symptoms, involving muscles, heart, liver, and the nervous system, which can overlap with those of other diseases and make them extremely difficult to diagnose. A presymptomatic identification of patients with β-oxidation disorders can, in many instances, prevent catastrophic events. This points to the importance of newborn screening for these disorders, which has been implemented in several countries and is being evaluated in a number of others. Such screening remains controversial, as it has raised concerns about cost-benefit ratios. Savings in medical expenses related to the evaluation and treatment of children in much more severe condition who would have been identified by newborn screening must be considered as a benefit of such screening [85].

Newborn screening would be a great opportunity for a vast statistical evaluation of the incidence of the different mutations in the human population and it could open the way for specific genetic tests for β-oxidation disorders besides LCHAD and MCAD deficiencies. Moreover, the variability of these disorders, often ascribed to the effects of various mutations in the same gene affecting the genotype/phenotype correlation, still needs to be assessed since such data are available only for several errors in the metabolism of very-long-, long-, and medium-chain-length fatty acids. The role of additional genetic and environmental factors in this variability also remains under investigation.

Subsequently, the belief that all defects of mitochondrial fatty-acid metabolism identified thus far are inherited in an autosomal recessive fashion is being undermined by recent research evaluating enzymatic activity in heterozygous animal models and patients. A suitable investigation to settle the question has just been started at the Mayo Clinic College of Medicine in Rochester, USA.

Lastly, the long-term consequences of β-oxidation disorders in the perspective of tens of years and their influence on health, quality of life, and life expectancy are completely unknown because of the very few diagnosed adult patients. Therefore, increased detectability of β-oxidation disorders by newborn screening would perhaps provide a possibility for long-term research.

## References

[1] Amendt B.A., Greene C., Sweetman L., Cloherty J., Shih V., Moon A., Teel L., Rhead W.J.: Short-chain acylcoenzyme A dehydrogenase deficiency: clinical and biochemical studies in two patients. J. Clin. Invest., 1987; 79: 1303–1309

[2] Andresen B.S., Bross P., Udvari S., Kirk J., Gray G., Kmoch S., Chamoles N., Knudsen I., Winter V., Wilcken B., Yokota I., Hart K., Packman S., Harpey J.P., Saudubray J.M., Hale D.E., Bolund L., Kølvraa S., Gregersen N.: The molecular basis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in compound heterozygous patients: is there correlation between genotype and phenotype? Hum. Mol. Genet., 1997; 6: 695–707

[3] Andresen B.S., Dobrowolski S.F., O'Reilly L., Muenzer J., McCandless S. E., Frazier D.M., Udvari S., Bross P., Knudsen I., Banas R., Chace D.H., Engel P., Gregersen N.: Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by ms/ms-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. Am. J. Hum. Genet., 2001; 68: 1408–1418

[4] Andresen B.S., Olpin S., Poorthuis B.J., Scholte H.R., Vianey-Saban C., Wanders R., Ijlst L., Morris A., Pourfarzam M., Bartlett K., Baumgartner E.R., deKlerk J.B., Schroeder L.D., Corydon T.J., Lund H., Winter V., Bross P., Bolund L., Gregersen N.: Clear correlation of genotype with disease phenotype in very- long-chain acyl-CoA dehydrogenase deficiency. Am. J. Hum. Genet., 1999; 64: 479–494

[5] Arenas J., Rubio J.C., Martin M.A., Campos Y.: Biological roles of L-carnitine in perinatal metabolism. Early Hum. Dev., 1998; 53(Suppl.): S43–S50

[6] Bartlett K., Eaton S.: Mitochondrial β-oxidation. Eur. J. Biochem., 2004; 271: 462–469

[7] Bennett M.J., Rinaldo P., Strauss A.W.: Inborn errors of mitochondrial fatty acid oxidation. Crit. Rev. Clin. Lab. Sci., 2000; 37: 1–44

[8] Bertrand C., Largilliere C., Zabot M.T., Mathieu M., Vianey-Saban C.: Very long chain acyl-CoA dehydrogenase deficiency: identification of a new inborn error of mitochondrial fatty acid oxidation in fibroblasts. Biochim. Biophys. Acta, 1993; 1180: 327–329

[9] Bonnefont J.P., Demaugre F., Prip-Buus C., Saudubray J.M., Brivet M., Abadi N., Thuillier L.: Carnitine palmitoyltransferase deficiencies. Mol. Genet. Metab., 1999; 68: 424–440

[10] Bonnefont J.P., Djouadi F., Prip-Buus C., Gobin S., Munnich A., Bastin J.: Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. Mol. Aspects Med., 2004; 25: 495–520

[11] Bougneres P.F., Saudubray J.M., Marsac C., Bernard O., Odievre M., Girard J.: Fasting hypoglycemia resulting from hepatic carnitine palmitoyl transferase deficiency. J. Pediatr., 1981; 98: 742–746

[12] Cairns A.P., O'donoghue P.M., Patterson V.H., Brown J.H.: Very-long-chain acyl-coenzyme A dehydrogenase deficiency – a new cause of myoglobinuric acute renal failure. Nephrol. Dial. Transplant., 2000; 15: 1232–1234

[13] Carpenter K.H., Wiley V.: Application of tandem mass spectrometry to biochemical genetics and newborn screening. Clin. Chim. Acta, 2002; 322: 1–10

[14] Chakrapani A., Wraith J.E.: Principles of management of the more common metabolic disorders. Current Paediatrics, 2002; 12: 117–124

[15] Clayton P.T., Eaton S., Aynsley-Green A., Edginton M., Hussain K., Krywawych S., Datta V., Malingre H.E., Berger R., van den Berg I.E.: Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. J. Clin. Invest., 2001; 108: 457–465

[16] Corydon M.J., Vockley J., Rinaldo P., Rhead W.J., Kjeldsen M., Winter V., Riggs C., Babovic-Vuksanovic D., Smeitink J., De Jong J., Levy H., Sewell A.C., Roe C., Matern D., Dasouki M., Gregersen N.: Role of common gene variations in the molecular pathogenesis of short-chain acyl-CoA dehydrogenase deficiency. Pediatr. Res., 2001; 49: 18–23

[17] Darras B.T., Friedman N.R.: Metabolic myopathies: a clinical approach; part I. Pediatr. Neurol., 2000; 22: 87–97

[18] Darras B.T., Friedman N.R.: Metabolic myopathies: a clinical approach; part II. Pediatr. Neurol., 2000; 22: 171–181

[19] de Lonlay P., Giurgea I., Touati G., Saudubray J.M.: Neonatal hypoglycaemia: aetiologies. Semin. Neonatol., 2004; 9: 49–58

[20] den Boer M.E., Wanders R.J., Morris A.A., IJlst L., Heymans H.S., Wijburg F.A.: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: clinical presentation and follow-up of 50 patients. Pediatrics, 2002; 109: 99–104

[21] Deschauer M., Chrzanowska-Lightowlers Z.M., Biekmann E., Pourfarzam M., Taylor R.W., Turnbull D.M., Zierz S.: A splice junction mutation in muscle carnitine palmitoyltransferase II deficiency. Mol. Genet. Metab., 2003; 79: 124–128

[22] DiMauro S., DiMauro P.M.: Muscle carnitine palmityltransferase deficiency and myoglobinuria. Science, 1973; 182: 929–931

[23] Eaton S.: Control of mitochondrial beta-oxidation flux. Prog. Lipid Res., 2002; 41: 197–239

[24] Eaton S., Chatziandreou I., Krywawych S., Pen S., Clayton P.T., Hussain K.: Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with hyperinsulinism:a novel glucose–fatty acid cycle? Biochem. Soc. Trans., 2003; 31: 1137–1139

[25] Ferdinandusse S., Meissner T., Wanders R.J., Mayatepek E.: Identification of the peroxisomal beta-oxidation enzymes involved in the degradation of leukotrienes. Biochem. Biophys. Res. Commun., 2002; 293: 269–273

[26] Fontaine M., Briand G., Largilliere C., Degand P., Divry P., Vianey-Saban C., Mousson B., Vamecq J.: Metabolic studies in a patient with severe carnitine palmitoyltransferase type II deficiency. Clin. Chim. Acta, 1998; 273: 161–170

[27] Frerman F.E., Goodman S.I.: Defects of electron transfer flavoprotein and electron transfer flavoprotein-ubiquinone oxidoreductase:glutaric aciduria type II. In: Metabolic and Molecular Basis of Inherited Disease. Eds: C.R.Scriver, A.L. Beaudet, D. Valle, McGraw-Hill, New York 2001

[28] Ghisla S.: β-Oxidation of fatty acids: a century of discovery. Eur. J. Biochem., 2004; 271: 459–461

[29] Glatz J.F., Luiken J.J., van Nieuwenhoven F.A., Van der Vusse G.J.: Molecular mechanism of cellular uptake and intracellular translocation of fatty acids. Prostaglandins Leukot. Essent. Fatty Acids, 1997; 57: 3–9

[30] Gregersen N., Andresen B.S., Corydon M.J., Corydon T.J., Olsen R.K., Bolund L., Bross P.: Mutation analysis in mitochondrial fatty acid oxidation defects exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationship. Hum. Mutat., 2001; 18: 169–189

[31] Gregersen N., Bross P., Andresen B.S.: Genetic defects in fatty acid beta-oxidation and acyl-CoA dehydrogenases: Molecular pathogenesis and genotype-phenotype relationships. Eur. J. Biochem., 2004; 271: 470–482

[32] Gregersen N., Lauritzen R., Rasmussen K.: Suberylglycine excretion in the urine from a patient with dicarboxylic aciduria. Clin. Chim. Acta, 1976; 70: 417–425

[33] Haunerland N.H., Spener F.: Fatty acid-binding proteins – insights from genetic manipulations. Prog. Lipid Res., 2004; 43: 328–349

[34] Hintz S.R., Matern D., Strauss A., Bennett M.J., Hoyme H.E., Schelley S., Kobori J., Colby C., Lehman N.L., Enns G.M.: Early neonatal diagnosis of long-chain 3-hydroxyacyl-coenzyme a dehydrogenase and mitochondrial trifunctional protein deficiencies. Mol. Genet. Metab., 2002; 75: 120–127

[35] Hsu B.Y., Iacobazzi V., Wang Z., Harvie H., Chalmers R.A., Saudubray J.M., Palmieri F., Ganguly A., Stanley C.A.: Aberrant mRNA splicing associated with coding region mutations in children with carnitine-acylcarnitine translocase deficiency. Mol. Genet. Metab., 2001; 74: 248–255

[36] Ibdah J.A., Paul H., Zhao Y., Binford S., Salleng K., Cline M., Matern D., Bennett M.J., Rinaldo P., Strauss A.W.: Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and sudden death, J. Clin. Invest., 2001; 107: 1403–1409

[37] Jones P.M., Butt Y.M., Bennett M.J.: Effects of odd-numbered medium-chain fatty acids on the accumulation of long-chain 3-hydroxy-fatty acids in long-chain L-3-hydroxyacyl CoA dehydrogenase and mitochondrial trifunctional protein deficient skin fibroblasts. Mol. Genet. Metab., 2004; 81: 96–99

[38] Kamijo T., Indo Y., Souri M., Aoyama T., Hara T., Yamamoto S., Ushikubo S., Rinaldo P., Matsuda I., Komiyama A., Hashimoto T.: Medium chain 3-ketoacyl-coenzyme A thiolase deficiency: a new disorder of mitochondrial fatty acid beta-oxidation. Pediatr. Res., 1997; 42: 569–576

[39] Kamijo T., Wanders R.J., Saudubray J.M., Aoyama T., Komiyama A., Hashimoto T.: Mitochondrial trifunctional protein deficiency: catalytic heterogeneity of the mutant enzyme in two patients. J. Clin. Invest., 1994; 93: 1740–1747

[40] Karpati G., Carpenter S., Engel A.G., Watters G., Allen J., Rothman S., Klassen G., Mamer O.A.: The syndrome of systemic carnitine deficiency. Clinical, morphologic, biochemical, and pathophysiologic features. Neurology, 1975; 25: 16–24

[41] Kerner J., Hoppel C.: Fatty acid import into mitochondria. Biochim. Biophys. Acta, 2000; 1486: 1–17

[42] Kottlors M., Jaksch M., Ketelsen U.P., Weiner S., Glocker F.X., Lucking C.H.: Valproic acid triggers acute rhabdomyolysis in a patient with carnitine palmitoyltransferase type II deficiency. Neuromuscul. Disord., 2001; 11: 757–759

[43] Liang X., Le W., Zhang D., Schulz H.: Impact of the intramitochondrial enzyme organization on fatty acid oxidation. Biochem. Soc. Trans., 2001; 29: 279–282

[44] Lund A.M., Leonard J.V.: Feeding difficulties in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Arch. Dis. Child., 2001; 85: 487–488

[45] Mansouri A., Fromenty B., Durand F., Degott C., Bernuau J., Pessayre D.: Assessment of the prevalence of genetic metabolic defects in acute fatty liver of pregnancy. J. Hepatol., 1996; 25: 781–782

[46] Marin-Garcia J., Goldenthal M.J.: Fatty acid metabolism in cardiac failure: biochemical, genetic and cellular analysis. Cardiovasc. Res., 2002; 54: 516–527

[47] Molven A., Matre G.E., Duran M., Wanders R.J., Rishaug U., Njølstad P.R., Jellum E., Søvik O.: Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. Diabetes, 2004; 53: 221–227

[48] O'Reilly L., Bross P., Corydon T.J., Olpin S.E., Hansen J., Kenney J.M., McCandless S.E., Frazier D.M., Winter V., Gregersen N., Engel P.C., Andresen B.S.: The Y42H mutation in medium-chain acyl-CoA dehydrogenase, which is prevalent in babies identified by MS/MS-based newborn screening, is temperature sensitive. Eur. J. Biochem., 2004; 271: 4053–4063

[49] Odaib A.A., Shneider B.L., Bennett M.J., Pober B.R., Reyes-Mugica M., Friedman A.L., Suchy F.J., Rinaldo P.: A defect in the transport of long-chain fatty acids associated with acute liver failure. N. Engl. J. Med., 1998; 339: 1752–1757

[50] Okun J.G., Kolker S., Schulze A., Kohlmuller D., Olgemoller K., Lindner M., Hoffmann G.F., Wanders R.J., Mayatepek E.: A method for quantitative acylcarnitine profiling in human skin fibroblasts using unlabelled palmitic acid: diagnosis of fatty acid oxidation disorders and differentiation between biochemical phenotypes of MCAD deficiency. Biochim. Biophys. Acta, 2002; 1584: 91–98

[51] Olpin S.E.: Implications of impaired ketogenesis in fatty acid oxidation disorders. Prostaglandins Leukot. Essent. Fatty Acids, 2004; 70: 293–308

[52] Olsen R.K., Andresen B.S., Christensen E., Bross P., Skovby F., Gregersen N.: Clear relationship between ETF/ETFDH genotype and phenotype in patients with multiple acyl-CoAdehydrogenation deficiency. Hum. Mutat., 2003; 22: 12–23

[53] Pierpont M.E., Breningstall G.N., Stanley C.A., Singh A.: Familial carnitine transporter defect: A treatable cause of cardiomyopathy in children. Am. Heart J., 2000; 139: S96–S106

[54] Pollitt R.J.: Defects in mitochondrial fatty acid oxidation: clinical presentations and their role in sudden infant death. Padiatr. Padol., 1993; 28: 13–17

[55] Pollitt R.J., Leonard J.V.: Prospective surveillance study of medium chain acyl-CoA dehydrogenase deficiency in the UK. Arch. Dis. Child., 1998; 79: 116–119

[56] Przyrembel H., Wendel U., Becker K., Bremer H.J., Bruinvis L., Ketting D., Wadman S.K.: Glutaric aciduria type II: report on a previously undescribedmetabolic disorder. Clin. Chim. Acta, 1976; 66: 227–239

[57] Rinaldo P., Matern D., Bennett M.J.: Fatty acid oxidation disorders. Annu. Rev. Physiol., 2002; 64: 477–502

[58] Roe C.R.: Inherited disorders of mitochondrial fatty acid oxidation: a new responsibility for the neonatologist. Semin Neonatol, 2002; 7: 37–47

[59] Roe D.S., Vianey-Saban C., Sharma S., Zabot M.T., Roe C.R.: Oxidation of unsaturated fatty acids by human fibroblasts with very-long-chain acyl-CoA dehydrogenase deficiency: aspects of substrate specificity and correlation with clinical phenotype. Clin. Chim. Acta, 2001; 312: 55–67

[60] Romero-Falcón A., de la Santa-Belda E., Garcia-Contreras R., Varela J.M.: A case of valproate-associated hepatotoxicity treated with L-carnitine. Eur. J. Intern. Med., 2003; 14: 338–340

[61] Röschinger W., Muntau A.C., Duran M., Dorland L., IJlst L., Wanders R.J., Roscher A.A.: Carnitine-acylcarnitine translocase deficiency: metabolic consequences of an impaired mitochondrial carnitine cycle. Clin. Chim. Acta, 2000; 298: 55–68

[62] Rubio-Gozalbo M.E., Bakker J.A., Waterham H.R., Wanders R.J.: Carnitine-acylcarnitine translocase deficiency, clinical, biochemical and genetic aspects. Mol. Aspects Med., 2004; 25: 521–532

[63] Schuler A.M., Barnes S., Gower B.A., Wood P.A.: Dietary phytoestrogens increase metabolic resistance (cold tolerance) in long-chain acyl-CoA dehydrogenase-deficient mice J. Nutr., 2004; 134: 1028–1031

[64] Schulze A., Schmidt C., Kohlmuller D., Hoffmann G.F., Mayatepek E.: Accurate measurement of free carnitine in dried blood spots by isotope-dilution electrospray tandem mass spectrometry without butylation. Clin. Chim. Acta, 2003; 335: 137–145

[65] Sim K.G., Hammond J., Wilcken B.: Strategies for the diagnosis of mitochondrial fatty acid beta-oxidation disorders, Clin Chim Acta, 2002; 323: 37–58

[66] Spiekerkoetter U., Khuchua Z., Yue Z., Bennett M.J., Strauss A.W.: General mitochondrial trifunctional protein (TFP) deficiency as a result of either α- or β-subunit mutations exhibits similar phenotypes because mutations in either subunit alter TFP complex expression and subunit turnover. Pediatr. Res., 2004; 55: 190–196

[67] Stanley C.A., Hale D.E., Berry G.T., Deleeuw S., Boxer J., Bonnefont J.P.: Brief report: a deficiency of carnitineacylcarnitine translocase in the inner mitochondrial membrane. N. Engl. J. Med., 1992; 327: 19–23

[68] Te Sligte K., Bourass I., Sels J.P., Driessen A., Stockbrugger R.W., Koek G.H.: Non-alcoholic steatohepatitis: review of a growing medical problem. Eur. J. Intern. Med., 2004; 15: 10–21

[69] Tein I., De Vivo D.C., Hale D.E., Clarke J.T., Zinman H., Laxer R., Shore A., DiMauro S.: Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency in muscle: a new cause for recurrent myoglobinuria and encephalopathy. Ann. Neurol., 1991; 30: 415–419

[70] Vianey-Saban C., Divry P., Brivet M., Nada M., Zabot M.T., Mathieu M., Roe C.: Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. Clin. Chim. Acta, 1998; 269: 43–62

[71] Vockley J.: The changing face of disorders of fatty acid oxidation. Mayo Clin. Proc., 1994; 69: 249–257

[72] Vockley J., Whiteman D.A.: Defects of mitochondrial beta-oxidation: a growing group of disorders. Neuromuscul. Disord., 2002; 12: 235–246

[73] Wanders R.J., Duran M., IJlst L., de Jager J.P., van Gennip A.H., Jakobs C., Dorland L., van Sprang F.J.: Sudden infant death and long-chain 3-hydroxyacyl-CoA dehydrogenase. Lancet, 1989; 2: 52–53

[74] Wanders R.J., Jansen G.A., Lloyd M.D.: Phytanic acid alpha-oxidation, new insights into an old problem: a review. Biochim. Biophys. Acta, 2003; 1631: 119–135

[75] Wanders R.J., Tager J.M.: Lipid metabolism in peroxisomes in relation to human disease. Mol. Aspects Med., 1998; 19: 69–154

[76] Wang Y., Korman S.H., Ye J., Gargus J.J., Gutman A., Taroni F., Garavaglia B., Longo N.: Phenotype and genotype variation in primary carnitine deficiency. Genet. Med., 2001; 3: 387–392

[77] Westin M.A., Hunt M.C., Alexson S.E.: The identification of succinyl-CoA thioesterase suggests a novel pathway for succinate production in peroxisomes, J. Biol. Chem., 2005; 280: 38125–38132

[78] Wiley V., Carpenter K., Wilcken B.: Newborn screening with tandem mass spectrometry: 12 months experience in NSW Australia. Acta Paediatr Suppl., 1999; 88: 48–51

[79] Wilson C.J., Champion M.P., Collins J.E.: Outcome of medium chain acyl-CoA dehydrogenase deficiency after diagnosis. Arch. Dis. Child, 1999; 80: 459–462

[80] Winter S.C., Buist, N.R.: Cardiomyopathy in childhood, mitochondrial dysfunction, and the role of L-carnitine. Am. Heart J., 2000; 139: S63–S69

[81] Wraige E., Champion M.P., Turner C., Dalton R.N., Galloway P.J., Robinson P.H.: Fat oxidation defect presenting with overwhelming ketonuria. Arch. Dis. Child., 2002; 87: 428–429

[82] Yang B.Z., Ding J.H., Dewese T., Roe D., He G., Wilkinson J., Day D.W., Demaugre F., Rabier D., Brivet M., Roe C.: Identification of four novel mutations in patients with carnitine palmitoyltransferase II (CPT II) deficiency. Mol. Genet. Metab., 1998; 64: 229–236

[83] Yang S.Y., He X.Y., Schulz H.: 3-Hydroxyacyl-CoA dehydrogenase and short chain 3-hydroxyacyl-CoA dehydrogenase in human health and disease. FEBS J., 2005; 272: 4874–4883

[84] Young S.P., Matern D., Gregersen N., Stevens R.D., Bali D., Liu H.M., Koeberl D.D., Millington D.S.: A comparison of *in vitro* acylcarnitine profiling methods for the diagnosis of classical and variant short chain acyl-CoA dehydrogenase deficiency. Clin. Chim. Acta, 2003; 337: 103–113

[85] Ziadeh R., Hoffman E.P., Finegold D.N., Hoop R.C., Brackett J.C., Strauss A.W., Naylor E.W.: Medium chain acyl-CoA dehydrogenase deficiency in Pennsylvania: neonatal screening shows high incidence and unexpected mutation frequencies. Pediatr. Res., 1995; 37: 675–678

The authors have no potential conflicts of interest to declare.