Chapter 53
Diseases Associated with Defects of Beta-Oxidation

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Mitochondrial β-oxidation of the long-chain fatty acids is a complex and finely tuned biochemical pathway present in most mammalian cells. It is crucial for energy production in organs that perform conspicuous mechanical work, such as skeletal muscle and the heart. It is essential for the synthesis of ketone bodies in the liver. Known genetic dysfunctions of this pathway cause a dozen human diseases that are associated with a wide spectrum of clinical presentations from birth to adulthood, though in most patients the first presentation is in infancy because of autosomal recessive inheritance. Noteworthy inborn errors of fatty acid metabolism cause important alterations in metabolic homeostasis expressed as variable combinations of hypoglycemia, inappropriately low synthesis of ketones, vomiting, drowsiness, and coma, in addition to signs and symptoms of skeletal muscle and heart dysfunction.

MITOCHONDRIAL METABOLISM OF FATTY ACID

Plasma free fatty acids, derived from adipose tissue, with a small contribution from intracellular lipid, are the main lipid fuel for peripheral organs. Once delivered into the cytosol, however, free fatty acids cannot pass across the mitochondrial membrane as such. First, they must undergo a series of enzymatic reactions to enter the mitochondrial matrix, where β-oxidation occurs.

First, long-chain acyl coenzyme A (acyl-CoA) esters are synthesized, from the corresponding long-chain fatty acids and the cytosolic free coenzyme A pool, by the long-chain acyl-CoA synthetase of the outer mitochondrial membrane. Because the inner mitochondrial membrane is impermeable to acyl-CoA esters, the acyl groups are then transferred into mitochondria as acyl-carnitine esters. L-carnitine, two carnitine palmitoyltransferases (CPT), located to the inner aspect of the outer mitochondrial membrane (CPT I) and to the matrix side of the inner mitochondrial membrane (CPT II), and a carnitine-acylcarnitine translocase (CT), embedded in the inner mitochondrial membrane, are required in mammalian tissues to transfer long-chain acyl-CoAs across the inner membrane (Figure 53.1) [1,2]. To generate acetyl CoA, fatty acyl-CoA esters undergo a process characterized by repeated cycles of four concerted reactions involving flavin adenine dinucleotide (FAD) dependent dehydrogenation of acyl-CoAs, hydratation of 2-enoyl CoAs, nicotinamide adenine dinucleotide (NAD) dependent oxidation of 3-hydroxyacyl-CoAs, and coenzyme A (CoA-SH)–dependent thiolysis of 3-ketoacyl-CoAs [3]. The first step of the β-oxidation spiral is catalyzed by FAD-dependent dehydrogenases, the fatty acyl-CoA dehydrogenases present in mammalian mitochondria in four molecular forms: The very-long-chain acyl-CoA dehydrogenase (VLCAD) of the inner mitochondrial membrane; the long-chain, medium-chain, and short-chain acyl-CoA dehydrogenase (LCAD, MCAD, and SCAD) of the mitochondrial matrix VLCAD [4,5]; and the recently discovered trifunctional protein (TP) [6,7] are integral proteins of the inner mitochondrial membrane active toward fatty acid of chain length up to 20–22 carbon atoms. TP is a multifunctional protein that exibits long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activities in addition to enoyl CoA hydratase and 3-oxoacyl-CoA thiolase activities, with substrate specificities that differ from those of the corresponding matrix enzymes. Notably, VLCAD activity with palmitoyl CoA is almost 10 times that of LCAD. VLCAD and TP are thought to carry on all steps of β-oxidation coordinately and to release shortened acyl-CoAs, which are further metabolized by the corresponding acyl-CoA dehydrogenases in the matrix [4–7].

LCAD, MCAD, and SCAD have partially overlapping substrate specificities for fatty acyl-CoAs of C4 to C14 carbon length and coordinately act on acyl-CoA substrates of different chain length, giving rise to reduced flavin adenine dinucleotide ($FADH_2$) and enoyl CoAs. A short-chain 2-enoyl CoA hydratase catalyzes the step leading to 3-hydroxyacyl-CoA, which is then oxidized to ketoacyl-CoA by the short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD). The final step is the conversion of the 3-ketoacyl-CoA esters to acetyl CoA and a fatty acyl-CoA that is two carbon atoms shorter. This step is accomplished by 3-ketoacyl CoA thiolase (3-KT, a potassium-sensitive acetoacetyl CoA thiolase of the matrix distinct from the long-chain thiolase activity, which is the TP protein). Therefore, complete catalysis of long-chain acyl-CoAs to acetyl CoA in mitochondria is accomplished by the action of two coordinated β-oxidation systems, one in the inner membrane, the other in the matrix (see Figure 53.1). Long-chain fatty acids with double bonds require additional enzymes, such as Δ^3–Δ^4-enoyl CoA isomerase and 2,4-dienoyl CoA reductase, for their oxidation to acetyl CoA [8].

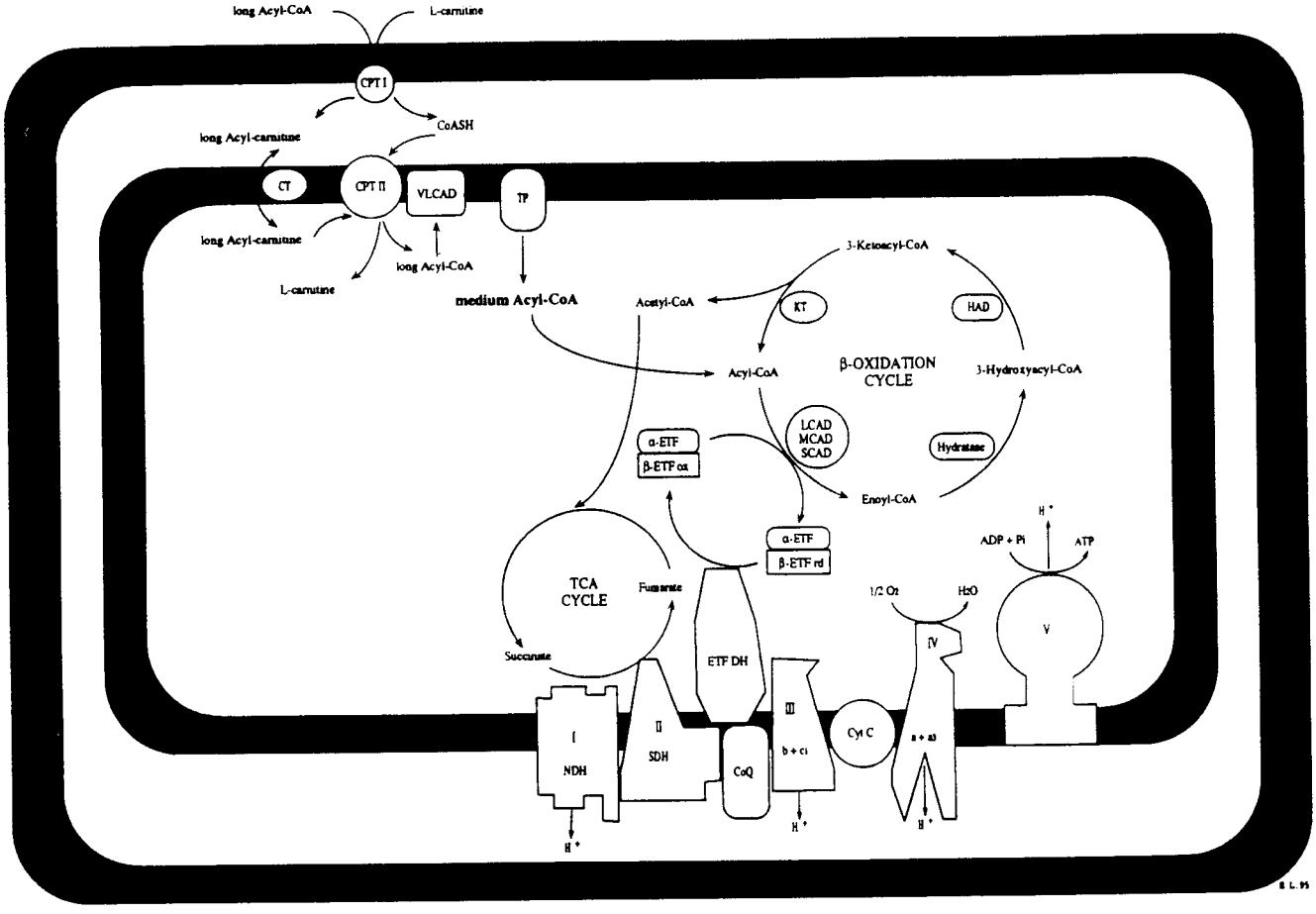

FIGURE 53.1 Schematic representation of mitochondrial fatty acid β-oxidation. (CPT I = carnitine palmitoyltransferase I [outer membrane], CPT II = carnitine palmitoyltransferase II [inner membrane], and CT = carnitine-acylcarnitine translocase [inner membrane] [CPT I, CPT II, and CT represent the fatty acyl-CoAs transport system]; VLCAD = very long chain acyl-CoA dehydrogenase [inner membrane] and TP = mitochondrial trifunctional protein [MTP, inner membrane] [TP is composed of an α-subunit and a β-subunit; VLCAD and TP represent the membrane-associated β-oxidation spiral]; LCAD = long-chain acyl-CoA dehydrogenase, MCAD = medium-chain acyl-CoA dehydrogenase, SCAD = short-chain acyl-CoA dehydrogenase [matrix], HAD = 3-hydroxyacyl-CoA dehydrogenase [matrix], and KT = 3-keto thiolase [matrix] [LCAD, MCAD, SCAD, HAD, and KT represent the matrix β-oxidation spiral]; α-ETF = α-subunit of the electron transfer flavoprotein, β-ETF = β-subunit of the electron transfer flavoprotein, and ETF-DH = electron transfer flavoprotein dehydrogenase [ETF and ETF-DH represent the electron transferring system from reduced flavin coenzymes]; I, II, III, IV, and V = the five complexes of the respiratory chain; NDH = NADH dehydrogenase; SDH = succinate dehydrogenase; CoQ = coenzyme Q; CytC = cytochrome C.)

During fatty acid oxidation the electrons are transferred to the respiratory chain. The electrons of the FAD-dependent acyl-CoA dehydrogenases are transferred from $FADH_2$ to coenzyme Q through two flavoproteins of the mitochondrial inner membrane: the electron-transferring flavoprotein (ETF), and the ETF-coenzyme Q-oxidoreductase (ETF-QO). The NAD-dependent 3-hydroxyacyl-CoA dehydrogenases transfer their electrons from NADH to complex I of the respiratory chain [1,9].

Fatty Acid Oxidation in Skeletal and Heart Muscles

The immediate source of chemical energy for muscle contraction is the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). ATP can be regenerated from ADP and the high-energy compound phosphocreatine, but during long-term exercise the rephosphorylation of ADP to ATP requires the utilization of other fuels, such as carbohydrate, fatty acid, and ketones, which are degraded in muscle mitochondria. The main carbohydrate fuel is intracellular glycogen, derived from blood glucose. The main lipid fuel is plasma free fatty acids [10,11].

The respiratory quotient of resting muscle is close to 0.8, indicating an almost total dependence on the oxidation of fatty acids. Indeed, approximately 70% of the energy requirement of resting muscle is met by the oxidation of long-chain fatty acids. During aerobic exercise, muscle performance relies on increasing supply as well as improved utilization of oxidizable substrates. Breakdown of stored glycogen and triglyceride sustains the initial steps of physical exercise. Within minutes of the onset of exercise, muscle blood flow increases, thus delivering to the muscle fibers larger amounts of both oxygen and nutrients (i.e., glucose

and fatty acids). In fact, in the first hours of exercise, glucose becomes the main fuel used aerobically. If physical effort continues, however, particularly at times of stress or fasting, long-chain fatty acids become the main source of energy for the working muscle [12]. This type of exercise is totally aerobic, and accumulation of lactate and pyruvate in peripheral blood is minimal. Conversely, the accelerated mobilization of lipid substrates results in progressively rising blood levels of free fatty acids and glycerol, whereas the accumulation of circulating β-hydroxybutyrate provides evidence that enhanced oxidation of fatty substrates is also occurring in the liver [12]. Although the duration and intensity of exercise strongly influence muscle energy metabolism, other factors contribute to the relative proportion of glucose and fatty acid in the combustion mixture of mitochondria, such as blood concentration of fatty acids, muscle blood flow, and blood oxygen concentration. As with skeletal muscle, heart is also substantially dependent on fatty acids for its high energy–demanding functional activity [12,13].

Fatty Acid Oxidation in Liver and Metabolic Homeostasis

Liver fatty acid oxidation is regulated by the fed or fasting state because prolonged fasting stimulates the release of fatty acids from adipose tissue, thus increasing delivery to the liver, where increased uptake of fatty acids is accompanied by a stoichiometric increase in the synthesis of ketone bodies. Blood ketones, in turn, are delivered to peripheral tissues, including skeletal muscle, where they provide auxiliary fuel, reducing glucose utilization and providing metabolic homeostasis during fasting [13].

Metabolic homeostasis means maintenance of a constant supply of substrates that can be oxidized to provide energy [14]. The main metabolic fuels of the body are glucose, fatty acids, and ketone bodies, and the main energy stores are liver glycogen, adipose tissue triglycerides, and muscle protein. In metabolic homeostasis, liver occupies a central position. In the fed state, the liver builds up energy stores in the form of glycogen and triglyceride, the latter being exported to adipose tissue. During fasting, it releases glucose and ketone bodies. Blood glucose derives from the degradation of liver glycogen; de novo synthesis of glucose in the liver occurs via gluconeogenesis from gluconeogenic amino acids derived from muscle. Blood ketones are produced from fatty acids (coming to the liver from adipose tissue) and from ketogenic amino acids. The major signals controlling the transition between fed and fasting state are glucose, insulin, and glucagon. In the fed state, the liver utilizes energy coming from glucose oxidation to build up triglycerides; in the fasted state, it utilizes energy generated by fatty acid oxidation to synthesize glucose [14].

The importance of fatty acid β-oxidation and the specific roles of carnitine and of the mitochondrial carnitine-palmitoyltransferases (CPT I and CPT II) in intermediary metabolism have been revealed by the important work of McGarry et al. [2,15]. These authors proposed that carnitine and malonyl CoA (which is a potent inhibitor of CPT I) might exert a reciprocal control on hepatic fatty acid oxidation and biosynthesis in ketotic and normal states. With carbohydrate feeding, when the plasma ratio of glucagon to insulin is low, the malonyl CoA concentration rises, enhancing fatty acid synthesis and suppressing fatty acid oxidation. Conversely, in the fasting state or in patients with uncontrolled diabetes, the plasma glucagon to insulin ratio is high, malonyl CoA levels fall, and carnitine levels increase in liver. In these conditions, fatty acid synthesis is diminished and CPT becomes derepressed , favoring fatty acid oxidation and ketogenesis [2,13,15]. Notably, there is some evidence that regulation of CPT activity might also include covalent phosphorylation of CPT, possibly under insulin control [16,17].

CLINICAL FEATURES

Genetic diseases of fatty acid mitochondrial β-oxidation are autosomal recessive disorders of infancy and childhood, though some patients present later in life. Although these disorders are multisystemic, the presenting symptoms tend to be homogeneous, and in only a few diseases is the diagnosis suggested by the clinical features alone (Table 53.1).

Recurrent episodes of hypoketotic hypoglycemia, with or without concomitant brain involvement, are the most common presentation in the newborn or infant. Nausea and vomiting, hypotonia, drowsiness, and coma are also frequent. Sometimes, attacks are triggered by fasting or minor viral infections. Probably, this is due to the increased fatty acid catabolism needed to maintain homeostasis in some physiopathologic conditions, such as prolonged physical exercise, fasting, or disease. Infants and young children are at greater risk of having problems with fasting adaptation because their basal metabolism is higher than in adults, brain energy needs are elevated and highly dependent on glucose availability, and some of the key enzymes involved in energy production and glucose homeostasis work at rates lower than in adults.

The acute and frequently life-threatening presentation in early infancy requires differential diagnosis from other encephalopathies of infancy, such as hypoxia, space-occupying lesions, and drug or toxin ingestions, because (1) a few defects of β-oxidation can be effectively cured, such as primary carnitine deficiency (CD) and riboflavin-responsive glutaric aciduria type II, and (2) early diagnosis may prevent acute metabolic attacks, mental retardation, epilepsy, severe brain damage, and death [1]. Conditions such as hypoglycemia with the blood glucose concentration less than 2.0 mmol/liter, metabolic acidosis with blood pH lower than 7.30, or hyperammonemia with blood ammonia of 100 mmol/liter or more must alert the physician because acute hypoglycemia with acidosis can cause death. Some infants with these diseases can also die abruptly without any evident preceding symptomatology. It is important to notice that similar features are also part of two relatively

Table 53.1 Clinical features in diseases of mitochondrial β-oxidation

Hepatic signs
Hypoglycemia associated with low ketones (hypoketotic hypoglycemia)
Reye's-like syndrome
Steatosis
Acute hepatic failure
Sudden infant death syndrome
Muscle signs
Hypotonia
Weakness and wasting
Proximal myopathy with lipid storage
Exercise intolerance and muscle pain with increased levels of creatine kinase
Episodic rhabdomyolysis (with occasional paroxystic myoglobinuria)
Cardiac signs
Hypertrophic and dilated cardiomyopathy
Progressive heart failure
Arrhythmias
Cardiac arrest
Sudden infant death syndrome
Nervous system signs
Permanent brain damage due to hypoglycemia, arrhythmias, or cardiac arrest
Microgyria, cortical atrophy, and neuronal heterotopia
Pigmentary retinopathy
Peripheral sensorimotor neuropathy
Malformations
Renal dysplasia and nephromegaly*
Polycystic kidney
Facial dysmorphism
Brain malformations

*Proximal and distal tubulopathy is observed in carnitine palmitoyltransferase I deficiency.

common but pathogenetically ill-defined disorders of infancy: Reye's hepatic encephalopathy and the sudden infant death syndrome (SIDS). Both syndromes, currently viewed as nongenetic disorders, might in fact be related to inherited disorders of fatty acid metabolism because dicarboxylic aciduria has been reported in Reye's syndrome, and has been observed in patients with β-oxidation defects, such as medium-chain acyl-CoA dehydrogenase deficiency [18]. Some infants survive the acute metabolic attacks but show poor growth, impaired psychomotor development, dystonia, spastic tetraplegia, and intractable seizures—the devastating effects of acidosis and acute energy shortage on the developing brain [1]. Nervous system involvement is rare and usually secondary to severe acidotic and hypoglycemic attacks, though patients with trifunctional protein deficiency may have retinitis pigmentosa and peripheral neuropathy. Infants with severe defects of CPT II, ETF, or ETF-QO, however, can present multiple congenital malformations involving the brain (microgyria, neuronal heterotopia) and sometimes facial dysmorphism reminiscent of Zellweger syndrome. In addition to metabolic symptoms, patients often have cardiomyopathy: Primary CD, CT deficiency, CPT II deficiency, VLCAD deficiency, and TP deficiency are all associated with various forms of heart disease.

Patients with onset in late infancy, childhood, or adulthood tend to have more chronic disorders characterized by progressive myopathy or cardiomyopathy, sometimes associated with mild metabolic symptoms, such as nausea and drowsiness, or with altered laboratory tests, such as hypoglycemia or poor rise of blood ketone concentrations in provocative tests. A subgroup of these patients experience exercise intolerance and exercise-induced rhabdomyolysis with myoglobinuria. Usually, early-onset forms are devastating diseases, whereas late-onset forms are more benign disorders. Different degrees of residual enzyme activity often underlie this heterogeneous clinical presentation. CPT II deficiency exemplifies this concept because different mutations in the CPT II gene cause biochemical phenotypes retaining different residual enzyme activities, which are associated with different clinical presentations (see the section on carnitine palmitoyltransferase II deficiency).

Because clinical presentation seldom helps in discriminating these diseases from nongenetic disorders, such as Reye's syndrome, the only way to reach a definitive diagnosis is to analyze body fluids for accumulating metabolites and to study the patient's cells for specific enzymes of fatty acid metabolism. Biochemical and histochemical analyses of muscle biopsies can also help in the differential diagnosis. Molecular genetic analyses of mutations in appropriate genes generally follow tentative biochemical diagnoses; molecular studies are less important for the early diagnosis of acute cases, except in diseases such as medium-chain acyl-CoA dehydrogenase deficiency, in which a common founder mutation underlies most cases [8]. Because most

Table 53.2 Defects of fatty acid transport

	Phenotype			
Enzyme/Cofactor	*Myopathy/ Hypotonia*	*Cardiomyopathy*	*Myoglobinuria*	*Hypoglycemia Hypoketonemia*
Carnitine	+	++	−	++
Carnitine palmitoyltransferase I	±	±	−	++
Carnitine palmitoyltransferase II*	−	++	++	++
Carnitine acylcarnitine translocase	±	+	+	+

++ = present; + = sometimes present; ± = rarely observed; − = absent.
*Neonatal forms with multiple organ malformations.

organic acids accumulating in β-oxidation defects are effectively cleared from the blood by the kidneys, gas chromatography-mass spectrometry (GC-MS) analysis of 24-hour urine specimens usually reveals a pattern of metabolites characteristic of a specific disease and is therefore the test of choice [1,8].

DEFECTS OF LONG-CHAIN FATTY ACID TRANSPORT

Because of the rather stereotypic clinical presentation, accurate metabolic, biochemical, and molecular investigations are necessary to pinpoint the site of the block in inborn errors of fatty acid mitochondrial oxidation. Accordingly, classification of these diseases is based on knowledge of the fundamental biochemical defects (Tables 53.2 and 53.4).

Carnitine Deficiency

L-Carnitine has an essential role in the transport of long-chain fatty acids into mitochondria for β-oxidation. Carnitine is present in tissues and biological fluids in free and esterified forms. In humans, acylcarnitine esters account for about 25% of total carnitine in serum and for approximately 15% of total carnitine in liver and skeletal muscle. Total carnitine concentration in adult human tissues is higher in heart and skeletal muscle than in liver, kidney, or brain. Carnitine in blood is 20–60 times less concentrated than in tissues. Therefore, carnitine must be actively transported from the blood into fatty acid–metabolizing organs [13]. Plasma cell receptors with high affinity for carnitine (Michael's constant, or Km 2 to 6 μM) have been identified in cultured muscle [13–19], heart cells [20], and cultured fibroblasts [19,21], whereas the kidney [22], and probably other organs, have receptors with lower affinity. Roughly 25% of the carnitine in the body is endogenously synthesized from the immediate precursor γ-butyrobetaine, and the remaining 75% comes from the diet [13].

Since the identification in 1973 of a patient with progressive lipid myopathy and CD in muscle [23], more than 100 patients with various forms of CD have been reported. As carnitine determination became widespread, however, it grew apparent that CD was a relatively common finding in patients with diverse inherited and acquired diseases, and many authors started to separate primary CD from secondary CD [13,19,24].

Primary Carnitine Deficiency with Cardiomyopathy

The first description of a disorder characterized by systemic CD, lipid myopathy, and cardiomyopathy has been reported by Morand et al. [25] in a girl that completely recovered from heart failure after carnitine supplementation. Familial forms of the disease were reported later [26–28], but the disease was identified as a carnitine membrane transport defect only in 1988 by Eriksson et al. [21,29] and by Treem and colleagues [30]. The association of cardiomyopathy, hypoglycemic attacks, systemic CD, and defective carnitine transport has now been reported in about 20 patients [31,32].

The disease presents in familial form, suggesting autosomal recessive inheritance.

The presenting symptom is a progressive dilated cardiomyopathy in about half of the patients, whereas the rest have a more acute presentation with hypoglycemia and hypoketonemia; a few patients start with myopathic symptoms [32]. Carnitine content is low in muscle, heart, liver, and plasma. Plasma total and free carnitines are less than 10% of normal, but carnitine esters are not increased. Total carnitine is reduced to 1–2% of the normal mean in skeletal muscle; it is also markedly low when measured in heart [33]. Morphologic features include lipid storage in skeletal muscle, heart, and liver. Lipid accumulation in skeletal muscle is characterized by small and numerous lipid droplets in type I muscle fibers, whereas large perimitochondrial vacuoles described in the first cases of muscle CD and chronic myopathy are not seen [1,32]. GC-MS analysis of urine does not reveal dicarboxylic aciduria, a useful diagnostic clue because most other defects of β-oxidation are associated with organic aciduria [13].

Patients respond well to carnitine supplementation (usually 2–6 g/day of oral L-carnitine). Heart function progressively returns to normal, hence cardiokinetic therapy can be discontinued [31,32]. Some young patients were enlisted for heart transplant before the correct diagnosis of CD was made [33]. Also, patients gradually recover muscle strength, and attacks of hypoglycemia tend to disappear. Excellent therapeutic results with carnitine supplementation have been reported in American [32], European [33], and Japanese families [34].

Table 53.3 Defects of enzymes of the β-oxidation spiral

	Phenotype			
Enzyme/Cofactor	*Myopathy/ Hypotonia*	*Cardiomyopathy*	*Myoglobinuria*	*Hypoglycemia Hypoketonemia*
Very long-chain acyl-CoA dehydrogenase	+	++	++	+
Long-chain 3-hydroxyacyl-CoA dehydrogenase; trifunctional protein[a]	++	++	+	++
Long-chain acyl-CoA dehydrogenase[b]	+	++	−	++
Medium-chain acyl-CoA dehydrogenase	±	±	±	++
Short-chain acyl-CoA dehydrogenase	+	±	−	+
Short-chain 3-hydroxyacyl-CoA dehydrogenase	+	++	++	++
2-4 dienoyl CoA reductase	+	−	−	−

++ = present; + = sometimes present; ± = rarely observed; − = absent.
[a]Peripheral neuropathy and pigmentary retinopathy is present in a subgroup of patients.
[b]The very existence of this disease has not yet been proved.

Pathogenesis and Etiology

Primary CD is due to a defect of carnitine transport in tissues. The defect in carnitine transport, as measured by carnitine concentrations in target organs, involves primarily the heart and skeletal muscles. Defective intestinal uptake and impaired renal readsorption of carnitine were documented in a few patients [27,28], suggesting that carnitine depletion in tissues might be due to reduced blood availability of carnitine. It has been suggested that the depletion of tissue carnitine stores has a dual pathogenesis, involving both impaired renal handling of carnitine and reduced carnitine uptake in tissues [32]. Defective carnitine transport across the cell membrane has since been demonstrated in cultured fibroblasts [29–33]. In patients' cells carnitine uptake is negligible, whereas in obligate heterozygotes the Km allotype for carnitine transport is normal (3.24 ± 0.57 SD mM for L-carnitine), but the maximal rate, or V_{max}, is 50% of control values [29–33], suggesting autosomal recessive inheritance. These data suggest that patients homozygous for the putative mutation lack functionally active plasma-membrane high-affinity carnitine receptors, whereas heterozygotes retain approximately 50% of the receptor pool [33].

Additional investigations aimed at isolating the receptor protein or proteins in different tissues and at cloning the corresponding gene or genes are required to understand the pathogenesis of this disease.

The Mitochondrial Carnitine Palmitoyltransferase System

There are two different CPTs in mitochondria. CPT I is loosely bound to the inner aspect of the outer membrane [35,36]. According to McGarry and colleagues [2,36,37], this enzyme has regulatory properties, and there are isoforms of different molecular weight in liver mitochondria (88 kd) and in skeletal muscle mitochondria (82 kd) [36–39]. CPT II, a different protein from CPT I [2,40,41], is tightly bound to the inner mitochondrial membrane. This enzyme has housekeeping functions, and there is no evidence of tissue-specific isoenzymes [39–41]. There was, however, some doubt whether the two CPT activities represented two distinct enzymes or a single enzyme in two locations, and it was suggested that CPT I could be composed of two subunits: a malonyl CoA–binding protein lacking CPT activity and a catalytic protein with CPT activity [42–44]. Recent data have provided conclusive evidence for the existence in rat liver mitochondria of CPT I as a distinct protein from CPT II. This protein contains both regulatory properties and CPT activity [36,38]. Furthermore, following the molecular cloning of a complementary DNA (cDNA) encoding the entire sequence of rat liver CPT II [40], a distinct cDNA encoding rat liver CPT I has also been cloned [37]. Finally, the human counterparts of rat CPT I and CPT II cDNAs have been recently obtained [45,46], and the corresponding genes have been mapped to human chromosome 11q [45] and 1p32 [47], proving conclusively that CPT I and CPT II are two separate proteins encoded by two different genes.

Human Defects of Carnitine Palmitoyltransferases

CPT deficiency has been formerly described to occur with two distinct clinical and biochemical phenotypes: an adult-onset muscular form due to CPT II deficiency and an infantile hepatic form due to CPT I deficiency [48]. More recently, it became clear that the spectrum of CPT II defi-

Table 53.4 Defects of the electron-transferring flavoproteins

	Phenotype			
Enzyme/Cofactor	*Myopathy/ Hypotonia*	*Cardiomyopathy*	*Myoglobinuria*	*Hypoglycemia Hypoketonemia*
ETF*	±	±	−	++
ETF coenzyme Q reductase*	±	−	−	++
Riboflavin-sensitive forms	++	−	−	++

++ = present; + = sometimes present; ± = rarely observed; − = absent; ETF = electron-transferring flavoprotein.
*Glutaric aciduria type II causes congenital malformations in patients with neonatal severe forms. Also, a few patients with progressive dystonia-dyskinesia have been reported.

ciency is wider and includes a hepatomuscular infantile disease [49], and a neonatal lethal disease with disorganogenesis [50]. Both clinical presentations and molecular aspects of CPT deficiencies have been recently reviewed [51].

Carnitine Palmitoyltransferase I Deficiency

Eleven infants with recurrent attacks of fasting-induced life-threatening hypoketotic hypoglycemia and CPT I deficiency have been reported [48,52–55]. Hypoglycemic attacks may be associated with lethargy, coma, and seizures; they may cause death or result in psychomotor developmental delay, hemiplegia, or generalized epilepsy. Hepatomegaly and liver steatosis are typical findings [53]. One patient had nephromegaly [54], and two other patients had proximal and distal tubulopathy that led to tubular acidosis [52,55].

Notably, plasma carnitine levels tend to be increased rather than low, as in most defects of β-oxidation; also, patients do not excrete high amounts of dicarboxylic acids in the urine [56]. CPT activity was barely detectable in liver specimens from these infants: In cultured fibroblasts, the malonyl-sensitive and detergent-labile CPT activity was decreased, but the malonyl-insensitive CPT activity was normal, pointing to CPT I deficiency [48]. Biochemical and immunologic studies in skeletal muscle, however, showed normal activity of both CPT I and CPT II enzymes, as well as a normal amount of CPT II antigen [57], suggesting that CPT I is present in human skeletal muscle as a different isoform from the liver isoenzyme [39]. It has recently been shown that human cultured fibroblasts express the liver but not the muscular isoform of CPT I [45].

Carnitine Palmitoyltransferase II Deficiency

The clinical spectrum of CPT II deficiency varies from a neonatal form with hypoglycemic attacks and organ malformations to infantile and late-infantile forms with hypoketotic hypoglycemia and cardiomyopathy to juvenile forms with paroxysmal myoglobinuria [51].

Carnitine Palmitoyltransferase II Deficiency: Lethal Neonatal–Early Infantile Phenotype. A rare severe infantile form of CPT II deficiency with neonatal manifestation has been reported in a handful of patients [51,58–60]. These neonates present at birth with severe hypoketotic hypoglycemia and generalized steatosis, and they usually die within a few days. An important feature of these infants is that they, like babies with glutaric aciduria type II (GA II), often show multiple organ malformations, including renal cystic dysplasia, nephromegaly, microgyria, subarachnoid hemorrhages, neuronal heterotopia in the brain, and facial dysmorphism. CPT II enzyme activity in patients' cells was either barely detectable or reduced to less than 10% of normal. Prenatal diagnosis was accomplished in one family with this disease [60].

Carnitine Palmitoyltransferase II Deficiency: Infantile Hepatomuscular Phenotype. As described for CPT I deficiency, acute episodes of fasting hypoglycemia with inappropriate levels of blood ketones, leading to lethargy, coma, and death, are also a feature of CPT II deficiency [49]. Seven infants with this disease suffering from severe hypoketotic hypoglycemia, lethargy, seizures, hepatomegaly, liver failure, cardiomegaly, and arrhythmias have been reported [51,61–63]. Recurrent pancreatitis has been described in one case [64]. Arrhythmias, rather uncommon in β-oxidation diseases, are frequent in these babies and have been ascribed to the accumulation of long-chain acylcarnitines [65]. Both acute hypoglycemia and prolonged arrhythmias can result in permanent brain damage. Biochemically, these patients have normal CPT I activity in cultured fibroblasts, but CPT II activity is reduced to less than 10% of normal. Cells from these infants lack CPT II protein, as in patients with the adult muscular phenotype [49].

Carnitine Palmitoyltransferase II Deficiency: Adult Muscular Presentation. Since the first description in 1973 by DiMauro and Melis DiMauro [66] of a young adult with exercise-induced myoglobinuria due to CPT deficiency in muscle, more than 100 patients complaining of muscle pain, rhabdomyolysis, and myoglobinuria after prolonged exercise have been reported worldwide [52,67,68]. The disease is inherited in an autosomal recessive fashion but is most frequently seen in young adult males, probably because environmental and hormonal factors influence the expression of the defect. Pseudodominant inheritance has been reported in some families [69]. In typical patients, the attacks are triggered by prolonged exercise in fasting conditions and consist of pain, stiffness, and discomfort of skeletal muscles, without clinical and neurophysiologic evidence of cramps. Sometimes, these metabolic attacks end in massive rhabdomyolysis

with myoglobinuria, evidenced by dark (cola color) urine, and often resulting in acute renal failure. In a few cases, characterized by the involvement of the chest muscles, respiratory failure may ensue [70]. Although the disease is typical of young adults, some infants with CPT II deficiency have rhabdomyolytic attacks associated with recurrent myalgias; these attacks are induced by fever rather than prolonged exercise [71]. In fact, cold, fever, or other stress conditions may also induce metabolic crises in adult patients [69]. Accordingly, it has been proposed to distinguish an exertional form, or type I, from a toxic form, or type II, of myoglobinuria due to CPT II deficiency [72].

Typically, serum creatine kinase (CK) levels in patients tend to increase enormously during attacks and may peak up to 100,000 mU/liter, reflecting massive muscle necrosis. Between acute episodes, however, the serum CK concentration is either normal or only slightly elevated, up to 100 mU/liter. Accordingly, the morphology of interictal muscle biopsy is normal, but transient fiber necrosis and lipid storage can be seen after acute episodes [69–72]. Lipid storage in myocardium has been reported in a few patients with congestive cardiomyopathy [13,51].

CPT activity in patients' muscle ranges from undetectable to 30% of normal. Although the enzyme defect could be demonstrated in several tissues, such as leukocytes and fibroblasts [73], the clinical expression of the disease is generally limited to muscle. A few patients, however, exibit a mild deficiency of CPT activity in the liver, as deduced by impaired ketogenesis on fasting or after lipid loading tests [74].

Biochemistry and Molecular Genetic Studies in Carnitine Palmitoyltransferase Deficiency

CPT II is a homotetrameric enzyme of 68-kd subunits [41]. A 2.2-kb cDNA encoding the full-length CPT II subunit of 658 amino acids was cloned [46]. The corresponding gene *(CPT1)* has been mapped to human chromosome 1p32 [47]. More recently, the genomic organization of the gene has been elucidated. *CPT1* spans approximately 20 kb of genomic DNA and is composed of 5 exons ranging from 81 to 1,305 bp [75]. Further studies of the CPT II promoter region suggest that the gene expression might be hormonally regulated [76]. CPT I is expressed in at least two different isoforms: an 88-kd liver isoenzyme present also in fibroblasts, and an 82-kd muscular isoenzyme, probably expressed also in the heart [45].

The first mutation in the CPT II gene was described in an infant with hypoketotic hypoglycemia and cardiomyopathy (hepatomuscular form) [77]: It consisted of a C-to-T transition at nucleotide 1992, causing an arginine-to-cysteine substitution at codon 631 (R631C). A different missense mutation in the carboxy-terminal of CPT II was reported later in another patient with the same phenotype [51]. In patients with the adult muscular presentation, the prevalent mutation is a C-to-T transition at nucleotide 439, changing a highly conserved serine into leucine (S113L) [78]. Subsequently, other mutations were shown to segregate with the adult muscular form of CPT II deficiency [52], including a C-to-A transversion at nucleotide 665 in exon 1, resulting in a proline to histidine substitution at residue 50 of the protein (P50H) [75]. The latter mutation is interesting because the amino acid substitution occurs within a leucine-proline motif that is highly conserved among acyltransferases of different species [46]. Kinetic characterization of this mutation showed that substrate binding sites were not affected, however, whereas V_{max} was dramatically reduced. Accordingly, transfection experiments in COS cells of R631L, S113L, and P50H mutations showed that all these mutations drastically decrease the catalytic activity of CPT II, both V_{max} and the steady-state level of the protein [51]. In three families with the lethal infantile form of CPT II deficiency, different mutations were found: one frameshift mutation, a deletion, and two missense mutations. All these mutations were associated with negligible residual enzyme protein and activity [79].

Carnitine-Acylcarnitine Translocase

CT deficiency has been suspected in patients with defective fatty acid oxidation and high plasma levels of long-chain acylcarnitine [80]. Stanley and coworkers [81] reported a young boy who had since birth stunted growth, recurrent vomiting, and coma. At age 2½ years, he also had muscle weakness, cardiomyopathy, and fasting hypoglycemia. Urinary excretion of medium-chain dicarboxylic acids was high, but ketones were inappropriately low. Plasma free carnitine was very low. By contrast, the plasma levels of long-chain acylcarnitines were markedly increased. Oleate oxidation in the patient's cultured fibroblasts was low; CPT activity was normal. CT activity in fibroblasts was less than 5% of controls, whereas intermediate values were found in cells from the patient's parents, suggesting heterozygosity [81].

A second patient with CT deficiency was a neonate who died 8 days after birth with severe hypoketotic hypoglycemia, hypothermia, hyperammonemia, and a high plasma CK concentration [82]. Notably, he suffered from a cardiomyopathy characterized by heartbeat abnormalities, and his carnitine ester fraction was unusually high in plasma, a feature consistent with the putative cardiotoxicity of long-chain acylcarnitines [65]. Total lack of CT activity was found in the patient's fibroblasts [82]. Since the first report [81], a handful of infants with extreme neonatal hypoglycemia, cardiorespiratory insufficiency, and CT deficiency have been reported [83,84].

DEFECTS OF ENZYMES OF THE β-OXIDATION SPIRAL

Biochemical, molecular, and genetic data on mitochondrial fatty acid oxidation, including the recent discovery of the

inner membrane VLCAD and TP proteins, suggest that the mitochondrial β-oxidation pathway is organized into two distinct but interconnected systems: the inner membrane β-oxidation spiral active on long-chain acyl-CoAs, and the matrix β-oxidation spiral active on medium- and short-chain acyl-CoAs. The classification reported below is based on these recent findings.

Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

The first description of this disease regards a neonate who presented at age 2 days with ventricular fibrillation and respiratory arrest. Notably, her only sibling had died suddenly at 2 days of age; massive hepatic steatosis was found at autopsy. The proband, however, was resuscitated and underwent biochemical analysis, which showed metabolic acidosis, massive dicarboxylic aciduria, and increased serum CK levels [85]. After recovering from the acute episode, she was put on a low-lipid diet and carnitine supplementation. At age 2 years, she is a perfectly normal child. Biochemical analysis of her cultured fibroblasts documented a marked deficiency of the membrane-bound LCAD activity, whereas soluble activity was within the range of controls; the oxidation of palmitate was defective. These data suggested a new defect of mitochondrial oxidation involving VLCAD.

Independent studies by Aoyama et al. [86] in patients with undefined defects of fatty acid oxidation identified two infants with very low palmitoyl-CoA dehydrogenase activity. The two infants, ages 4 and 5 months, had hypoketotic hypoglycemia, hepatocellular disease, cardiomyopathy, and very high plasma levels of long-chain acylcarnitines. Biochemical investigations with antibodies raised against purified VLCAD showed absence of protein immunoreactivity in cultured fibroblasts, pointing to VLCAD deficiency [86]. Further evidence for VLCAD deficiency came from studies in three other patients previously diagnosed as having LCAD deficiency on the basis of activity assays. Analysis of the LCAD cDNA revealed perfectly normal sequence in the patients, whereas immunoblot analysis for VLCAD showed absence of immunoreactive protein, suggesting VLCAD deficiency [87].

Biochemistry and Molecular Genetics

The cDNA encoding human VLCAD has been recently cloned: It encompasses 2,177 bp, which encode the entire 655-amino acid sequence of VLCAD, including a 40-amino acid-long leader peptide [88]. Genetic analysis in two unrelated patients previously reported to have absence of VLCAD cross-reacting material (CRM) in cultured fibroblasts [86,87], demonstrated that both patients carried a 105-bp deletion encompassing bases 1,078–1,182 of the wild-type cDNA. Molecular genetic data suggested homozygosity for the deletion in the two infants [88].

These findings, besides proving that VLCAD deficiency is a cause of hypoglycemia and cardiomyopathy in infancy, also suggested an important role for this membrane-bound enzyme in human mitochondrial oxidation of fatty acid, in agreement with data in rats showing that VLCAD was a rate-limiting enzyme in long-chain fatty acid β-oxidation [89]. Further functional studies in mutant human cultured cells proved that fibroblasts from patients with VLCAD deficiency accumulate fatty acyl intermediates 14 carbon atoms in length after palmitate feeding. This suggested a crucial role of VLCAD and TP in the first catalytic steps of long-chain fatty acid oxidation [90–92].

Trifunctional Protein Deficiency

Since the first report of an infant with sudden death and massive urinary excretion of long-chain 3-hydroxydicarboxylic acids [93], LCHAD deficiency has been identified as a disorder of fatty acid mitochondrial β-oxidation in about 40 patients [94–96]. Clinical features in these patients include Reye's-like episodes; hypoketotic hypoglycemia and hepatic dysfunction; progressive myopathy, recurrent myoglobinuria, or both; cardiomyopathy; and SIDS. A few patients presented peripheral sensorimotor neuropathy, sometimes associated with pigmentary retinopathy [97]. Cardiomyopathy was characterized by cardiac dilatation and fibrosis.

After the discovery that LCHAD was part of a multienzyme complex also harboring enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activity, clinical and biochemical studies made clear that most patients with LCHAD deficiency suffered in fact from TP deficiency. The vast majority of patients have isolated LCHAD deficiency, whereas the mitochondrial TP protein is present in immunoblot analysis, albeit at a reduced level [98]. A small group of patients, however, shows a combined defect of the three enzymes, and the mitochondrial TP protein is completely missing in immunoblots [99,100].

The first clear documentation of combined enzyme defect was reported in a young girl who suffered from a progressively severe and eventually fatal myopathy [100]. The myopathy occurred after a brief prodromal illness characterized by nausea and anorexia. She was the youngest of three children born to healthy nonconsanguineous parents. The first sibling died at 2.5 years of age after a history of episodic vomiting, hypotonia, and lethargy, culminating in rapid physical deterioration and profound muscle weakness. The patient herself had normal physical and intellectual development except for two episodes of anorexia, hypotonia, and weakness after minor infections at age 3 years. At age 4.5 years, after a few days of vomiting and anorexia, she was admitted to the hospital because of rapidly progressive muscle weakness. Her blood glucose concentration was normal, but her lactate concentration was moderately increased; the serum CK concentration was

disproportionately increased to 33,000 U/liter. Urinalysis revealed only moderate amounts of ethylmalonic, adipic, and suberic acids. She died a few days after admission. Autopsy showed fatty liver and moderate lipid storage in muscle. The activity of LCHAD was severely decreased in her muscle, heart, liver, and fibroblasts; also, long-chain 2-enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase were markedly low in muscle, liver, and heart, pointing to a combined defect of these enzymes. A second patient with this disease, a 1.5-year-old girl born of nonconsanguineous parents, had experienced in her first months several episodes of vomiting, drowsiness, and hypotonia after minor infections [99]. Notably, her eldest sibling died suddenly at age 7 months after a few days of anorexia. The proband had hypoglycemia, weakness, and cardiomegaly. Concentrations of ketones were inappropriately low. After a diagnosis of long-chain 3-hydroxy acyl-CoA dehygrogenase (HAD) deficiency was reached, she received a diet supplemented with medium-chain triglycerides. The patient, however, died suddenly at home. Biochemical studies in fibroblast homogenates and muscle mitochondria showed a combined deficiency of long-chain HAD, long-chain enoyl-CoA hydratase, and long-chain 3-oxoacyl-CoA thiolase. Studies in cultured cells showed accumulation of abnormal intermediates of β-oxidation, including acylcarnitine ester derivatives from 3-hydroxyacyl-CoA, 2-enoyl CoA, and 3-oxoacyl CoA.

Biochemistry and Molecular Genetics

TP is a hetero-octamer that consists of four moles of a larger α-subunit of approximately 80 kd and four moles of a smaller β-subunit of 50 kd. The α-subunit is assumed to possess enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, whereas the β-subunit has 3-ketoacyl CoA activity [101].

Biochemical and immunologic studies in one infant with hypoglycemic attacks and cardiomyopathy, and in a second infant with hypoketotic hypoglycemia and 3-hydroxydicarboxylic aciduria, showed heterogeneity of the enzyme defect in the two patients. All three enzyme activities associated with TP were markedly reduced in the first patient, and the content of TP protein was reduced by more than 90% in immunoblots. By contrast, in the second patient, enzyme deficiency was restricted to LCHAD, with minor impairment of the other two activities, and the amount of TP in pulse-chase experiments was roughly 60% of normal [98].

After the cDNAs encoding both subunits of TP were cloned [101], molecular genetic studies identified the first mutation in a patient with enzyme deficiency restricted to LCHAD: A single nucleotide substitution at position 1528 of the cDNA of the α-subunit of TP changed a highly conserved glutamate to a glutamine [101]. Further studies showed that this mutation is very frequent among LCHAD-deficient patients [94]. More recently, full deletion of exon 3 in the α-subunit messenger RNA (mRNA), causing premature termination of the protein, was found in another infant, who died at age 18 months after a history of hypotonia, lethargy, hypoglycemia, and dilated cardiomyopathy [102]. Sequencing of genomic DNA from this patient showed that he was a compound heterozygote with two different mutations in the 5-donor splice site following exon 3: a G-to-A transversion at the invariant position +1 in one allele, and an A-to-G transversion at position +3 in the other allele. Molecular studies in two additional patients studied by Hashimoto and colleagues [101] identified missense mutations in the gene for the β-subunit. Notably, studies of the biosynthesis and processing of TP subunits and of heteromeric complex formation in the three patients showed that both the deletion in the α-subunit and the missense mutations in the β-subunit alter the stability of the heteromeric complex and result in the rapid degradation of both subunits [102].

Long-Chain Acyl-CoA Dehydrogenase, Medium-Chain Acyl-CoA Dehydrogenase, and Short-Chain Acyl-CoA Dehydrogenase

LCAD is active on fatty acyl-CoA substrates of 8–16 carbon atoms, MCAD acts on fatty acids of 4–14 carbon atoms, and SCAD acts on those with 4–6 carbon atoms. Hence, the three enzymes have overlapping chain-length specificities and coordinately act on the acyl-CoAs previously shortened by the combined action of VLCAD and TP. They have been purified to homogeneity from human liver [103]. They are homotetramers with molecular weights of 160–180 kd: The molecular masses of the mature subunits for LCAD, MCAD, and SCAD are 45, 45, and 41 kd, respectively. Each subunit is synthesized in the cytosol as a precursor protein holding a 24–30 amino acid leader peptide. The precursor is imported into the mitochondrial matrix, and the leader is proteolytically cleaved to yield the mature enzyme subunit. The mature subunit is then assembled as a tetramer, and the attachment of FAD occurs after mitochondrial import, with 1 molecule of FAD per subunit [104].

Long-Chain Acyl-CoA Dehydrogenase Deficiency

Hale et al. [105] first reported three unrelated children with nonketotic hypoglycemia and LCAD deficiency. All patients were infants with failure to thrive, hepatomegaly, cardiomegaly, hypotonia, and metabolic crises characterized by hypoglycemia and low ketones. GC-MS analysis of the patients' urine showed marked C_6–C_{16} dicarboxylic aciduria. Cardiac involvement was prominent, and cardiorespiratory arrest might ensue during hypoglycemic attacks. Additional patients with LCAD deficiency were described later [106].

The cDNA encoding full-length LCAD has been cloned and characterized: It encodes a 430-amino acid polypep-

tide, including a leader peptide of 30 amino acids. The corresponding gene is localized on human chromosome 2, bands q34–q35 [107]. Analysis of LCAD-deficient patients did not disclose mutations in the corresponding gene. By contrast, after the discovery of the VLCAD, Yamaguchi et al. [87] proved that three patients with a previous diagnosis of LCAD had, in fact, VLCAD deficiency. Currently, there is general agreement that most, if not all, patients with LCAD deficiency suffer from VLCAD deficiency.

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

MCAD deficiency is, together with CPT deficiency, the most frequent disease of β-oxidation. Since the first report of Kolvraa et al. in 1982 [108], more than 200 patients have been identified worldwide, with a striking prevalence of whites of Anglo-Saxon origin [106]. Typical symptoms include fasting intolerance, nausea, vomiting, hypoketotic hypoglycemia, lethargy, and coma beginning within the first 2 years of life [109,110]. Clinical manifestations, however, are variable, and some patients may be asymptomatic: They are identified through screening of relatives of clinically affected subjects. On the other hand, some patients have died suddenly, suggesting SIDS [106]. Patients have dicarboxylic aciduria. Adipic, suberic, and sebacic acids are detected in their urines, but C_{12}–C_{14} dicarboxylic acids, the hallmarks of LCAD-VLCAD deficiency, are absent. Carnitine and β-hydroxybutyrate tend to be inappropriately low in plasma [106].

Early diagnosis and treatment of MCAD deficiency can result in good long-term outlook. Noninvasive diagnosis is greatly facilitated by fast-atom-bombardment (FAB)-MS analysis showing in MCAD patients increased urinary excretion of hexanoyl glycine, suberyl glycine, and phenylpropionyl glycine, in addition to the most common dicarboxylic acids [111]. Other FAB-MS studies indicate, however, that increased urinary octanoylcarnitine is probably the most useful diagnostic marker of MCAD, because it is detectable in high amounts in blood even between metabolic attacks [112]. Avoidance of fasting and maintenance of adequate caloric intake may prevent life-threatening metabolic attacks.

Deficiency of MCAD has been documented in most tissues, including cultured fibroblasts, lymphocytes, and liver [106]. The residual enzyme activity is 2–10% of normal. Cultured cells from patients do not oxidize properly medium-chain fatty acid, whereas long- and short-chain substrates are oxidized normally.

Molecular Genetics. The gene for MCAD is located on human chromosome 1p31; it is organized in 12 exons and spans 44 kb of genomic DNA [113,114]. Preliminary studies by immunoprecipitation assays in cultured fibroblasts from 13 patients with MCAD deficiency showed that the size of the defective MCAD protein was indistinguishable from the normal human enzyme, suggesting point mutations at the MCAD locus [115]. Other immunologic and cellular mRNA studies led to different conclusions. Cells from different patients showed either reduced synthesis of a normal-sized MCAD precursor or synthesis of an MCAD enzyme of abnormal size, suggesting that both point mutations and aberrant splicing or premature termination of transcription products might underlie such pathologies [116]. Subsequent molecular genetic analysis proved, however, that most MCAD patients carry a point mutation at nucleotide 985 of the coding region [117,118].This mutation is an A-to-G transition, which changes a highly conserved lysine at position 304 of the mature MCAD subunit into a glutamate. The mutation causes impairment of tetramer assembly and instability of the protein [119]. Most MCAD patients were shown to inherit this mutation in the homozygous configuration, suggesting a high frequency of the G985 mutation in the general population [120]. In a worldwide study of 172 unrelated patients, each representing an independent pedigree, a total of 8 different mutations have been identified [120,121], but the 985 A-to-G transition was found in 90% of the 344 variant alleles.

Because of the 985 A-to-G mutation an Nco I restriction site can be introduced in the mutant copies, facilitating the identification of the mutated allele or alleles through polymerase chain reaction. A retrospective study of the incidence of MCAD deficiency in infants who died of SIDS could be performed in postmortem fixed tissue. Seven families, in which a total of nine infants had died suddenly from unexplained causes, were studied. The diagnosis of MCAD deficiency had been established in subsequent live siblings on the basis of urine analysis or enzyme assay in fibroblasts. In all postmortem samples from deceased infants, mutational analysis revealed homozygous A-to-G transition at nucleotide 985, whereas parents were heterozygous for the same mutation [122].

A single patient had a mutation changing a highly conserved arginine into a cysteine at position 28 (R28C). This mutation may lead to the formation of an enzymatically active protein [123].

Short-Chain Acyl-CoA Dehydrogenase Deficiency

This disease has been documented only in a few patients. The first patient was an adult with progressive myopathy and massive lipid storage in type I muscle fibers [124]. Two unrelated infants had failure to thrive and nonketotic hypoglycemia and died at 20 and 30 months, respectively [125]. Still another infant had failure to thrive, vomiting, and severe lipid storage myopathy, with decreased carnitine levels in skeletal muscle [126]. All SCAD-deficient patients showed increased urinary excretion of ethylmalonic and methylsuccinic acids and, in some instances, of butyryl glycine. Although myopathy may be a prominent sign of SCAD deficiency, lipid accumulation in muscle is minimal and restricted to scattered type I muscle fibers. Carnitine content is reduced in skeletal muscle. Deficiency of SCAD

activity was found in cultured fibroblasts in all patients except a woman described by Turnbull [124] who had lipid storage myopathy and normal enzyme activity in fibroblasts, suggesting that she could suffer from SCAD deficiency restricted to skeletal muscle. It is still controversial, however, whether a muscle-specific SCAD isoenzyme exists [1].

Molecular Genetics. Early studies with anti-SCAD antibodies in three unrelated infants with SCAD deficiency provided evidence for molecular heterogeneity of the enzyme defect. The enzyme was labile in fibroblasts from one patient, but it had normal stability in cell lines from the other two patients [127]. More information on the molecular pathology of SCAD deficiency came after the identification of a cDNA encoding the precursor of human SCAD. The 1,852 bases of the full-length clone encode a 412–amino acid–long protein, including the 24 amino acids of the leader peptide [128]. The SCAD clone was utilized for studies in human SCAD-deficient cell lines. In Northern blot analysis, no differences were observed in the size of transcripts between normal and SCAD-deficient cell lines. Moreover, Southern blots showed no difference in the restriction pattern after digestion of genomic DNA with four different endonucleases. These data suggested that SCAD deficiency might be due to point mutations. Accordingly, in a single SCAD patient, DNA sequencing showed two different mutations in the two alleles: A C-to-T transition at codon 136 caused an arginine-to-tryptophan (R22W) substitution, and a C-to-T transition at codon 319 caused an arginine-to-cysteine substitution [129]. Notably, the R22W substitution affects the same conserved arginine that was mutated in a single patient with MCAD deficiency (an R28C mutation), supporting the hypothesis that the carboxy-terminal conserved arginine is crucial for the activity of straight-chain acyl-CoA dehydrogenases [123].

Short-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

Recurrent episodes of hypoglycemia, Reye's-like encephalopathy, and deficiency of mitochondrial SCHAD were first demonstrated in a 9-month-old child [130]. The patient's mildly retarded only brother had experienced episodes of irritability and ataxia and died at 8 years of age in status epilepticus from encephalopathy. At age 16 years, the proband had a dramatic attack of hypoketotic hypoglycemia, acute myoglobinuria, and encephalopathy: She was admitted to an intensive care unit; however, arrhythmia and cardiac failure ensued, and she died of a dilated cardiomyopathy. Her serum free carnitine concentration was low, but concentrations of carnitine esters were increased. Urinary organic acids included small amounts of adipic, suberic, sebacic, and 3-hydroxydodecanoic acids. SCHAD activity was markedly reduced in skeletal muscle, whereas other enzymes of β-oxidation, including LCHAD, were in the normal range. Notably, SCHAD activity was reduced in the patient's muscle, but it was normal in her cultured fibroblasts, a finding reminiscent of the myopathic form of SCAD deficiency described by Turnbull and coworkers [124].

β-Ketothiolase Deficiency

β-Ketothiolase deficiency is a rare inherited metabolic disorder characterized by ketoacidotic attacks, with headache, vomiting, and coma. Ketoacidosis is associated with normal or elevated blood glucose levels (ketotic hyperglycemia). The most frequent clinical presentation is in late childhood and is characterized by acidosis and protein intolerance. The course of the disease is relentlessly downhill, and patients die of ketoacidotic coma; severe psychomotor retardation may ensue in surviving patients. On a restricted protein diet, however, one patient had favorable evolution of the disease [131]. Muscle pathology was never reported in this disease.

The laboratory hallmark of the disease is urinary excretion of high amounts of 2-methyl-3-hydroxybutyric and 2-methylacetoacetic acids and tiglyl glycine as a consequence of the block of isoleucine catabolism. Four different thiolase activities are known in mammalian cells: cytosolic and mitochondrial acetoacetyl-CoA thiolases, and mitochondrial and peroxisomal long-chain 3-ketoacyl-CoA thiolases (the mitochondrial long-chain activity is part of the TP). The human disease is due to deficiency of the mitochondrial potassium-dependent acetoacetyl-CoA thiolase or (short-chain) 3-ketothiolase (3-KT), which plays a major role in isoleucine and ketone body metabolism. Its etiologic role was first proved by the demonstration of a specific defect in the biosynthesis of this enzyme in cultured fibroblasts of one patient [132].

The cDNA encoding mitochondrial 3-KT has been cloned, and Fukas and coworkers have described several mutations in patients from six different families. Most of these mutations are at the 5' and 3' splice sites, causing exon skipping [133].

2,4 Dienoyl-CoA Reductase Deficiency

One 4-month-old infant with 2,4 dienoyl-CoA reductase deficiency in muscle and liver has been reported. The clinical history was characterized by infantile hypotonia and sudden death at age 4 months. Plasma carnitine level was low [8].

MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY

Multiple acyl-CoA dehydrogenase deficiency (MAD), or GA II, is a genetic disorder first described by Przyrembel et al. [134] and characterized clinically by metabolic acidosis, hy-

poketotic hypoglycemia, strong sweaty-feet odor, and early death. Pathologically, there is fatty degeneration of multiple organs, including liver, kidney, heart, and skeletal muscle [135]. Biochemically, the disease is characterized by impaired activity of various acyl-CoA dehydrogenases; its laboratory hallmark is the urinary excretion of massive amounts of numerous organic acids that derive from different acyl-CoA substrates. On biochemical and genetic grounds, MAD is heterogeneous because it can be associated to a deficiency of either ETF or ETF-QO [136]. A third type of MAD (riboflavin-responsive MAD) has no identified etiology and is treatable with riboflavin [137]. The clinical and molecular aspects of these disorders have been reviewed [138].

Electron-Transferring Flavoprotein and the Electron-Transferring Flavoprotein–Coenzyme Q-Oxidoreductase Deficiencies: Clinical Presentation

At least three groups of patients with GA type II have been identified [135]: (1) patients with a neonatal form, characterized by hypotonia, hepatomegaly, severe hypoglycemia and metabolic acidosis, multiple congenital anomalies, typical sweaty-feet odor, and early death; (2) infants without congenital anomalies but with a clinical course as severe as that of patients with the neonatal form (these patients survive up to a few months of age and may develop rapidly progressive cardiomyopathy); and (3) patients with later onset and variable clinical presentation, frequently characterized by vomiting, hypoglycemia, hepatomegaly, and proximal myopathy. The last group of patients characteristically excrete predominantly ethylmalonic and adipic acids (ethylmalonic-adipic aciduria) [139]. There is no evidence that these patients can be distinguished on a biochemical genetic basis from those patients with GA II who excrete very large amounts of glutaric, ethylmalonic, isovaleric, isobutyric, 2-methyl-butyric, saturated and unsaturated dicarboxylic acids, and sarcosine. The amount of 3-hydroxybutyric and acetoacetic acid is not prominent. Carnitine content in plasma, muscle, and liver may be low. Radiologic examination can show cardiac enlargement, and echocardiography may demonstrate hypertrophic cardiomyopathy and renal cysts. Severe forms of GA II with congenital anomalies have been more frequently associated to ETF-QO deficiency [135].

Clinical heterogeneity is exemplified by a few patients with late onset and a milder course who have progressive proximal myopathy, hepatopathy, and diffuse tissue steatosis [140]. Some other patients present a progressive extrapyramidal disorder similar to that found in GA type I, with no, or little, organic aciduria even in the presence of severe enzyme deficiency [135,138].

Biochemistry and Molecular Genetics

In patients' cells, oxidation of several metabolites is decreased, including [1-^{14}C]palmitate, [1-^{14}C]octanoate, [1-^{14}C]butyrate, [1,5-^{14}C]glutarate, [2-^{14}C]lysine, and [2-^{14}C]leucine, which are oxidized by different acyl-CoA dehydrogenases (i.e., SCAD, MCAD and LCAD, isovaleryl CoA dehydrogenase, 2-methyl branched-chain acyl-CoA dehydrogenase, glutaryl CoA dehydrogenase, dimethylglycine dehydrogenase, and sarcosine dehydrogenase) [135]. This is due to the primary deficiency of either ETF or ETF-QO, which represent a metabolic bottleneck in the final oxidation of numerous oxidative reactions.

ETF α-subunit and ETF-QO deficiencies have been the first causes of GA II to be identified. In the case of ETF deficiency, studies with antibodies directed against ETF subunits showed that biosynthesis of the α-subunit precursor in cultured cells from three affected infants was either absent or resulted in a polypeptide of abnormal size [141]. Biosynthesis of ETF β-subunit was normal. These studies suggested that GA II with ETF deficiency was mainly associated with mutations of the gene encoding α-ETF. However, additional studies in 23 cell lines from GA II infants illustrated the biochemical heterogeneity of MAD [142]. Fibroblasts from all patients had severe deficiency of ETF or ETF-QO, but immunotitration gave variable results, showing absence of CRM for either α-ETF subunit or both subunits. One cell line with undetectable ETF enzyme activity had negligible β-ETF CRM [142], however. In cultured cells from two other Japanese infants with GA II and low ETF activity, β-ETF protein was also not detectable, whereas α-ETF was synthesized normally [143].

After the cloning of ETF genes [144–146], molecular studies in GA II with ETF deficiency showed a mutation of the α-ETF gene in a cell line derived from a GA II boy with hypoglycemia, hepatomegaly, and cardiomyopathy. Sequencing showed on one allele a glycine-for-valine 157 substitution, which, according to computer-assisted predictions, could cause a significant change in the secondary structure of this polypeptide, whereas the other allele carried an 18-bp deletion in the same region [147].These same authors investigated the α-ETF sequence in 6 more patients and found that the most common mutation was a methionine-for-threonine substitution at codon 266. Molecular investigation of the two Japanese siblings with GA II and β-ETF deficiency showed a single missense mutation, predicting a methionine-for-threonine substitution at codon 154 in one allele, whereas the other allele carried a G-to-C transversion at the first nucleotide of the intron donor site, which resulted in skipping of one exon during the splicing event [148].

In GA II infants with early death due to ETF-QO deficiency, enzyme activity is almost undetectable and, in most cases, associated with absence of ETF-QO CRM [149]. Preliminary molecular studies in three unrelated infants with ETF-QO deficiency have also been reported [94]. In one case, a deletion of 200 bp encompassed the most upstream cysteine-rich region in the iron-sulfur center binding domain. A unique single base deletion in a lysine codon close to the Fe_4S_4 cluster of ETF-QO was found in another family. This frameshift mutation results

in a protein that is not incorporated into mitochondria and appears to be rapidly degraded [94].

Riboflavin-Responsive Glutaric Aciduria Type II

Riboflavin-responsive GA II has been described both in infants with acute metabolic attacks and in young adults with progressive myopathy and lipid storage in type I and type II muscle fibers [150,151]. The urinary pattern of organic acid is compatible with either GA II or ethyl-melonic aciduria. Carnitine content in plasma and tissues is variably reduced. The clinical, morphologic, biochemical, and physiologic responses to oral riboflavin supplementation are dramatic [152,153]. Biochemically, the disease is characterized by multiple deficits of several flavin-dependent acyl-CoA dehydrogenases. Recent studies in muscle mitochondria isolated from myopathic patients showed that the defect involves the primary dehydrogenases active on fatty acyl-CoA substrates, notably SCAD and, to a lesser extent, MCAD [152]. Riboflavin (100 mg/day oral riboflavin) normalizes the activities of SCAD and MCAD and restores to normal the amount of anti-SCAD and anti-MCAD CRM, which are typically absent or markedly reduced before treatment [152]. A few patients have combined deficiency of acyl-CoA dehydrogenases and respiratory chain complexes [154]; these patients respond favorably to riboflavin supplementation, suggesting that riboflavin deficiency in humans might impair FAD-dependent electron transport in addition to FAD-dependent fatty acid oxidation [155].

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