

14.1 Introduction

Long-chain (C_{16} – C_{20}) fatty acids, which are stored in adipose tissue, can be β -oxidised for the production of energy. The main part of the β -oxidation takes place in the mitochondria, a small part of the fatty acids is metabolised in the peroxisomes. Energy production from fatty acids becomes crucial during prolonged fasting. It has been known for a long time that the fasting brain depends on two fuels, i.e. glucose derived from gluconeogenesis and ketone bodies derived from mitochondrial fatty acid β -oxidation. Both processes mainly reside in the liver. Entry of the long-chain fatty acids into the mitochondrion requires the formation of fatty acyl carnitine esters by carnitine palmitoyltransferase 1 (CPT1) at the mitochondrial outer membrane (Fig. 1). The carnitine esters are then shuttled across the inner membrane by the carnitine acylcarnitine carrier protein (CAC). Attached to the inner side of the inner mitochondrial membrane are three proteins with all together five enzyme activities, i.e. carnitine palmitoyltransferase 2 (CPT2), very long-chain acyl-CoA dehydrogenase (VLCAD) and the so-called mitochondrial trifunctional protein (MTP) with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), long-chain enoyl-CoA hydratase and long-chain 3-oxothiolase activities. Although there is no hard evidence yet, it is likely that there is channeling of substrates between these proteins. Each cycle of β -oxidation results in the production of a two-carbon chain-shortened acyl-CoA molecule and one molecule of acetyl-CoA. As soon as the chain length of the acyl-CoA precludes handling by the long-chain enzymes, the acyl-CoA's are released into the mitochondrial matrix where they are further metabolized by a series of soluble enzymes, amongst which are medium-chain acyl-CoA dehydrogenase (MCAD), short-chain acyl-CoA dehydrogenase (SCAD), enoyl-CoA hydratase or crotonase, short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), and one or more oxothiolases. Each action of an acyl-CoA dehydrogenase produces two electrons which are transferred to electron transfer flavoprotein (ETF) and subsequently to ETF-dehydrogenase (ETF-DH). Flavin adenine dinucleotide (FAD), which is derived from riboflavin, acts as a cofactor in these reactions. The electrons are eventually handled by the respiratory chain.

The acetyl-CoA molecules are the immediate sources of the ketone bodies. The actual formation of acetoacetate proceeds via the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) cycle with HMG-CoA synthetase (HMGS) and HMG-CoA lyase (HMGL, see Sect. 6.8) as the key enzymes. Extrahepatic tissues are able to utilize the ketone bodies and require for this the action of succinyl-CoA: 3-oxoacid CoA transferase (SCOT) and 3-oxothiolase (see Sect. 7.4).

Failure of any of the steps of the β -oxidation cascade or the ketone body pathway will result in an inadequate utilisation of fatty acids as an energy source. Because gluconeogenesis alone is not sufficient to meet the total energy demand, the patient will end up with fasting hypoglycemia. Defective long-chain and medium-chain enzymes as well as defects of ketogenesis will result in hypoketotic hypoglycemia and increased plasma free fatty acid levels. Disorders of short-chain enzymes and of ketone body utilisation result in ketotic hypoglycemia. A shortage of acetyl-CoA, as observed in the fatty acid oxidation disorders, has additional effects.

N-acetylglutamate is not formed in adequate amounts, hence the patient will develop hyperammonemia. Due to the accumulation of fatty acids hepatic steatosis may develop. This combination of symptoms is generally named "Reye-like syndrome".

Carnitine uptake defect (CUD, 14.1) is due to a defect in the high-affinity carnitine transporter [1]. Plasma free and total carnitine levels are extremely low. Clinically two forms exist: an early childhood-onset cardiomyopathic form and a hepatic form with recurrent crises of Reye-like syndrome. In general the response to oral carnitine supplements is very good. Plasma acylcarnitines are normal, there are scattered reports on moderate dicarboxylic aciduria.

CPT1 deficiency (14.2) has only hepatic involvement; cardiomyopathy is absent. The clinical presentation is rather homogenous with recurrent episodes of hepatic dysfunction, often accompanied by hypoglycemia. Many patients develop renal tubular acidosis (unexplained) [2]. This is one of the defects in which the mother of a patient may experience acute fatty liver of pregnancy [3]. Plasma-free carnitine is reportedly increased, but normal values have been observed. Dicarboxylic aciduria is a rarity.

Carnitine acylcarnitine carrier deficiency (CAC, 14.3) has two forms, i.e. a severe form with a high incidence of sudden childhood death and a mild form. Both cardiomyopathic acid hepatopathic features occur. All patients have a very low plasma free carnitine and increased C₁₆–C₁₈ acylcarnitines [4]. A subtle dicarboxylic aciduria may be present. A number of patients initially presented with coma and marked hyperammonemia.

CPT2 deficiency (14.4) is divided into a neonatal form, a childhood-onset form and an adolescent-adult form [5]. The latter form was characterized by exercise intolerance and muscle pain. The neonatal form is generally lethal and may include renal cysts. Plasma free carnitine is very low in the young patients, it may increase with age. Accumulation of C₁₆–C₁₈ acylcarnitines in plasma is the diagnostic hallmark; however, this may almost

normalize with age. Dicarboxylic aciduria is the exception, notable is the occurrence of C₁₂-dicarboxylic acid.

Three forms of VLCAD deficiency (14.5) are known, i.e. a (lethal) neonatal cardiomyopathic form, a childhood form with recurrent Reye-like episodes, and an adolescent/adult muscular form [6]. The latter may be accompanied by severe rhabdomyolysis and sometimes renal failure. Diagnostic criteria are the abnormal plasma acylcarnitine and organic acid profile (C_{14:1}, C_{14:2} and C_{16:1}) and the C₆–C₁₀ dicarboxylic aciduria, especially in young patients.

MCAD-deficiency (14.6) is the most frequent fatty acid oxidation disorder. Its incidence may be as high as 1:15 000 in North-Western European communities [7]. Approximately 25% of the patients have died suddenly and unexpectedly. The same percentage of patients may remain free of symptoms throughout life. Ninety percent of all alleles bears the same A985G mutation. The diagnosis is made by the finding of: C₈, C_{10:1}, C_{10:0} acylcarnitines in plasma, C_{10:1} fatty acid in plasma and C₆–C₁₀ dicarboxylic acids together with medium-chain acylglycines in the urine. Between the hypoketotic hypoglycemic episodes the metabolite profiles can be virtually normal.

SCAD deficiency (14.7) is a complicated disorder. Its biochemical hallmark is an increased urinary excretion of ethylmalonic acid (EMA), the carboxylation product of butyryl-CoA. Plasma C₄-carnitine is moderately increased. Literally hundreds of patients with an increased EMA excretion are known; yet only 20 or so proven SCAD-deficient patients have been reported [8]. There is a high frequency of two mutations of the SCAD-gene (C511T and G625A) in the general population, both (or together) conferring susceptibility to EMA-uria. The correct diagnosis relies on the measurement of SCAD in fibroblasts or muscle using the ETF-reduction assay *and* an anti-MCAD antiserum. The fibroblast C₄-acylcarnitine production from labelled fatty acids is an adjacent diagnostic tool. Formally tested patients developed hypoglycemia with ketosis upon fasting. Otherwise the clinical spectrum is wide with a high frequency of hypotonia. Maternal illness during pregnancy has been reported. EMA-uria with a normal SCAD-activity has been described in the so-called EMA-encephalopathy and in some respiratory chain defects [9].

The mitochondrial trifunctional protein (MTP) is membrane-bound; it has three enzyme activities, viz. long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), long-chain enoyl-CoA hydratase, and long-chain 3-oxoacyl-CoA thiolase. The protein consists of four α -subunits and four β -subunits. Depending on the position of the mutation, the disorder is named LCHAD- α or LCHAD- β (14.8A and 14.8B). The majority of the patients do have cross-reacting MTP, they have only LCHAD deficiency [10]. Patients lacking cross-reacting material do not possess hydratase and thiolase activities [11]. No clinical differences exist between LCHAD- α and LCHAD- β patients. In general Reye-like episodes with (cardio)myopathy are the main symptoms. Most patients develop neuropathy and retinopathy. There is a

high incidence of maternal illness during pregnancy [12]. Diagnostic features are long-chain hydroxy fatty acids and acylcarnitines in plasma (not universally increased) and hydroxydicarboxylic aciduria. The latter can also be observed as a secondary phenomenon in respiratory chain disorders, coeliac disease or abetalipoproteinemia. LCHAD- α patients often have a common G1528A mutation [10].

Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (SCHAD, 14.9) is perhaps the most difficult disorder for diagnosis. Affected patients may have episodes of ketotic hypoglycemia, whereas their urinary organic acid profile shows ketonuria and only moderate dicarboxylic aciduria. The plasma acylcarnitine profile may not be conclusive in all cases (3-hydroxybutyrylcarnitine) and there is considerable heterogeneity of tissues expressing the enzyme defect [13]. It is not even sure how many short/medium-chain hydroxyacyl-CoA dehydrogenases actually exist. Mutations have been reported in a single patient.

The multiple acyl-CoA dehydrogenation defect (MAD, 14.10A, 14.10B and 14.10C) and its riboflavin-responsive form (MAD/RR, 14.11) show an extremely wide clinical variability from lethal neonatal acidosis with renal cysts and brain dysplasia to mild adult lipid storage myopathy and exercise intolerance. The function of all acyl-CoA dehydrogenases is compromised, including those of glutaryl-CoA dehydrogenase and the branched-chain acyl-CoA dehydrogenases. The urine and plasma metabolite profiles reflect the generalized defect [14]. Mutations may reside in the α -subunit (14.10A) of electron transfer flavoprotein (ETF), the β -subunit (14.10B) or ETF-dehydrogenase (14.10C). No clinical differences exist between the three disorders. A primary defect underlying the riboflavin-responsive MAD has yet to be found [15].

The inability to form ketone bodies as a result of 3-hydroxy-3-methylglutaryl-CoA synthase deficiency (HMGs, 14.12) leads to hypoketotic hypoglycemia with unusually high plasma free fatty acid levels. Urine organic acid profiles are non-descript, the plasma acylcarnitine pattern is normal. Thus far only four patients were reported at ages between 1 and 6 years; their outcome was favourable. Mutations were found in most alleles [16].

Succinyl-CoA: 3-oxoacid-CoA-transferase deficiency (SCOT, 14.13) was already described in the early seventies. More than 10 patients are known now; they all had multiple episodes of severe ketoacidosis, similar to the patients with β -ketothiolase deficiency (see Sect. 7.4). Whenever there is hypoglycemia, this is denoted hyperketotic. Urine organic acids and plasma acylcarnitines are non-informative. Various mutations have been reported [17]. Growth and development of the patients was reported to be normal.

Not all enzymes and proteins associated with mitochondrial fatty acid β -oxidation have been fully characterized. It is to be expected that more defects will be discovered. The recognition of unusual presentation in a neurological, muscular, or hepatic sense will help in guiding us to the delineation of novel disorders.

14.2 Nomenclature

No.	Disorder	Protein/ synonym	Tissue	Chromosome	MIM
14.1	Carnitine uptake	OCTN2	FB, kidney, heart, liver	5q31	212140
14.2	Carnitine palmitoyltransferase 1	CPT1	FB, liver	11q22.23	255120
14.3	Carnitine acylcarnitine carrier	CAC	FB, liver, heart, muscle, WBC	3p21.31	212138
14.4	Carnitine palmitoyltransferase 2	CPT2	FB, liver, heart, muscle, WBC	1p32	255110
14.5	Very long-chain acyl-CoA dehydrogenase	VLCAD	FB, liver, heart, muscle, WBC	17p11.2–11.1	201475
14.6	Medium-chain acyl-CoA dehydrogenase	MCAD	FB, liver, muscle, WBC	1p31	201450
14.7	Short-chain acyl-CoA dehydrogenase	SCAD	FB, WBC, liver, muscle	12q22-ter	201470
14.8A	Long-chain 3-hydroxyacyl-CoA dehydrogenase- α	LCHAD- α	FB, WBC, liver, muscle	2p23	600890
14.8B	Long-chain 3-hydroxyacyl-CoA dehydrogenase- β	LCHAD- β	FB, WBC, liver, muscle	2p23	143450
14.9	Short-chain 3-hydroxyacyl-CoA dehydrogenase	SCHAD	FB, muscle, liver, WBC	4q22–q26	601609
14.10A	Multiple acyl-CoA dehydrogenation defect (electron transfer flavoprotein- α)	ETF- α	FB	15q23–q25	231680
14.10B	Multiple acyl-CoA dehydrogenation defect (electron transfer flavoprotein- β)	ETF- β	FB	19q13.3	130410
14.10C	Multiple acyl-CoA dehydrogenation defect (electron transfer flavoprotein dehydrogenase)	ETF-DH	FB	4q32-ter	231675
14.11	Riboflavin-responsive multiple acyl-CoA dehydrogenation defect				
14.12	3-Hydroxy-3-methylglutaryl-CoA synthase	HMG-CS2	Liver	1p13-p13	600234
14.13	Succinyl-CoA: 3-oxoacid-CoA transferase	OXCT	FB	5p13	245050

14.3 Metabolic Pathway

The conversion of long-chain fatty acids into ketone bodies is schematically drawn in Fig. 14.1. The whole process takes place in the liver cell. Long-chain fatty acids are taken up by the cell and are converted into coenzyme A esters (AS). Carnitine has to be brought into the cell by its plasma membrane transporter. Once inside the intermembrane space, acyl-CoA and carnitine react under the influence of CPT1, a membrane-bound enzyme, the activity of which is regulated by the levels of malonyl-CoA and glucagon. This is the key regulating step of the fatty acid β -oxidation.

Acylcarnitines cross the inner mitochondrial membrane (I.M.M.) via a carrier, after which the acyl-CoA is reformed by CPT2, again membrane-bound. The first few rounds of β -oxidation are catalyzed by VLCAD and MTP (containing LCHAD, enoyl-CoA hydratase and long-chain oxothiolase

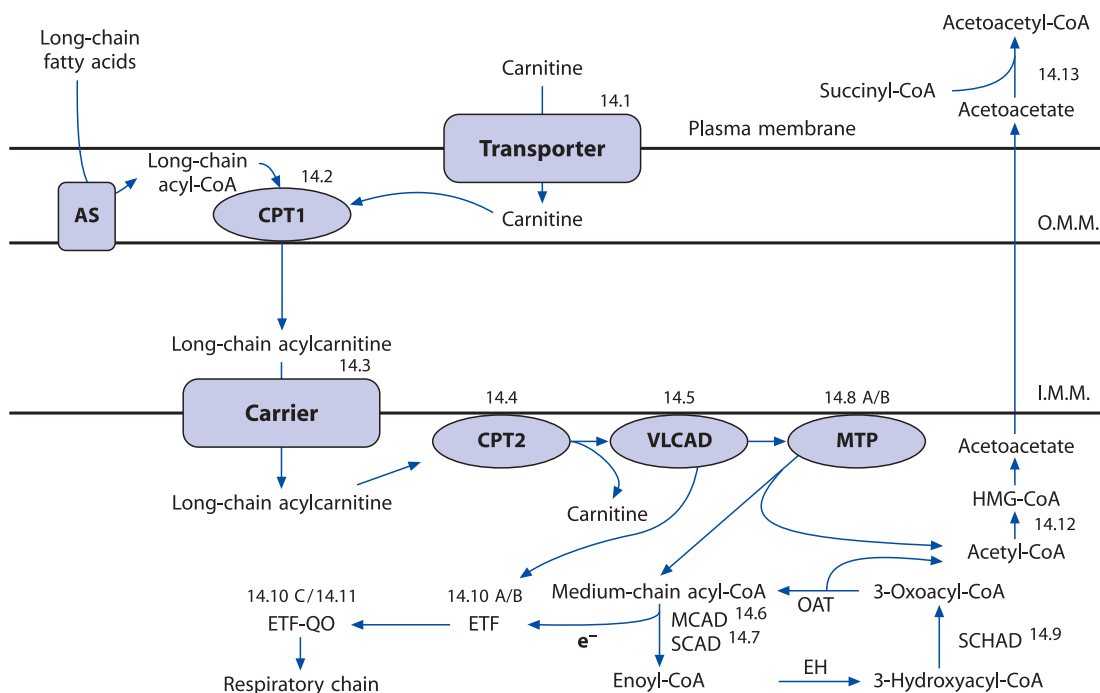


Fig. 14.1. Schematic diagram of the conversion of fatty acids into ketone bodies. The numbers refer to the defects described in this chapter. *O.M.M.*, outer mitochondrial membrane; *I.M.M.*, inner mitochondrial membrane; AS, acyl-CoA synthetase

activities). These enzymes are membrane-bound; there is probably channeling of substrates between the enzyme proteins. The final medium-chain acyl-CoA's are further degraded by a set of matrix enzymes. Every acyl-CoA dehydrogenation step yields electrons, which are transported by ETF and ETF-DH to the respiratory chain.

Each cycle of β -oxidation produces one molecule of acetyl-CoA. This cannot leave the cell, but has to be transformed to acetoacetate (and D-3-hydroxybutyrate) by a series of reactions in the so-called hydroxymethylglutaryl cycle. This includes HMG-CoA synthase and HMG-CoA lyase (see Sect. 6.8). Ketone bodies need several enzymes for their utilization in peripheral tissues, i.e. 3-oxothiolase (Sect. 7.4) and succinyl-CoA: 3-oxoacid CoA transferase (SCOT).

Acyl-CoA's can back-react with carnitine and leave the cell in the form of acylcarnitines. Similar processes occur for some acyl-CoA's in the formation of acylglycines. Finally excess acyl-CoA's may be subjected to ω -oxidation in the microsomal fraction to yield dicarboxylic acids.

14.4 Signs and Symptoms

Table 14.1. Carnitine uptake defect (25 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Cardiomyopathy	+	+	+	(+)	(-)
	Hypotonia	+	+	+	(+)	(-)
	Coma	+	+	+		
	Liver dysfunction		+	+		
	Hepatomegaly		+	+		
Routine laboratory	Glucose (B)	↓	↓	↓		
	Ammonia (B)	↑	↑	↑		
	Acidosis	+	+	+		
	Ketones (B)	↓	↓	↓-n		
	Liver enzymes (P)	↑	↑	↑		
	Creatine kinase (P)	↑	↑	↑		
	Myoglobin (U)		↑	↑		
Special laboratory	Dicarboxylic acids (U)	n-↑	n-↑	n-↑		
	Acylglycines (U)	n	n	n	n	n
	Free carnitine (P)	↓	↓	↓	↓	↓
	Long-chain acylcarnitines (P)	↓	↓	↓	↓	↓
Other	Sudden death	+	+			
	Effect of carnitine	+	+	+	+	+
	Anemia		(+)	(+)		

Table 14.2. Carnitine palmitoyltransferase 1 deficiency (<20 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	Coma/lethargy	(+)	+	+	
	Liver dysfunction	(+)	+	+	
	Hepatomegaly	(+)	+	+	
	Vomiting/diarrhoea	(+)	+	+	
	Hypotonia		+	+	
Routine laboratory	Glucose (B)	↓	(↓)	(↓)	
	Ketones (B)	↓	↓	↓	
	Acidosis	+	+	+	+
	Ammonia (B)	n-↑	n-↑	n-↑	
	Liver enzymes (P)	n	(↑)	(↑)	
	Creatine kinase (P)	n	n	n	n
Special laboratory	Dicarboxylic acids (U)	-	(-)	-	-
	Free carnitine (P)	n-↑	n-↑	↑	↑
	Long-chain acylcarnitines (P)	↓	↓	↓	↓
CNS	Seizures		+		
Other	Renal tubular acidosis	+	+	+	+
	Maternal disease of pregnancy	+			
	Symptom free	+	+	+	

Table 14.3. Carnitine acylcarnitine carrier defect (<20 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Coma/lethargy	+	+	(-)
	Cardiac abnormality	+		-
	Liver dysfunction	+		(-)
	Hypotonia	+		-
Routine laboratory	Glucose (B)	↓-n	↓	
	Ketones (B)	↓		
	Acidosis	+		
	Lactate (B)	↑	n	
	Ammonia (B)	↑	↑	
	Liver enzymes (P)	↑		
	Creatine kinase (P)	↑		
	Uric acid (P)	↑		
	Dicarboxylic acids (U)	n-↑	n-↑	
	Acylglycines (U)	n	n	
Special laboratory	Free carnitine (P)	↓	↓	
	C ₁₆ -C ₁₈ acylcarnitines (P)	↑	↑	
	Seizures	+	+	
	Microcephaly		(+)	
CNS				
Other	Sudden death	+	+	-

Table 14.4. Carnitine palmitoyltransferase 2 deficiency (<100 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Coma/lethargy	+	+			
	Hepatic dysfunction	+	+			
	Hepatomegaly	+	+			
	Hypotonia		+	+	+	+
	Exercise intolerance			+	+	+
	Muscle pain			+	+	+
	Cardiomyopathy	+	+			
Routine laboratory	Glucose (B)	↓	↓			
	Ketones (B)	↓	↓			
	Liver enzymes (P)	↑	↑			
	Creatine kinase (P)	↑	↑			
	Myoglobin (U)		+	+	+	+
Special laboratory	Dicarboxylic acids (U)	(-)	(-)	(-)	-	-
	Free carnitine (P)	↓	↓			
	C ₁₆ -C ₁₈ acylcarnitine (P)	↑	↑	↑		(↑)
	Acylglycines (U)	n	n	n	n	n
Other	Sudden death	+	+			
	Pancreatitis				+	
	Renal cysts	+				

Table 14.5. Very long-chain acyl-CoA dehydrogenase deficiency (>100 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Coma/lethargy	+	+			
	Cardiomyopathy	+	+	+	(+)	(+)
	Vomiting	+	+	+		
	Diarrhoea	+	+	+		
	Hepatic dysfunction	+	+	+	-	-
	Rhabdomyolysis	-	(+)	+	+	+
	Renal failure				(+)	(+)
	Hypotonia	+	+	+	(-)	(-)
	Muscle pain				+	+
Routine laboratory	Glucose (B)	↓	↓	↓	↓-n	n
	Ketones (B)	↓	↓	↓	↓	↓
	Acidosis	+	+	+		
	Liver enzymes (P)	+	+	+		
	Ammonia (B)	n-↑	n-↑	n-↑		
	Lactate (B)	n-↑	n-↑	n-↑		
	Creatine kinase (P)	+	+	+	+	+
	Myoglobin (U)				+	+
	Dicarboxylic acids (U)	↑	↑	↑	n-↑	n
Special laboratory	Acylglycines (U)	n	n	n	n	n
	Free carnitine (P)	↓	↓	↓	↓-n	↓-n
	Long-chain acylcarnitines (P)	↑	↑	↑	↑	↑
	14:1 Fatty acid/acylcarnitine (P)	+	+	+	+	+
	Sudden death	+	+			

Table 14.6. Medium-chain acyl-CoA dehydrogenase deficiency (>500 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Coma/lethargy	+	+	+	+	
	Liver dysfunction	+	+	+	+	
	Hypotonia	+	+	+	+	+
	Respiratory arrest	+	+	+		
	Vomiting	+	+	+		
Routine laboratory	Cardiomyopathy	–	(–)	(–)	(–)	–
	Glucose (B)	↓	↓	↓	↓–n	↓–n
	Ketones (B)	↓–n	↓–n	↓–n	↓–n	↓–n
	Acidosis	+	+			
	Liver enzymes (P)	↑	↑	↑	↑	↑
Special laboratory	Ammonia (B)	↑	↑	↑		
	Uric acid (P)	↑	↑	↑	↑	↑
	Creatine kinase (P)	n	n	n	n	n
	Dicarboxylic acids (U)	↑	↑	↑	↑	↑
	Acylglycines (U)	↑	↑	↑	↑	↑
CNS	Free carnitine (P)	↓–n	↓–n	↓–n	↓–n	↓–n
	C ₈ –C ₁₀ acylcarnitines (P)	↑	↑	↑	↑	↑
	Cis-4-decenoic acid (P)	↑	↑	↑	↑	↑
	Seizures		(+)	(+)	(+)	
	Mental retardation			(+)		
Pathology	Attention deficit				(+)	(+)
	Fatty infiltration of liver	+	+	+		
Other	Symptom-free	(+)	(+)	(+)	(+)	(+)
	Exercise intolerance				(+)	(+)
	Sudden death	+	+	+	–	–

Table 14.7. Short-chain acyl-CoA dehydrogenase deficiency (<20 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Hypotonia	+	+	+	+	–
	Vomiting	+		+		
	Poor feeding	+		+		
	Developmental delay			(+)		
Routine laboratory findings	Glucose (B)		↓	↓		
	Ketones (P)		↑	↑		
	Free fatty acids (P)		↑	↑		
	Creatine kinase (P)		n	n		
Special laboratory findings	Ethylmalonate (U)	↑	↑	↑	↑	(↑)
	Methylsuccinate (U)	(↑)	(↑)	(↑)	(↑)	(↑)
	Butyrylglycine (U)		(↑)			
	Butyrylcarnitine (P)	(↑)	(↑)	(↑)	(↑)	(↑)
Other findings	Free carnitine (P)	↓–n	↓–n	↓–n	↓–n	n
	Pneumonia		(+)			
	Symptom-free	(+)	(+)	(+)	(+)	+
	Maternal disease of pregnancy	(+)				

Table 14.8 A, B. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD- α and LCHAD- β) (>100 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Coma/lethargy	+	+	+		
	Cardiomyopathy	+	+	+		
	Liver dysfunction	+	+	+		
	Hypotonia	+	+	+	+	+
	Neuropathy		+	+	+	+
	Retinopathy		+	+	+	+
	Vomiting	+	+	+		
Routine laboratory	Glucose (B)	↓	↓	↓		
	Ketones (B)	↓	↓	↓		
	Lactate (B)	↑	↑	↑	↑	↑
	Ammonia (B)	↑	↑	↑		
	Liver enzymes (P)	↑	↑	↑		
	Creatine kinase (P)		↑	↑	↑	
	Acidosis	+	+	+		
Special laboratory	Dicarboxylic acids (U)	↑	↑	n-↑	n-↑	n-↑
	Hydroxydicarboxylic acids (U)	↑	↑	↑	↑	↑
	Acylglycines (U)	n	n	n	n	n
	Free carnitine (P)	↓	↓	↓-n	↓-n	↓-n
	Long-chain acylcarnitines (P)	↑	↑	n-↑	n-↑	n-↑
	Long-chain 3-hydroxyfatty acids (P)	↑	↑	↑	↑	↑
CNS	Seizures	+	+			
Other	Maternal disease of pregnancy	+				
	Sudden death	+	+	+		

Table 14.9. Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (<10 patients)

System	Symptoms/markers	Neonatal	Infancy	Adolescence
Characteristic clinical findings	Coma/lethargy		+	+
	Vomiting		+	+
	Liver steatosis		+	
	Muscle weakness		+	+
	Cardiomyopathy			+
Routine laboratory	Glucose (B)	↓	↓	↓
	Ketones (U)		(+)	+
	Creatine kinase (P)			↑
	ASAT/ALAT (P)			↑
	Myoglobin (U)		(+)	+
Special laboratory	Dicarboxylic acids (U)		(+)	+
	3-OH-C ₄ acylcarnitine (P)	n-↑	n-↑	
	Enzyme L		↓	
	Enzyme M		n	↓
	Enzyme FB		↓-n	n
CNS	Convulsions	+	+	
Other	Sudden death		+	

Table 14.10. Multiple acyl-CoA dehydrogenation defect (<100 patients) (ETF α , ETF β , ETF-DH)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Coma/lethargy	+	+	+		
	Vomiting	+	+	+		
	Hepatic dysfunction	+	+	+		
	Hypotonia	+	+	+	+	+
	Odor of sweaty feet	+	(+)			
Routine laboratory	Exercise intolerance			(+)	+	+
	Glucose (B)	↓	↓	↓		
	Ketones (B)	↓	↓	↓		
	Acidosis	+	+	+		
	Ammonia (B)	↑	↑	n-↑	n-↑	
Special laboratory	Liver enzymes (P)	↑	↑	↑		
	Creatine kinase (P)	↑	↑	↑		
	Glutaric and/or ethylmalonic acid (U)	↑	↑	↑	↑	↑
	Dicarboxylic acids (U)	↑	↑	↑	↑	
	Acylglycines (U)	↑	↑	↑	↑	
Other	Free carnitine (P)	↓	↓	↓	↓	↓
	All acylcarnitines (P)	↑	↑	↑	↑	↑
	Sarcosine (U)	n-↑	n-↑			
	Ataxia			±		
	Renal cysts	(+)				
Other	Malformations	(+)				
	Stroke-like episodes			(+)		

Table 14.11. Riboflavin-responsive multiple acyl-CoA dehydrogenation defect (>20 patients)

System	Symptoms/markers	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Lethargy		±	+	+
	Hepatic dysfunction	±	±	±	
	Muscle weakness	+	+	+	+
	Exercise intolerance		+	+	+
	Psychiatric behavior				+
Routine laboratory	Glucose (B)	n	↓-n	↓-n	↓-n
	Ketones (B)	↓-n	↓-n	↓-n	↓-n
	Lactate (B)	n	↑	↑	↑
	Liver enzymes (P)	n-↑	n-↑	↑	↑
	Creatine kinase (P)		n-↑	↑	↑
Special laboratory	Glutaric and/or ethylmalonic acid (U)	↑	↑	↑	↑
	Dicarboxylic acids (U)	n-↑	n-↑	n-↑	n-↑
	Acylglycines (U)	↑	↑	↑	↑
	Free carnitine (P)	↓	↓	↓	↓
	All acylcarnitines (P)	↑	↑	↑	↑
Other	Stridor	+			
	Lipid storage myopathy		+	+	+

Table 14.12. 3-Hydroxy-3-methylglutaryl-CoA synthase deficiency (4 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	Coma/lethargy		+	+	–
	Vomiting		+	+	–
	Diarrhoea		+	+	–
	Hepatomegaly		+	+	–
Routine laboratory	Glucose (B)		↓	↓	
	Ketones (B)		↓	↓	
	Free fatty acids (P)		↑	↑	
	Liver enzymes (P)		↑	↑	
Special laboratory	Dicarboxylic acids (U)		±	–	
	Acylglycines (U)		n	n	
	Free carnitine (P)		n	n	
	Long-chain acylcarnitines (P)		n	n	
CNS	Seizures			+	
	Mental development	n	n	n	n
Other	Ketonemia after leucine load		+		

Table 14.13. Succinyl-CoA: 3-oxoacid-CoA transferase deficiency (<20 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Coma/lethargy	+	+	+
	Tachypnoea	+	+	+
	Hypotonia	(+)	(+)	
	Cardiomegaly	(+)		
Routine laboratory	Glucose (B)	n	n	n
	Ketones (B)	↑	↑	↑
	Acidosis	+	+	+
	Free fatty acids (P)	n	n	n
	Ammonia (B)	n	n	n
	Lactate (B)	n	n	n
	Dicarboxylic acids (U)	–	–	–
Special laboratory	Acylglycines (U)	–	–	–
	Free carnitine (P)		↓	
	Acylcarnitines (P)		n	
CNS	Mental development	n	n	n
Other	Sudden death	+	+	

14.5 Reference Values

■ Plasma (μmol/l)

	C ₀ (free)	C ₂	C ₄	C ₅	C ₆	C ₈	C _{10:1}	C _{10:0}	C _{12:0}	C _{14:1}
Acylcarni- tine	21–54	3.4–13	0.07–0.58	0.04–0.22	0.02–0.12	0.04–0.22	0.04–0.22	0.04–0.30	0.04–0.14	0.02–0.18
Free fatty acid					0.5–4.2	0.1–10	0.04–0.6	0.6–4	0.96–12	<0.38
3-OH-fatty acid					<1	<1		<0.27	<0.15	<0.09

	C _{14:0}	C _{16:1}	C _{16:0}	C _{18:2}	C _{18:1}	C _{18:0}	3-OH-C _{16:0}	3-OH-C _{18:2}	3-OH-C _{18:1}
Acylcarni- tine	0.02–0.15	0.02–0.08	0.06–0.24	0.02–0.18	0.06–0.28	0.02–0.1	<0.02	<0.02	<0.02
Free fatty acid	3.23–23	1.8–19	58–221	13–167	37–264	17–75			
3-OH-fatty acid	<0.12	<0.02	<0.29	<0.10	<0.13	<0.20			

All unsaturated long-chain substances (C₁₂–C₁₆) increase during prolonged fasting [18].

■ Urine (mmol/mol Creatinine)

Substance	Fasting level	Substance	Fasting level
3-Hydroxybutyrate	160–6460	Acetylglycine	2.5–47.6
Ethylmalonate	<10	Isobutyrylglycine	0.003–1.5
Methylsuccinate	<3	Butyrylglycine	0.01–0.12
Glutarate	<10	Isovalerylglycine	0.2–0.9
Adipate	74–610	Hexanoylglycine	0.2–0.8
Suberate	19–230	Phenylpropionylglycine	0.002–0.15
Decanedioate	90–310	Suberylglycine	0.02–0.52
Dodecanedioate	10–200		
3-Hydroxyadipate	10–100		
3-Hydroxysuberate	5–50		
3-Hydroxydecanedioate	10–200		
3-Hydroxydodecanedioate	10–200		

14.6 Pathological Values

■ Plasma (μmol/l)

● *Acylcarnitines*

	14.1 CUD	14.2 CPT1	14.3 CAC	14.4 CPT2	14.5 VLCAD	14.6 MCAD	14.7 SCAD	14.8A/B LCHAD	14.9 SCHAD	14.10 MAD	14.11 MAD/RR	14.12 HMGS	14.13 SCOT
C ₀ =free	0–5	50–230	0–5	5–40	0–13	3–24	15–34	10–18	18	4–23	17	n	n
C ₂			4.4				0.8–5				7.85		
C ₄			1.03				0.64–6.0		+	0.12–15	0.80		
C ₅										0.09–0.75	0.39		
C ₆			1.22			0.12–2.1				0.22–3.54	0.13		
C ₈			0.55			1.1–26				0.8–7.96	1.75		
C _{10:1}			0.22			0.26–2.2					0.93		
C _{10:0}			0.72			0.1–0.4				0.8–5.88	4.66		
C _{14:1}			0.67		0.76–19	0.01–0.04		0.1–0.25		0.24–2.70	0.28		
C _{14:0}			1.32		0.13–5.0	0.01–0.03		0.16–0.23			0.20		
C _{16:1}			1.94	0.5–0.9	0.16–9.1	<0.02		0.14–0.35		0.12–1.0	1.25		
C _{16:0}			18.7	2.1–3.9	0.33–14.4	0.20		1–2.2	+	0.12–1.14	0.71		
C _{18:2}			0.71	0.54–1.66	0.11–2.1			0.20–0.24		0.08–0.64			
C _{18:1}			3.50	1.56–4.32	0.14–6	0.14–0.32		0.35–1.1	+	0.18–0.88	1.31		
3-OH-C _{16:0}			0.16					0.12–0.60		0.02–0.04			
3-OH-C _{18:1}			0.11					0.14–0.86		0.02–0.06			

Non-characteristic profiles will be observed in 14.1, 14.2, 14.12 and 14.13.

● *Free Fatty Acids and 3-Hydroxyfatty Acids*

	14.1 CUD	14.2 CPT1	14.3 CAC	14.4 CPT2	14.5 VLCAD	14.6 MCAD	14.7 SCAD	14.8A/B LCHAD	14.9 SCHAD	14.10 MAD	14.11 MAD/RR	14.12 HMGS	14.13 SCOT
C ₆		8.1		3.9	2–7	61		0.7–14		1–154			
C ₈		10		10	2–8	8–394		2–21		10–110			
C _{10:1}		0.35		0.4	0.1–3	3–168	8	0.1–30		0.7–36			
C _{10:0}		4.5		3.6	5–20	7–96	19	2–31		5–71			
C _{12:0}		14		8.6	20–195	33	106	1–40		7–80			
C _{14:1}				0.74	16–191	0.72	+	1–8		0.2–82			
C _{14:0}		37		84	49–172	57	91	5–140		7–255			
C _{16:1}		70		148	21–120	39	130	3–107		7–145			
C _{16:0}		473		1349	223–1600	398	493	61–545		64–1128			
C _{18:2}		196		59	93–371	585	159	16–218		15–379			
C _{18:1}		170		277	217–1123	1573	1273	27–357		60–1612			
C _{18:0}		121		67	29–126	448	132	7–158		30–361			
3-OH-C _{16:0}		0.04		0.58				0.6–15		0.15–0.60			
3-OH-C _{18:2}								0.3–9		0.03			
3-OH-C _{18:1}								0.7–3.7		0.04			

■ Urine Organic Acids (mmol/mol Creatinine)

	14.1 CUD	14.2 ^a CPT1	14.3 CAC	14.4 CPT2	14.5 VLCAD	14.6 MCAD	14.7 SCAD	14.8A/B LCHAD	14.9 SCHAD	14.10 MAD	14.11 MAD/RR	14.12 HMGS	14.13 SCOT
3-OH-butyrate		8		n.d.	0–15	55–1625	160–6460	<24	+++	<29	8–682	<15	<125000
Adipate	(+)	44	5–138	262	178–2000	52–4700	3–750	517	+	<2580	<3970	+	N
3-OH-adipate	(+)	83	+		250–600		<180	664					
Suberate	(+)	25	2–168	94	40–410	80–290	<160	169	+	<460	<440	+	n
Δ-Suberate		23	3–187		86–560	15–260	<72	96		<170	<510		
3-OH-suberate							<28						
Decanedioate	(+)	75	7–266	111	88	<840			+	<30	<530	+	n
Δ-Decanedioate			0–205		<1000	20–760	<100			<210			
3-OH-decanedio- ate	(+)	51			120–500	10–310	<75	230					
Dodecanedioate		60	0–74							<34			
3-OH-dodecane- dioate	(+)	13			<40		<22						
Ethylmalonate							36–1150			85–394	9–139	(+)	
Methylsuccinate							22–100			12–152	11–23		
Glutarate							<23			<5700	<460	(+)	
Isobutyrylglycine						0.08–3.6				2–300			
Butyrylglycine						0.14–0.91				0.2–144			
Isovalerylglycine						0.28–6.7				1–216			
Hexanoylglycine						5.5–290				0.8–182		(+)	
Suberylglycine			(+)			5.2–359				0.3–50			
Phenylpropionyl- glycine						0.10–28				0.04–6.8			
5-OH-hexanoate					0–13	50–380	<45				<390	(+)	

n, normal; +, present, not quantitated; +++, increased; (), only in a limited number of patients. n.d., not detectable.

^a Only one observation.

14.7 Loading Tests

Fatty acid β -oxidation defects often have an episodic character. In between the attacks of metabolic decompensation, plasma and urine parameters tend to virtually normal. On the other hand, several conditions have been recognized to yield metabolite profiles similar to those of fatty acid oxidation defects. Amongst these are: feeding with medium-chain triglyceride containing feedstuffs, gastro-intestinal problems such as coeliac disease, and dysfunction of the respiratory chain.

In order to get a more clear expression of the metabolic abnormalities, one or more of the established *in vivo* or *in vitro* loading tests can be applied. A description and interpretation is given below.

■ Long-Chain Triglyceride (LCT) Test

- Patient condition: overnight fast.
- Test dose: 1.5 g/kg body weight of sunflower oil (mainly oleic and linoleic acids). Oral administration by gavage.

Assays:

	Time (h)				
	0	1	2	3	4
Blood glucose	X	X	X	X	X
Blood ketone bodies	X	X	X	X	X
Plasma FFA	X	X	X	X	X
Plasma acylcarnitines	X	X	X	X	X
Plasma organic acids				X	

Urine for organic acid analysis is collected at 0–3 h and 3–6 h after the load. Insufficient ketogenesis (less than twofold increase of the starting value) is observed in long-chain fatty acid oxidation defects and HMGS-deficiency. Controls have been shown to accumulate unsaturated C₁₂–C₁₆ acylcarnitines; this profile is different from any of the primary defects [18]. Patients with any of the primary defects will accumulate the relevant acylcarnitines and (hydroxy) fatty acids in plasma. Defects distal from CPT2-deficiency are additionally characterized by the excretion of dicarboxylic acids in the urines after loading.

When the suspicion of a long-chain fatty acid oxidation defect is substantial, additional proof can be found by a medium-chain triglyceride (MCT) loading test. This should result in normal ketogenesis, except in patients with MCAD and HMGS deficiency. The value of the latter test is limited.

● *Fasting Test*

Patient condition: prolonged fasting, depending on age. In infancy the test is carried out for 15–20 h, in childhood 24 h, adolescents take 30–48 h, and adults may require up to 72 h. Close monitoring of blood glucose *and* the clinical condition of the patient is essential. The test should be interrupted when the blood glucose drops below 3.0 mmol/l.

Assays:

Blood glucose (hourly towards end of test), ketone bodies, free fatty acids, acylcarnitines, organic acids, counterregulatory hormones at least two times during the final stage of the test (in childhood at 15, 20, and 24 h after the start).

A 4-h urine before *and* after refeeding is analyzed for dicarboxylic acids.

Hypo- or hyperketotic hypoglycemia can be diagnosed with certainty [19]. In addition all metabolic abnormalities (acylcarnitines, acylglycines, dicarboxylic acids) become much more prominent than during the LCT-load. The fasting test is also of clinical use, i.e. obtaining an estimate of the maximum safe fasting period of the patient at home.

Caution: FASTING CAN BE DANGEROUS!

● *Phenylpropionate Loading Test*

- Patient condition: no special requirements.
- Test dose: 25 mg/kg body weight (nasty smell and taste).

Assay:

A 6-h urine collection. Analysis of phenylpropionylglycine and hippuric acid. Phenylpropionyl-CoA is degraded by MCAD. Hence MCAD-deficient patients will excrete 5–70% of the ingested dose as phenylpropionylglycine [20]. Patients with any other defect will oxidize phenylpropionate completely (excretion of hippurate).

● *Leucine Loading Test*

- Patient condition: overnight fast.
- Test dose: 200 mg/kg body weight.

Assay:

Measurement of blood ketones during 3 h after the load. The test is only applicable to patients with HMGS deficiency, who will produce ketone bodies after leucine in contrast to LCT or MCT loading tests [21].

● *In Vitro Tests*

In vitro testing may involve the oxidation of ^{14}C -labelled or ^3H -labelled palmitate and/or myristate by lymphocytes or fibroblasts [22] or the oxidation of ^{13}C -labelled palmitate by fibroblasts with subsequent measurement of acylcarnitines in the supernatant [23]. Both approaches are for specialized labs only.

14.8 Diagnostic Flow Charts

Patients will be referred with a variety of symptoms, mainly hypoglycemia with hepatic dysfunction, cardiomyopathy, or muscular weakness. The first thing to know is the absence or presence of ketosis during energy demand. Two laboratory parameters are of prime importance, i.e. the analysis of organic acids in urine and the analysis of acylcarnitines in plasma. As the latter requires tandem mass spectrometry, it may be replaced by gas chromatography/mass spectrometry of plasma organic acids, yielding virtually the same information [24].

Figure 14.2 A and B shows the algorithm to arrive at the respective diagnoses. Note that it will be impossible to distinguish between CPT1 and HMGS on the one hand, and between CPT2 and CAC on the other hand. Direct enzyme assays and mutation screening will be needed to confirm each diagnosis. These are available now for all defects.

Symptom-free affected siblings may occur. It is thus indicated to screen siblings following the diagnosis of an index case.

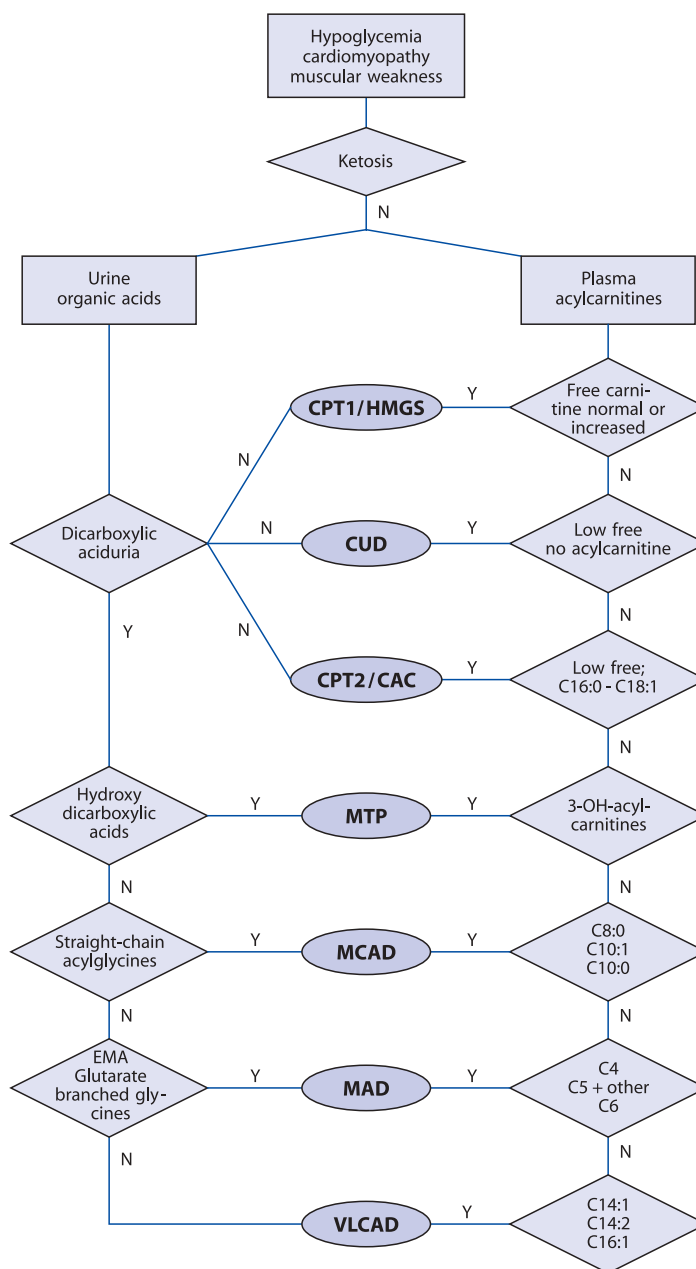


Fig. 14.2a. Diagnostic approach for patients with defects of fatty acid β -oxidation associated with non-ketotic presentation. Instead of plasma acylcarnitines, similar results may be obtained by analyzing plasma organic acids

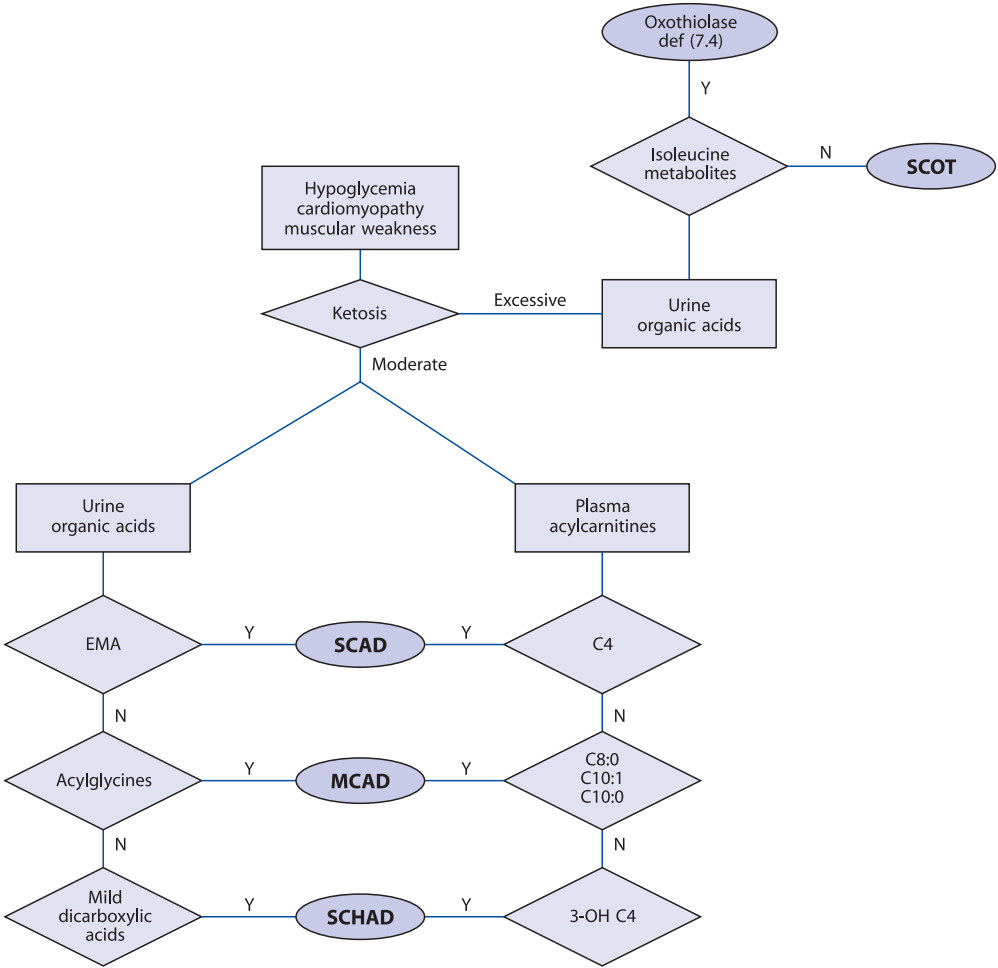


Fig. 14.2b. Diagnostic approach for ketotic patients. MCAD deficiency may either lead to hypoketotic or ketotic hypoglycemia

14.9 Specimen Collection

Test	Preconditions	Material	Handling	Pitfalls
Dicarboxylic acids (U)	Fasting	Spot urine	Keep frozen	MCT diet Glucose infusion
Carnitine and acylcarnitines	Fasting	Plasma Blood spot Bile	Keep frozen	Hemolysis MCT diet Pivampicillin
Free fatty acids (+ hydroxyacids)	Fasting	Plasma	Keep frozen	Glucose infusion
Ketone bodies	Fasting	Blood	Immediate deproteinization	Glucose infusion

14.10 Prenatal Diagnosis

Disorder	Material	Timing, trimester	Remarks
14.1	CVB; amniocytes	I; II	
14.2	CVB; amniocytes	I; II	
14.3	CVB; amniocytes	I; II	
14.4	CVB; amniocytes	I; II	
14.5	CVB; amniocytes	I; II	
14.6	CVB; amniocytes	I; II	Frequent A985G mutation
14.7			
14.8A, B	CVB; amniocytes	I; II	Frequent G1528A mutation
14.9			
14.10A, B, C	CVB; amniocytes	I; II	
14.11			
14.12	CVB; amniocytes	I; II	
14.13	CVB; amniocytes	I; II	

CVB, chorionic villus biopsy.

14.11 DNA Analysis

Disorder	Tissue/specimen	Method	Remarks
14.1	WBC; FB	PCR, sequencing	Multiple mutations
14.2	WBC; FB		
14.3	WBC; FB		
14.4	WBC; FB		
14.5	WBC; FB		
14.6	WBC; FB		
14.7	WBC; FB		
14.8A, B	WBC; FB		Frequent A985G mutation
14.9	WBC; FB		Frequent G625A, C511T polymorphism
14.10A, B, C	WBC; FB; muscle		Frequent G1528A mutation
14.11	Not available		
14.12	WBC; FB		
14.13	WBC; FB		

14.12 Initial Treatment

The hallmark of fatty acid oxidation defects is the onset of symptoms following either prolonged fasting or extreme muscular exercise. The logical treatment will be the supply of glucose. Adolescent/adult patients may develop renal failure and rhabdomyolysis. This should be treated symptomatically.

Plasma-free carnitine is aimed to be normal or close to normal. Supplementation of carnitine is therefore guided by the plasma levels.

Follow-up treatment relies on the correct diagnosis; as an example MCT-diets will only be administered to patients with long-chain defects. It may take a long time to obtain satisfying clinical results, especially of the cardiac and the muscular system.

14.13 Summary/Comments

The inherited defects of mitochondrial fatty acid β -oxidation are considered an important – and frequent – cause of disturbed energy homeostasis throughout all ages. Unfortunately, the clinical spectrum of this group of disorders is extremely variable: from a lethal neonatal presentation via relatively mild lipid storage myopathy to completely symptom-free subjects.

Fatty acid oxidation disorders should be considered in any case of unexplained hypoglycemia and/or myopathy. The laboratory diagnosis relies on the measurement of plasma acylcarnitines and urine organic acids, mainly the alternative fatty acid oxidation products. Novel defects may be expected to be delineated, especially the short- and medium-chain enzymes have not

yet all been fully characterized. As an example there is a single report only on medium-chain 3-oxoacyl-CoA thiolase deficiency, dealing with severely neurologically handicapped patients [25].

Furthermore, there may be variable tissue expression of enzyme defects, as demonstrated for SCHAD deficiency. This may also become valid for other defects.

The prognosis of the various defects cannot be given in a few sentences. Even patients with the same gene mutation may have entirely different symptoms, as exemplified by MCAD- and LCHAD deficiency. CAC deficiency was originally reported to have a poor prognosis, but milder patients are being reported. In every case immediate and aggressive treatment is always warranted. Insufficient data are available on the long-term outcome and the quality of life, especially of patients who were picked up by newborn screening programs.

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References

1. Nezu J, Tamai I, Oku A, Ohashi R, Yabuuchi H, Hashimoto N et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet* 1999; 21:91–94.
2. IJlst L, Mandel H, Oostheim W, Ruiter JP, Gutman A, Wanders RJ. Molecular basis of hepatic carnitine palmitoyltransferase I deficiency. *J Clin Invest* 1998; 102:527–531.
3. Innes AM, Seargeant LE, Balachandra K, Roe CR, Wanders RJ, Ruiter JP et al. Hepatic carnitine palmitoyltransferase I deficiency presenting as maternal illness in pregnancy. *Pediatr Res* 2000; 47:43–45.
4. Roschinger W, Muntau AC, Duran M, Dorland L, IJlst L, Wanders RJ et al. Carnitine-acylcarnitine translocase deficiency: metabolic consequences of an impaired mitochondrial carnitine cycle. *Clin Chim Acta* 2000; 298:55–68.
5. Bonnefont JP, Taroni F, Cavadini P, Cepanec C, Brivet M, Saudubray JM et al. Molecular analysis of carnitine palmitoyltransferase II deficiency with hepatocardiomyocardial expression. *Am J Hum Genet* 1996; 58:971–978.
6. Vianey-Saban C, Divry P, Brivet M, Nada M, Zabot MT, Mathieu M et al. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta* 1998; 269:43–62.
7. Tanaka K, Gregersen N, Ribes A, Kim J, Kolvraa S, Winter V et al. A survey of the newborn populations in Belgium, Germany, Poland, Czech Republic, Hungary, Bulgaria, Spain, Turkey, and Japan for the G985 variant allele with haplotype analysis at the medium chain acyl-CoA dehydrogenase gene locus: clinical and evolutionary consideration. *Pediatr Res* 1997; 41:201–209.
8. Corydon MJ, Vockley J, Rinaldo P, Rhead WJ, Kjeldsen M, Winter V et al. Role of common gene variations in the molecular pathogenesis of short-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 2001; 49:18–23.
9. Burlina AB, Dionisi-Vici C, Bennett MJ, Gibson KM, Servidei S, Bertini E et al. A new syndrome with ethylmalonic aciduria and normal fatty acid oxidation in fibroblasts. *J Pediatr* 1994; 124:79–86.

10. IJlst L, Wanders RJ, Ushikubo S, Kamijo T, Hashimoto T. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta* 1994; 1215:347–350.
11. Kamijo T, Wanders RJ, Saudubray JM, 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.
12. Treem WR, Rinaldo P, Hale DE, Stanley CA, Millington DS, Hyams JS et al. Acute fatty liver of pregnancy and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Hepatology* 1994; 19:339–345.
13. Bennett MJ, Spotswood SD, Ross KF, Comfort S, Koonce R, Boriack RL et al. Fatal hepatic short-chain L-3-hydroxyacyl-coenzyme A dehydrogenase deficiency: clinical, biochemical, and pathological studies on three subjects with this recently identified disorder of mitochondrial beta-oxidation. *Pediatr Dev Pathol* 1999; 2:337–345.
14. Rhead WJ, Wolff JA, Lipson M, Falace P, Desai N, Fritchman K et al. Clinical and biochemical variation and family studies in the multiple acyl-CoA dehydrogenation disorders. *Pediatr Res* 1987; 21:371–376.
15. Antozzi C, Garavaglia B, Mora M, Rimoldi M, Morandi L, Ursino E et al. Late-onset riboflavin-responsive myopathy with combined multiple acyl coenzyme A dehydrogenase and respiratory chain deficiency. *Neurology* 1994; 44:2153–2158.
16. Thompson GN, Hsu BY, Pitt JJ, Treacy E, Stanley CA. Fasting hypoketotic coma in a child with deficiency of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. *N Engl J Med* 1997; 337:1203–1207.
17. Fukao T, Mitchell GA, Song XQ, Nakamura H, Kassovska-Bratinova S, Orie KE et al. Succinyl-CoA:3-ketoacid CoA transferase (SCOT): cloning of the human SCOT gene, tertiary structural modeling of the human SCOT monomer, and characterization of three pathogenic mutations. *Genomics* 2000; 68:144–151.
18. Costa CC, De Almeida IT, Jakobs C, Poll-The BT, Duran M. Dynamic changes of plasma acylcarnitine levels induced by fasting and sunflower oil challenge test in children. *Pediatr Res* 1999; 46:440–444.
19. Bonnefont JP, Specola NB, Vassault A, Lombes A, Ogier H, de Klerk JBC et al. The fasting test in paediatrics: application to the diagnosis of pathological. *Eur J Pediatr* 1990; 150:80–85.
20. Seakins JW, Rumsby G. The use of phenylpropionic acid as a loading test for medium-chain acyl-CoA dehydrogenase deficiency. *J Inher Metab Dis* 1988; 11 Suppl 2:221–224.
21. Morris AA, Lascelles CV, Olpin SE, Lake BD, Leonard JV, Quant PA. Hepatic mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase deficiency. *Pediatr Res* 1998; 44:392–396.
22. Brivet M, Slama A, Saudubray JM, Legrand A, Lemonnier A. Rapid diagnosis of long chain and medium chain fatty acid oxidation disorders using lymphocytes. *Ann Clin Biochem* 1995; 32:154–159.
23. Ventura FV, Costa CG, Struys EA, Ruiter J, Allers P, IJlst L et al. Quantitative acylcarnitine profiling in fibroblasts using [U-13C] palmitic acid: an improved tool for the diagnosis of fatty acid oxidation defects. *Clin Chim Acta* 1999; 281:1–17.
24. Costa CG, Dorland L, Holwerda U, Tavares de Almeida I, Poll-The BT, Jakobs C et al. Simultaneous analysis of plasma free fatty acids and their 3-hydroxy analogs in fatty acid beta-oxidation disorders. *Clin Chem* 1998; 44:463–471.
25. Kamijo T, Indo Y, Souri M, Aoyama T, Hara T, Yamamoto S et al. Medium chain 3-ketoacyl-coenzyme A thiolase deficiency: a new disorder of mitochondrial fatty acid beta-oxidation. *Pediatr Res* 1997; 42:569–576.