CHAPTER 101

Mitochondrial Fatty Acid Oxidation Disorders

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1. **Mitochondrial β-oxidation plays a major role in energy production, especially during periods of fasting. The pathway is complex and includes as many as 20 individual steps: cellular uptake of fatty acids; their activation to acyl-CoA esters; transesterification to acylcarnitines; translocation across the mitochondrial membrane; re-esterification to acyl-CoA esters; and the intramitochondrial β-oxidation spiral, generating electrons that are transferred to electron transfer flavoprotein, and acetyl-CoA, which is converted to ketone bodies in the liver. Within the spiral, each step is catalyzed by enzymes with overlapping chain-length specificities. There is also a series of enzymes specifically required for the oxidation of unsaturated fatty acids.**
2. **Inherited defects of 11 proteins directly involved in this process have been identified in humans. These include defects of plasma membrane carnitine transport (MIM 212140); carnitine palmitoyltransferase (CPT) I (MIM 255120) and CPT II (MIM 255110); carnitine/acylcarnitine translocase (MIM 212138); very long-chain, medium-chain, and short-chain acyl-CoA dehydrogenases [VLCAD (MIM 201475), MCAD (MIM 201450), and SCAD (MIM 201470), respectively]; 2,4-dienoyl-CoA reductase (MIM 222745); and long- and short-chain 3-hydroxyacyl-CoA dehydrogenase [LCHAD (MIM 143450), SCHAD (MIM 601609)]; and mitochondrial trifunctional protein (MIM 600890).**
3. **MCAD deficiency is the most common defect in the pathway and highlights many of the features that characterize patients with disorders of β-oxidation. It has been described in patients worldwide, most of whom are of northwestern European origin. MCAD deficiency is a disease primarily of hepatic fatty acid oxidation. The most frequent presentation is episodic hypoketotic hypoglycemia provoked by fasting and beginning in the first 2 years of life. Accumulation of fatty acid intermediates results in plasma and urinary metabolites, some of which are general indicators of impaired function of the β-oxidation pathway (e.g., dicarboxylic acids), while others are unique and characteristic of MCAD deficiency (e.g., octanoylcarnitine). Although the first episode may be fatal, resembling sudden infant death syndrome (SIDS), patients with MCAD deficiency are normal between episodes. Therapy includes avoidance of fasting and treatment of acute episodes with IV glucose. Diagnosis can be made by analysis of blood acylcarnitines or, in many cases, by molecular analysis because a single MCAD missense allele accounts for nearly 90 percent of the mutant MCAD genes causing this disorder.**
4. **Other disorders of the β-oxidation pathway are characterized by skeletal and/or cardiac muscle weakness. These include deficiencies of VLCAD, LCHAD, trifunctional protein, CPT II, SCAD, and carnitine/acylcarnitine translocase deficiencies, as well as a carnitine transport defect. In some of these disorders unique metabolites can be identified in blood or urine; the exceptions are CPT I deficiency and the carnitine transport defect, in which no abnormal metabolites are excreted. In addition, hypoketotic hypoglycemia with increased blood carnitine levels occurs in CPT I deficiency.**
5. **VLCAD deficiency has two distinct clinical phenotypes: hypertrophic cardiomyopathy (VLCAD-C) and a milder form manifesting recurrent hypoglycemia (VLCAD-H). They can be distinguished biochemically by different acylcarnitine profiles following incubation of fibroblasts or amniocytes with 16-$^2H_3$-palmitate.**
6. **Carnitine deficiency is a primary manifestation of the carnitine transport defect; patients with this defect respond dramatically to carnitine therapy. Carnitine deficiency is a secondary feature of all other β-oxidation disorders, except CPT I deficiency which is characterized by increased plasma carnitine levels.**
7. **Syndromes of severe maternal illness (HELLP syndrome and AFLP) have been associated with pregnancies carrying a fetus affected by LCHAD, trifunctional protein, and CPT I deficiencies. These may require emergency delivery in the last trimester. The 1528G > C mutation observed in LCHAD deficiency can often identify a mother at risk for that disease.**

Disorders of fatty acid oxidation are relative newcomers to the arena of inborn errors of metabolism. The first well-documented disorders were described in the early 1970s in patients with skeletal muscle weakness or exercise-induced rhabdomyolysis and abnormalities in muscle fatty acid metabolism associated with decreased muscle carnitine[1] or carnitine palmitoyltransferase (CPT).[2] Shortly thereafter, the syndrome of "systemic carnitine deficiency" was identified; in this disorder, plasma, muscle, and liver carnitine levels were low and fatty acid oxidation in both muscle and liver was impaired.[3] Characterization of another group of inborn errors of mitochondrial fatty acid oxidation began in 1982–1983 with the description of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in patients with a disorder of fasting adaptation by several groups of investigators.[4–7] Altogether, 10 disorders affecting mitochondrial fatty acid

A list of standard abbreviations is located immediately preceding the index in each volume. Additional abbreviations used in this chapter include: CPT = carnitine palmitoyltransferase; EMG = electromyography, electromyogram; ETF = electron transfer flavoprotein; ETF:QO = electron transfer flavoprotein:ubiquinone oxidoreductase; LCAD = matrix long chain acyl coenzyme A dehydrogenase; LCHAD = long chain 3-hydroxyacyl coenzyme A dehydrogenase; MCAD = medium-chain acyl coenzyme A dehydrogenase; MCT = medium-chain triglycerides; MS/MS = tandem mass spectrometry; MTP = mitochondrial trifunctional protein; SCAD = short chain acyl coenzyme A dehydrogenase; SCHAD = short chain L-3-hydroxyacyl coenzyme A dehydrogenase; TCA = tricarboxylic acid; and VLCAD = very long chain acyl coenzyme A dehydrogenase.

oxidation and ketogenesis have been defined.[8] MCAD deficiency in particular, is a common metabolic disease, implicated in some cases of sudden infant death syndrome (SIDS) and Reye syndrome.

Fatty acid oxidation disorders may have escaped attention, in part because the pathway does not play a major role in energy production under nonfasting conditions.[9] Thus, defects in fatty acid oxidation may be clinically silent until relatively late in fasting. Another factor contributing to the delay in their recognition is that routine laboratory tests, other than qualitative urinary ketone analysis, often do not provide clues about potential defects in the fatty acid oxidation pathway. Methods to identify abnormal metabolites of fatty acids using gas chromatography coupled to mass spectrometry (GC-MS) have been available only since the mid-1970s; it is in large measure the availability of these analytic techniques, and others (tandem mass spectrometry) that have evolved more recently, that have permitted the identification of patients with fatty acid oxidation defects, even when they are well.

In this chapter, we describe the pathway of mitochondrial β-oxidation and its constituent enzymes. We then review the clinical, laboratory, pathologic metabolic, and molecular findings in patients with disorders of fatty acid oxidation resulting from deficiency of the four steps of the carnitine cycle—plasma membrane carnitine uptake, CPT I, mitochondrial membrane carnitine/acylcarnitine translocase, and CPT II; deficiency of each of the three acyl-CoA dehydrogenases—very long chain acyl-CoA dehydrogenase (VLCAD), MCAD, and short chain acyl-CoA dehydrogenase (SCAD); deficiency of three other enzymes of the β-oxidation spiral—long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), trifunctional protein deficiency and possible short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD); and deficiency of an enzyme required for unsaturated fatty acid oxidation—2,4-dienoyl-CoA reductase. Details of inherited defects in electron transfer (electron transfer flavoprotein [ETF] and ETF:ubiquinone oxidoreductase [ETF:QO] deficiencies) and ketogenesis (3-hydroxy-3-methylglutaryl [HMG]-CoA lyase deficiency) are given in Chaps. 102 and 103, respectively.

FATTY ACID OXIDATION

Fatty acid oxidation and ketogenesis have been reviewed extensively elsewhere.[10–12] In this section, we outline the major steps in these pathways (Fig. 101-1) including uptake and activation of fatty acids by cells; the carnitine cycle, required for mitochondrial entry of fatty acids; the β-oxidation spiral; and enzymes required for the oxidation of unsaturated fatty acids.

Mobilization, Tissue Uptake, and Activation

Long chain length fatty acids are mobilized from adipose tissue stores and transported in the circulation primarily bound to albumin. During periods of fasting, fatty acids become the predominant substrate for energy production via oxidation in liver, cardiac muscle, and skeletal muscle. During prolonged aerobic exercise, fatty acid oxidation accounts for 60 percent of muscle oxygen consumption.[13] The brain does not directly utilize fatty acids for oxidative metabolism, but readily oxidizes ketone bodies derived from the acetyl-CoA and acetoacetyl-CoA produced by β-oxidation of fatty acids in the liver. Fatty acids are taken up by the liver and other tissues by concentration-dependent mechanisms; these remain poorly understood, but apparently include both saturable carrier-mediated uptake and nonsaturable diffusion.[12] A 40-kDa plasma membrane fatty acid binding protein has been characterized in rat liver, which may function in the sodium-linked ATP-dependent uptake of fatty acids.[14] Long chain fatty acid uptake is inhibited by an antibody raised against the plasma membrane fatty acid binding protein.[15] Inside the cell, fatty acids may become associated with low-molecular-weight cytosolic fatty acid binding proteins. These have been extensively characterized in intestine, liver, heart, skeletal muscle, and other tissues,[16] but their role in mediating fatty acid transfer from the cell membrane to mitochondria remains uncertain. The gene for human-skeletal-muscle-soluble 15-kDa fatty acid binding protein is on chromosome 1 pter-q31.[17] Fatty acids are activated to form CoA thioesters through the action of a series of acyl-CoA synthetases. While long chain acyl-CoA synthetase activities have been characterized in endoplasmic reticulum, peroxisomes, and mitochondria, they are believed to be products of the same gene, which in humans has been localized to chromosome 4.[18] The acyl-CoA esters can serve as substrates for triglyceride, phospholipid, and cholesteryl ester synthesis, and can also be directed to peroxisomes for β-oxidation (Chap. 130). Under fasting conditions, however, they are channeled primarily toward mitochondria for β-oxidation.

The Carnitine Cycle

The carnitine cycle is required for the transport of long chain fatty acids into the mitochondrial matrix, and includes four steps: a plasma membrane carnitine transporter which maintains the intracellular supply of carnitine; an outer mitochondrial membrane

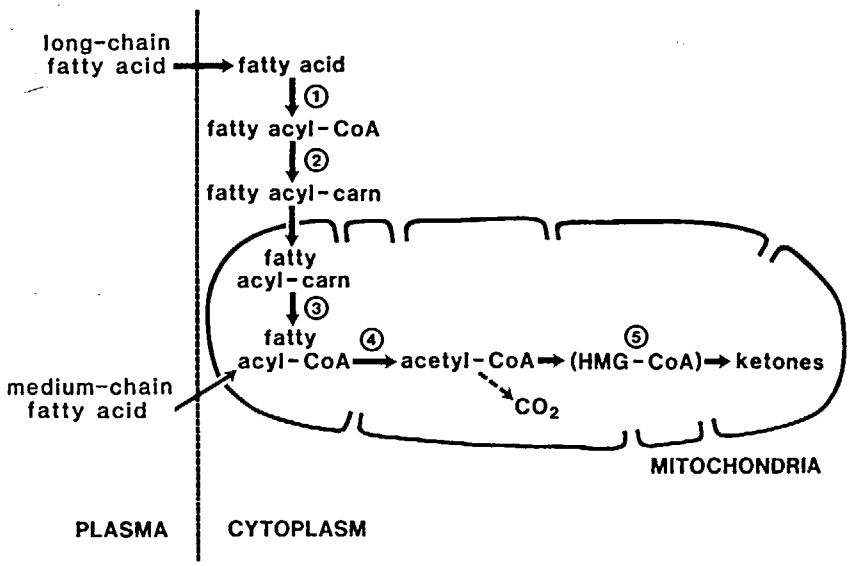


Fig. 101-1 Pathway of fatty acid entry, activation, mitochondrial uptake, β-oxidation, and ketogenesis in liver. The vertical dashed line denotes the liver-cell membrane. Numbers indicate the sequence of reactions: (1) fatty acid activation by acyl-CoA synthetase to form acyl-CoA esters; (2) transesterification of acyl-CoA by CPT I prior to mitochondrial translocation by carnitine/acylcarnitine translocase; (3) reesterification of acylcarnitine to acyl-CoA by CPT II; (4) β-oxidation spiral, each turn of which yields acetyl-CoA, which can be oxidized in the tricarboxylic acid (TCA) cycle to Co2 (broken line with arrow) or can become available for the reactions depicted by 5, the hydroxymethylglutaryl HMG-CoA pathway to form ketone bodies. Medium chain fatty acids can traverse the mitochondrial membrane without the need for carnitine-mediated transport. (*Courtesy of C.A. Stanley, M.D.*)

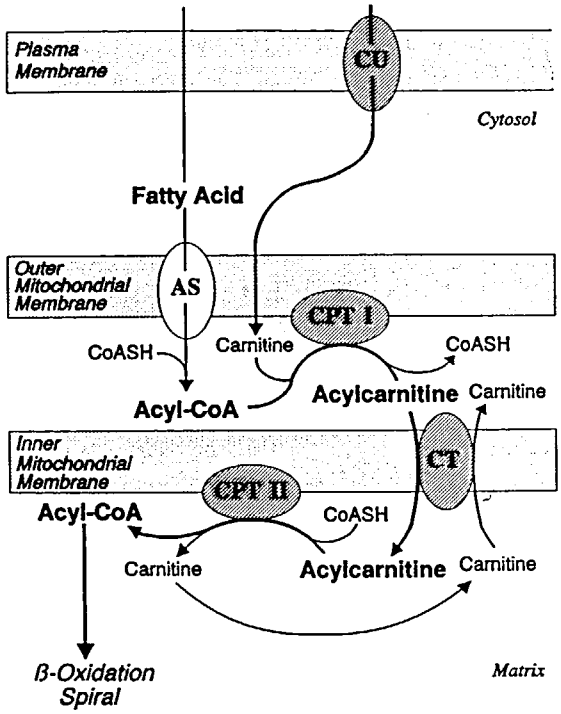


Fig. 101-2 The carnitine cycle. See text for details. AS = acyl-CoA synthetase; CoASH = coenzyme A; CPT = carnitine palmitoyltransferase; CT = carnitine/acylcarnitine translocase; CU = carnitine uptake.

CPT I that converts acyl-CoA compounds to their acylcarnitine analogues; the transmembrane transfer of acylcarnitines, mediated by carnitine/acylcarnitine translocase; and the reesterification of acylcarnitines to form acyl-CoA esters by CPT II within the mitochondrial matrix (Fig. 101-2). Fatty acids <8 carbons in length are believed to traverse the mitochondrial membrane as free acids without the need for carnitine esterification, and then are activated to form acyl-CoA esters within the matrix by the medium chain acyl-CoA synthetase.

Plasma Membrane Carnitine Uptake

There appear to be two distinct plasma membrane carnitine uptake mechanisms. In most tissues, a carnitine transporter has been identified which has a K_m for free carnitine that ranges from 2 to 10 mM in isolated cells and 20 to 200 mM in whole organs.[19] In the liver, by contrast, there is a low-affinity high-capacity transporter with a K_m for carnitine of 2 to 4 mM.[20] The liver, therefore, is dependent on the plasma level of carnitine to maintain normal tissue concentrations. There may be another low-affinity carnitine uptake system present in muscle cells,[21] which gradually develops during muscle maturation, at least in tissue culture, and which operates at carnitine concentrations between 25 and 200 mM.

Carnitine Palmitoyltransferase Activities

CPT I and CPT II are genetically and functionally distinct enzymes.[22]

CPT I is embedded in the outer mitochondrial membrane. In the liver, it is inhibited by malonyl-CoA, thereby providing the site for regulation of fatty acid oxidation and ketogenesis. By contrast, CPT II on the inner mitochondrial membrane is unaffected by malonyl-CoA. Both rat and human liver CPT II cDNAs have been cloned and sequenced, and they have remarkable similarities.[23,24] The gene in humans is on chromosome 1[24] and encodes a precursor polypeptide of 658 amino acids, including a leader peptide of 25 amino acids. The mature CPT II subunit has 633 amino acids, with a molecular weight of 66; the fully active enzyme is a homotetramer.[25] The development of specific antibodies against the individual CPT I and II proteins in rat liver[26–28] has elucidated the structural and functional relationships among the CPT proteins. Anti-CPT I antibody recognizes CPT I, but not CPT II, in rat liver. It recognizes CPT I only in liver and kidney, but not CPT I in muscle or heart. It therefore appears that CPT II is the same enzyme in all tissues of the rat, while CPT I has tissue-specific isoforms. It is likely that these results can be extrapolated to humans, given what we know about the human CPT deficiency states, described below.

Carnitine/Acylcarnitine Translocase

This enzyme facilitates the exchange of carnitine and acylcarnitines across the inner mitochondrial membrane; it also facilitates the unidirectional transport of both free carnitine and acylcarnitine across the membrane so that carnitine concentrations are the same in both cytosolic and mitochondrial matrix spaces. The translocase has been isolated from rat liver mitochondria as a 32.5-kDa protein.[29] It has highest affinity for 12- to 16-carbon acylcarnitines, lower affinity for short chain acylcarnitines, and little or no affinity for free carnitine in reconstituted liposomes.[30]

Mitochondrial β-Oxidation Spiral and Electron Transfer

It is the acyl-CoA ester that enters the pathway of mitochondrial β-oxidation. With each turn of the β-oxidation spiral, the chain length of a saturated acyl-CoA is shortened by two carbon atoms as an acetyl-CoA moiety is released. In most tissues, such as muscle and heart, acetyl-CoA is completely oxidized in the tricarboxylic acid (TCA) cycle, ultimately to carbon dioxide and water. In liver, and to a much smaller extent in kidney, acetyl-CoA produced from β-oxidation is largely converted to the ketone bodies β-hydroxybutyrate and acetoacetate via the HMG-CoA pathway (see "Hepatic Ketogenesis" below). These are then exported for final oxidation by other tissues such as brain and muscle.

Acyl-CoA compounds cycle through β-oxidation as many times as it is possible to generate two-carbon acetyl-CoA fragments; for example, palmitoyl (C16)-CoA makes seven cycles through β-oxidation. Each turn of the β-oxidation spiral is mediated by a sequence of enzymes, all of which exhibit some degree of specificity for the chain length of the acyl-CoA moiety. For a typical saturated acyl-CoA such as palmitoyl-CoA, the sequence of four enzyme steps is (a) acyl-CoA dehydrogenase, (b) 2-enoyl-CoA hydratase, (c) L-3-hydroxyacyl-CoA dehydrogenase, and (d) 3-ketoacyl-CoA (or 3-oxoacyl-CoA) thiolase.

Acyl-CoA Dehydrogenases

Acyl-CoA dehydrogenases mediate the reaction:

$$\mathrm{R{-}CH_2{-}CH_2{-}\overset{\overset{\mathrm{O}}{\|}}{C}{-}SCoA + FAD \longrightarrow R{-}\overset{\overset{\mathrm{O}}{\|}}{CH{=}CH{-}C}{-}SCoA + FADH_2}$$

These enzymes contain noncovalently bound FAD. In the process of inserting a double bond between the α and β (i.e., the second and third) carbons of the acyl-CoA, and forming the corresponding 2-trans-enoyl-CoA, the electrons generated by dehydrogenation are transferred from the FAD of the acyl-CoA dehydrogenase to the FAD of ETF.[31] In turn, reduced ETF is oxidized by ETF:QO and coenzyme Q, with the ultimate transfer of electrons to the electron transport chain.[32] For details of the reactions involving ETF and ETF:QO, see Chap. 103.

The acyl-CoA dehydrogenases represent the best example of the chain-length specificity of β-oxidation enzymes. Except for VLCAD, LCAD, MCAD, and SCAD and the related enzymes, isovaleryl-CoA dehydrogenase and 2-methyl branched-chain acyl-CoA dehydrogenase, are members of a family of enzymes that

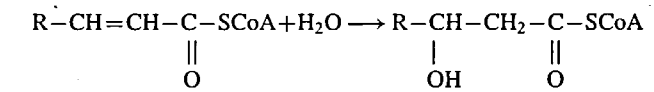
share many common features. They have been purified and characterized in several laboratories,[33–43] and their substrate specificities have been extensively studied.[33,37,39,40,43,44] They are homotetramers containing one molecule of FAD per subunit;[33] attachment of the FAD ligand occurs after mitochondrial import.[45] ETF is their physiological electron acceptor. In liver, VLCAD in the inner mitochondrial membrane catalyzes the first reaction in β-oxidation of acyl-CoA moieties ranging in length from 20 carbons down to 14 carbons.[46] LCAD was purified and characterized in rat liver mitochondria; it also catalyzes the dehydrogenation of acyl-CoA compounds from 12 to 18 carbons in length. MCAD acts on a broad range of acyl-CoA compounds from 12 carbons down to 4 carbons. SCAD acts only on 6- and 4-carbon compounds.

Although VLCAD also requires ETF as electron acceptor, it is structurally distinct from the other members of this family; it is a heterodimer, and it does not cross-react with antibodies raised against the other acyl-CoA dehydrogenases. The purified VLCAD enzyme has greatest activity toward palmitoyl (C16)-CoA, which overlaps with matrix LCAD. VLCAD has also been termed membrane LCAD, to distinguish it from the previously described matrix LCAD enzyme.[47]

Rat and human cDNAs for LCAD, MCAD, and SCAD have been cloned and sequenced.[48–54] Within the same species, LCAD, MCAD, and SCAD share 30 to 40 percent sequence identity, indicating that they evolved from a common ancestral gene, although they do not cross-react immunologically. This is in contrast to the same enzyme from different mammalian species, which share 87 to 90 percent sequence identity and which generally cross-react immunologically. This family of enzymes also shares similarities with the acyl-CoA oxidases that initiate peroxisomal β-oxidation.[55] The structure-function relationships of these enzymes have become increasingly understood as a result of the x-ray crystallographic detail now available with high resolution.[56] The MCAD monomer, for example, is folded into three domains: N-terminal, middle, and C-terminal. Both N- and C-terminal domains consist mainly of α-helixes; the middle domain is composed of β-sheets. FAD and substrate lay in a crevice between the N-terminal and middle domains, with the reactive side of the flavin ring buried in the crevice. The C-terminal domain forms the core of the tetramer.

2-Enoyl-CoA Hydratases

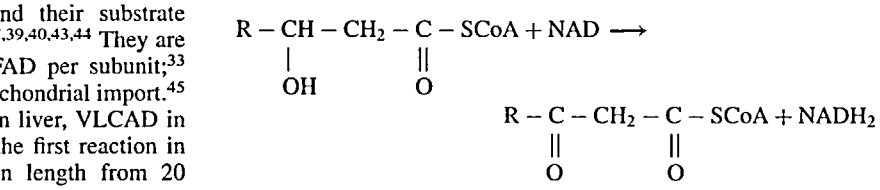
The second reaction is hydration of the 2-trans-enoyl-CoA catalyzed by 2-enoyl-CoA hydratases:



This is a reversible reaction, with equilibrium favoring the product, an L-3-hydroxyacyl-CoA. There is evidence for two different 2-enoyl-CoA hydratases in mammalian tissues, a short chain enzyme commonly known as crotonase, and a long chain enzyme.[57,58] Rat crotonase cDNA has been cloned and sequenced; it encodes a 290-amino-acid precursor that is subsequently processed to a 261-amino-acid mature subunit with a molecular weight of 28.3. The fully active crotonase enzyme is a homohexamer and shares considerable sequence similarity with the enoyl-CoA hydratase component of the peroxisomal multifunctional enzyme.[59] It acts on enoyl-CoA substrates with chain lengths of four or more carbons, although enzyme activity decreases with increasing chain length. Long chain 2-enoyl-CoA hydratase, partially purified from pig heart mitochondria,[58] is inactive toward crotonyl-CoA, but is active with all longer-chain substrates (see "Trifunctional Protein," below).

L-3-Hydroxyacyl CoA Dehydrogenases

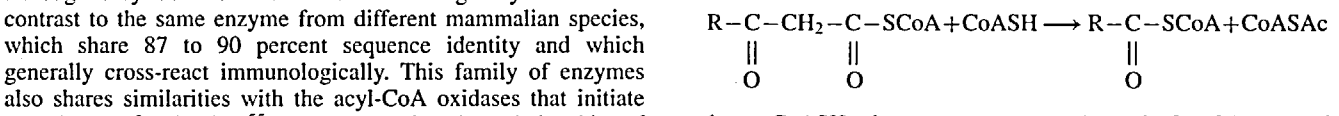
The freely reversible dehydrogenation of the hydroxy group to a keto group is catalyzed by L-3-hydroxyacyl-CoA dehydrogenases in an (NAD^+)-dependent reaction:



Two different L-3-hydroxyacyl-CoA dehydrogenases have been identified in mammalian tissues.[60] Short chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) from pig heart is a soluble matrix enzyme with a molecular weight of 65 and is composed of two identical subunits.[61] The rat cDNA has been cloned.[62] It encodes a 34-kDa precursor that is processed to a 31-kDa mature subunit. It is active toward 3-hydroxyacyl-CoA esters from C4 to C16, but its activity declines with increasing chain length. Given its broad substrate specificity, the designation "short-chain" is misleading. Long chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) has been partially purified and characterized in the rat liver inner mitochondrial membrane, with a native molecular weight of 230 kDa,[63] which is most active with long chain ester substrates (see "Trifunctional Protein," below).

3-Ketoacyl-CoA Thiolases

The final reaction is thiolytic cleavage of the a,b bond, catalyzed by 3-ketoacyl-CoA thiolases, in the presence of CoA:



where CoASH denotes coenzyme A and CoASAc acetyl coenzyme A. Although this is a reversible reaction, equilibrium considerably favors the products, acetyl-CoA and an acyl-CoA two carbons shorter than the original substrate (e.g., palmitoyl-CoA, a 16-carbon acyl-CoA, yields acetyl-CoA and myristoyl-CoA, with 14 carbons). Mitochondria contain at least two distinct 3-ketoacyl-CoA thiolases, one with a broad chain-length specificity and one specific for acetoacetyl-CoA.[64,65] Acetoacetyl-CoA thiolase is the enzyme involved in β-ketothiolase deficiency.[66] The molecular properties of the broad-specificity 3-ketoacyl-CoA thiolase are unknown (see "Trifunctional Protein" below).

Trifunctional Protein

A trifunctional protein bearing long-chain 2-enoyl-CoA hydratase, LCHAD, and long chain 3-ketoacyl-CoA thiolase activities has been purified from the inner mitochondrial membrane in both rat and human liver.[67,68] In rat liver mitochondria, the native enzyme is an octamer of 46 kDa that consists of α subunits (79 kDa) and two β-subunits called β_1 (51 kDa) and β_2 (49 kDa). The subunits are immunologically distinct. The trifunctional protein exhibits highest enoyl-CoA hydratase activity toward C10 and longer chain substrates. Its activity toward 3-hydroxyacyl-CoA compounds also differs from that of the corresponding short chain hydroxyacyl-CoA dehydrogenase: optimal activity is found with C12 to C16 compounds, in contrast to SCHAD, which has its optimum around C6. Similarly, ketoacyl-CoA thiolase activity of the trifunctional protein is considerably higher with C12 to C16 substrates than is the acetoacetyl-CoA thiolase. Thus, the membrane-bound trifunctional protein contains all three of the enzyme activities with long chain specificity that had been previously purified from various sources. It has been demonstrated that the LCHAD activity previously purified from human liver mitochondria[63] likewise resides in a trifunctional protein.[68]

Unsaturated Fatty Acid Oxidation

Unsaturated acyl-CoA compounds, such as the CoA esters of oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, are also oxidized by the same series of reactions, until the double bond is

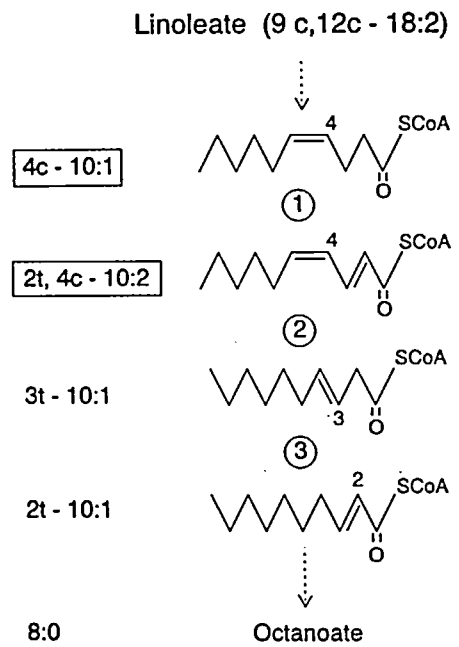


Fig. 101-3 Mitochondrial β-oxidation of linoleic acid. See text for details. Step 1: MCAD inserts a double bond in the C2 position of 4-*cis*-C10:1. Step 2: 2,4-Dienoyl-CoA reductase reduces the series of double bonds, producing the 3-*trans* intermediate. Step 3: D3, D2-Enoyl-CoA isomerase converts this to 2-*trans*-enoyl-CoA for further β-oxidation.

reached (Fig. 101-3).[12] The double bonds in these compounds are usually in the *cis* configuration and extend either from an even-numbered carbon (e.g., the 12-*cis* double bond of linoleic and linolenic acids) or an odd-numbered carbon (e.g., the 9-*cis* double bond of oleic, linoleic, and linolenic acids). Linoleic acid, with its double bonds in the 9- and 12-carbon positions, undergoes three cycles of β-oxidation until 3-*cis*,6-*cis*-dodecadienoyl (C12:2)-CoA is formed.

Δ^3, Δ^2-Enoyl-CoA isomerase converts it to 2-*trans*,6-*cis*-C12:2-CoA. One round of β-oxidation yields 4-*cis*-decenoyl (C10:1)-CoA, which is dehydrogenated by MCAD to 2-*trans*, 4-*cis*-decadienoyl (C10:2)-CoA. Then, the second auxiliary enzyme, 2-4-dienoyl-CoA reductase, catalyzes its reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction to 3-*trans*-C10:1-CoA. Isomerization of this compound by the Δ^3, Δ^2-enoyl-CoA isomerase yields an intermediate, 2-*trans*-C10:1-CoA, that reenters the β-oxidation spiral for complete degradation.

The degradation of fatty acids with odd-numbered double bonds, such as oleate (9-*cis*-C18:1), was thought to proceed via β-oxidation to 5-*cis*-tetradecenoyl (C14:1)-CoA, and then to 3-*cis*-dodecenoyl (C12:1)-CoA, with isomerization to 2-*trans*-C12:1-CoA and further oxidation to decanoate. In vitro studies with VLCAD deficient fibroblast lines incubated with oleate or linoleate reveal that all oxidation ceases at the level of 5-*cis*-tetradecenoyl (C14:1)-CoA and 5-*cis*-8-*cis*-tetradecadienoyl (C14:2)-CoA, respectively indicating that in human cells VLCAD is the enzyme responsible for dehydrogenation of substrates with the 5-*cis* double bond. It has also been suggested[69] that the 5-*cis* intermediate is directly reduced by an NADPH-dependent reductase; however, the "5-*cis* reductase" has not been purified or characterized. There appears to be another possible explanation, involving a newly identified NADPH-dependent dienoyl-CoA isomerase.[70] 2-*trans*,5-*cis*-C8:2-CoA produced by dehydrogenation of 5-*cis*-C8:1 is isomerized by enoyl-CoA isomerase to the 3-*trans*,5-*cis*-C8:2 intermediate. This is a substrate for dienoyl-CoA isomerase, producing 2-*trans*,4-*trans*-C8:2. The NADPH-dependent dienoyl-CoA reductase converts this to a 3-*trans* product that is isomerized by Δ^3, Δ^2-enoyl-CoA isomerase to the 2-*trans* intermediate for reentry into the β-oxidation spiral. These other possibilities do not seem to be operational in VLCAD deficient cell lines. Δ^3, Δ^2-Enoyl-CoA isomerase activity is found in two distinct mitochondrial proteins,[71,72] which differ in structure and in their relative activities toward substrates of different chain length. One is an enzyme with its greatest activity toward the 6-carbon 3-enoyl-CoA; it is a homodimer with a subunit molecular weight of 29.3 and has sequence similarities to the amino terminal half of the peroxisomal multifunctional enzyme. The other is a 200-kDa protein with a preference for 10- and 12-carbon substrates;[71] it does not cross-react with antibodies against the short chain enzyme. 2,4-Dienoyl-CoA reductase has been purified from a number of sources, including beef liver,[73] rat liver,[74] and *Escherichia coli*.[73] The gene encodes a monomer of 32 kDa.[74]

Odd-Chain Fatty Acid Oxidation. These acyl-CoA compounds are oxidized by the same series of reactions described above, until the 3-carbon moiety, propionyl-CoA, is formed. This is then degraded by the biotin-dependent enzyme, propionyl-CoA carboxylase.[11]

Hepatic Ketogenesis. Liver is virtually the only tissue which can channel the product of fatty acid β-oxidation, acetyl-CoA, into ketone body formation.[10,11] Especially under conditions of fasting, when carbohydrate stores are depleted, the rate of hepatic ketogenesis is increased. This provides an auxiliary source of substrate for brain oxidative metabolism, sparing glucose oxidation and preventing proteolysis. Acetoacetyl-CoA derived from the last turn of the β-oxidation spiral combines with acetyl-CoA to form HMG-CoA, catalyzed by HMG-CoA synthase. HMG-CoA lyase cleaves HMG-CoA to form acetyl-CoA and acetoacetate, which is reduced to D-3-hydroxybutyrate by the NAD^+-linked D-3-hydroxybutyrate dehydrogenase within mitochondria.

Peroxisomal and Microsomal Fatty Acid Oxidation

Peroxisomal β-oxidation closely parallels the mitochondrial process. It differs from mitochondrial oxidation, however, in some key features: transport of long chain acyl-CoA compounds into peroxisomes does not require carnitine; the first step is catalyzed by a long chain acyl-CoA oxidase (not a dehydrogenase), which does not use ETF as its electron acceptor; the 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase steps are carried out by a multifunctional enzyme which also has Δ^3, Δ^2-enoyl-CoA isomerase activity; and peroxisomal β-oxidation apparently proceeds only to the medium-chain acyl-CoA level. Methyl-branched fatty acids such as phytanate and pristanate are sequentially degraded first in the peroxisome down to the level of 2,4-dimethylnonanoyl-CoA, which is converted to an acylcarnitine that is exported. This compound requires carnitine acylcarnitine translocase and CPT II for further oxidation in the mitochondrion.[75] For details of peroxisomal metabolic pathways and the clinical disorders associated with peroxisomal dysfunction, see Chaps. 129 to 132.

Microsomal ω-oxidation of fatty acids is mediated by a cytochrome P450-linked mixed function oxygenase, which catalyzes ω-hydroxylation in the presence of molecular oxygen and NADPH.[76] An NAD^+-dependent oxidation subsequently converts the ω-hydroxy fatty acid into a dicarboxylic acid. The resulting dicarboxylic acid can be transported to the mitochondrial matrix or to peroxisomes for β-oxidation.[77] Dicarboxylic acids may be formed because mitochondrial β-oxidation is overloaded or genetically impaired, and are rapidly and quantitatively excreted in urine. They are also present in the urine of patients fed a diet high in medium-chain triglycerides (MCT).[78] A similar hydroxylation reaction occurs at the ω-1 position, probably using the same microsomal oxygenase pathway.[76] Microsomal α-oxidation degrades fatty acids one carbon at a time; 3-methyl-branched chain fatty acids (e.g., phytanic acid) require an α-oxidation step which uses NADPH and molecular oxygen.[11] For further details, see Chap. 130.

Regulation of Fatty Acid Oxidation

The regulation of fatty acid oxidation in mammals by hormones, competing substrates, cofactors, and diet has been reviewed extensively.[10] Numerous studies have related rates of fatty acid oxidation to the concentrations of free fatty acids mobilized from adipose tissue which are then available for oxidative metabolism. In the transition from the fed to the fasted state, the liver converts from glucose uptake and fatty acid synthesis to glucose production, fatty acid synthesis to glucose production, fatty acid oxidation, and ketogenesis.

Hormonal control of fatty acid oxidation is exerted at the level of substrate mobilization from adipose tissue and at the level of CPT I. Insulin inhibits lipolysis in adipose tissue, decreasing the level of free fatty acids available for oxidative metabolism; it also stimulates lipogenesis and synthesis of malonyl-CoA, an inhibitor of CPT I, and therefore inhibits fatty acid oxidation. Glucagon stimulates hepatic fatty acid oxidation indirectly, by inhibiting acetyl-CoA carboxylase and thereby reducing tissue levels of malonyl-CoA, which results in enhanced activity of CPT I. Therefore, in the fed state, in which the glucagon:insulin ratio is low, the liver directs fatty acid metabolism toward synthesis. In the fasting state, the elevated glucagon:insulin ratio directs fatty acids toward mitochondria for oxidation.

GENETIC DEFECTS OF MITOCHONDRIAL β-OXIDATION

The inherited disorders of mitochondrial β-oxidation are detailed in this section, with emphasis on the clinical, biochemical, and metabolic derangements associated with each of them, their pathogenesis, and when known, their molecular basis. The concluding section describes a general approach to patients in whom a fatty acid oxidation defect is suspected.

Defects of the Carnitine Cycle

Carnitine Transport Defect

Clinical Presentations. An inherited defect in the plasma membrane transport of carnitine was first described in 1988.[79–87] In one review,[82] the ethnic distribution included Caucasian, African-American, North African Arab, Asian Indian, Mexican, and Chinese; the parents were consanguineous in five families. There are two general types of clinical presentation associated with this defect, illustrated by the following case reports.

Case 1. A 3.5-month-old girl,[79] the first child of unrelated parents, previously had been in good health. Four weeks before her initial episode, she had been switched from a cow's-milk formula containing carnitine to a carnitine-free soy-protein formula. She was found limp, unresponsive, and apneic after an overnight fast. Blood glucose was 0.4 mM (7 mg%); amino acid and electrolyte levels were normal. Urine contained only trace ketones and no dicarboxylic acids. Her liver was enlarged, and liver function tests were abnormal [aspartate aminotransferase (AST), 248 IU/L alanine aminotransferase (ALT), 149 IU/liter]. Plasma carnitine was < 1 μM (normal, 40 to 60), and muscle and liver carnitine concentrations were < 5 percent of those found in comparably aged controls. Cardiac evaluation included a normal chest film, sinus tachycardia by ECG, and increased wall thickness of the left ventricle and septum; she had a normal shortening fraction. Her response to IV glucose therapy was rapid. She was treated with long-term oral L-carnitine (100 to 120 mg/kg day) and supplemental nasogastric feeding. Her parents were advised to prevent her from fasting. She had no further episodes of fasting hypoglycemia, but had myoclonic seizures, hypotonia, and delayed mental development. She died from complications of intestinal adhesions after placement of a feeding gastrostomy. There was no family history of SIDS, Reye syndrome, or unexplained cardiac, muscle, or neurologic disease. An unaffected sib had normal plasma carnitine levels.

Case 2. A boy, the second child of distantly related parents, presented at 3.5 years with cardiomegaly and a grade 2 systolic murmur.[85] The ECG was abnormal, and echocardiogram showed left ventricular hypertrophy and dilatation, with greatly reduced left ventricular ejection fraction. He had modest hepatomegaly. Muscle strength and tone were normal. Cardiomegaly increased over the next year and symptoms of congestive heart failure developed. He had muscle weakness; muscle biopsy showed lipid deposition in type 1 fibers with atrophy of type 2 fibers; electromyography (EMG) and nerve conduction studies were normal. He was anemic (hemoglobin [Hb], 10 g/dl) and had modest elevation of AST (31 IU/L) and CPK (138 IU/L). There were no episodes of hypoglycemia. Plasma carnitine was < 5 uM. He began to experience severe dyspnea, and had two episodes of cardiac decompensation with respiratory distress. L-Carnitine therapy (174 mg/kg/day) resulted in rapid and dramatic clinical improvement. His cardiothoracic ratio declined, physical activity increased, and exercise tolerance normalized. A previous male sib died at 2 years with cardiomegaly and anemia; postmortem examination showed fatty infiltration of liver and heart.

Half of the reported patients presented early (3 months to 2.5 years) with episodes characterized by hypoketotic hypoglycemia, hyperammonemia, and elevated transaminases, some with cardiomyopathy and/or skeletal muscle weakness. Cardiomyopathy alone was the presenting sign in the other half of cases; this was frequently of later onset (1 to 7 years), progressive, and associated with skeletal muscle weakness, but without evidence of hypoglycemia. These differences in presentation most likely reflect the occurrence of a period of fasting long enough to result in hypoglycemia (and hence early recognition) before the cardiac and skeletal muscle weakness became apparent. In one family, an affected sib presented early with hypoglycemia, while another presented later with cardiomyopathy and weakness.[82] Several patients were noted to have mild to moderate anemia which responded poorly to iron therapy.[81] The very low plasma carnitine level (< 10 μM) in patients with these clinical findings, especially in the absence of a significant dicarboxylic aciduria, is virtually pathognomonic of the carnitine transport defect. With the exception of one case,[81] pretreatment plasma carnitine levels have been < 10 μM.

Diagnosis. The defect in plasma membrane carnitine transport is expressed in muscle, kidney, leukocytes, and fibroblasts, and presumably in heart, although this has not been measured. Carnitine uptake by fibroblasts and leukocytes from patients is < 10 percent of control rates.[81–84,86,87] Fibroblasts from parents have intermediate rates of carnitine uptake, consistent with heterozygosity. The carnitine transporter is able to maintain a positive gradient across the plasma membrane at low (< 5 μM) extracellular carnitine concentrations in both controls and heterozygotes; above this concentration, there is a linear increase in carnitine uptake by cells, reflecting passive diffusion. Patient fibroblasts cannot maintain a gradient, so the intracellular carnitine level passively follows the extracellular concentration. The failure to transport carnitine into these cells means that intracellular carnitine concentrations are considerably reduced.[17,100] The carnitine transport system in fibroblasts is potently inhibited by medium and long chain acylcarnitines.[82] This may provide an explanation for the secondary carnitine deficiency noted in other β-oxidation defects; the acylcarnitines which accumulate in those disorders may induce a defect in the tissue uptake of free carnitine similar to but less severe than that found in patients with the genetic transport defect.

The oxidation of long chain fatty acids is reported to be low[83] or normal[86] in fibroblasts from a few patients. Addition of carnitine to the medium enhances fatty acid oxidation in all cases studied; under the same conditions there is little or no effect of exogenous carnitine on fatty acid oxidation in control cells.

Pathogenesis. This disorder is a true primary systemic carnitine deficiency for several reasons: It results in extremely low plasma and tissue carnitine levels; it is not secondary to a mitochondrial defect of organic acid oxidation; and patients with this disorder respond dramatically to carnitine therapy. It results from a failure of high-affinity carnitine uptake into several tissues, including muscle, heart, and kidney, but not liver. The failure to concentrate carnitine in cardiac and skeletal muscle means that there is insufficient carnitine to support fatty acid oxidation. The defect in renal transport is evident by studies of carnitine withdrawal. In normal individuals, efficient renal conservation of carnitine permits plasma levels to remain normal even in the face of extended periods of carnitine withdrawal. By contrast, levels of plasma carnitine in affected patients fall to near zero within a few days of stopping carnitine supplementation. During this time, carnitine excretion in urine remains high; even at low plasma levels, the fractional excretion of free carnitine remains at 100 percent of the filtered load (normal, <5 percent). Failure to reabsorb carnitine in the kidney results in very low plasma carnitine levels, which, in turn, diminish the hepatic uptake of carnitine by passive diffusion. Hence, ketogenesis is impaired; it is restored to normal on carnitine supplementation. The accumulated acyl-CoA compounds become substrates for other cellular processes, including peroxisomal β-oxidation and triglyceride synthesis. Peroxisomal β-oxidation produces intermediates such as medium chain fatty acids and dicarboxylic acids, which do not require carnitine for entry into mitochondria. Their complete oxidation in mitochondria explains the lack of dicarboxylic aciduria in patients with this disorder.

Treatment with L-carnitine restores plasma carnitine levels to nearly normal, but muscle carnitine levels rise very slightly, consistent with a failure of carnitine transport into this tissue. It is remarkable that muscle function can be nearly normalized in these patients when their muscle carnitine levels remain less than 10 percent of control levels. This suggests that the normal muscle carnitine level greatly exceeds that necessary to support fatty acid oxidation. While heart carnitine levels have not been measured, there was objective clinical improvement in cardiac ventricular function in one patient, although this remained subnormal even after months of therapy.[81] Therefore, the possibility of later recurrence of cardiomyopathy cannot be ruled out.

Molecular Aspects. Physiological studies suggested the participation of multiple sodium ion-dependent transporters in carnitine movement across cell plasma membranes.[11,79,88,89] Recently, a cDNA of a second member of the human organic cation transporter (OCTN) family, *OCTN2* (GenBank AB015050), has been cloned which has a high similarity (78.5 percent) to human *OCTN1*.[90,91] The full-length *OCTN2* cDNA encodes a polypeptide of 557 amino acids that has many of the characteristics of a high-affinity, sodium ion-dependent carnitine transporter.[91] Furthermore, the *OCTN2* structural gene mapped to 5q31.2-32, the same region shown to harbor the gene for systemic carnitine deficiency in a large Japanese pedigree.[92]

These results focused attention on *OCTN2* as a candidate gene for systemic carnitine. Subsequently, Tsuji and colleagues[309] and others[310,311] identified *OCTN2* mutations in patients with systemic carnitine deficiency including deletions and nonsense mutations. Additionally Tsuji and colleagues surveyed plasma carnitine levels in 973 unrelated Japanese white-collar workers. Fourteen consistently had values <5 percentile, and among these, *OCTN2* mutations were identified in nine. These included W132X (three individuals); S467C (four individuals); W283C (one individual); and M179L (one individual). All but M179L had functional significance when expressed in HEK cells. These results suggest a carrier frequency of about 1 percent in the Japanese population. Furthermore, echocardiographic studies suggested that these heterozygotes were predisposed to asymptomatic cardiac hypertrophy.[312]

Carnitine Palmitoyltransferase I (CPT I) Deficiency. Despite the existence of two isoforms of this enzyme, muscle and liver, mainly the hepatic form of the disease has been documented. This could be due, in part, to the fact that the muscle isoform is not expressed in fibroblasts or amniocytes making a direct muscle biopsy necessary to detect a form of the disease expressed only in heart, muscle, or both. Ten patients (five male, five female) with CPT I deficiency in eight families have been reported. The ethnic origins of patients with the hepatic form of the disease include Caucasian, Middle Eastern, Central American Indian, Inuit, and Asian Indian.[93,94–102]

Clinical Presentation. The first presenting illness is stereotypical and is usually associated with fasting (viral infection, diarrhea). Coma, seizures, hepatomegaly, and hypoketotic hypoglycemia dominate the picture. Elevated CPK, attributable to the MM isozyme, has been seen in acute episodes in two sibs,[99] but without myoglobinuria, and not in other patients. There is no evidence of chronic muscle weakness and cardiomyopathy has not been noted in any patients with CPT I deficiency. Initial illness has occurred between 8 and 18 months, except for one patient who presented as a newborn. In the absence of urinary ketones, there is little or no dicarboxylic aciduria. Plasma carnitine levels are normal to elevated (total, 55 to 141 μM; free, 45 to 93 μM). All but one patient is alive. Renal tubular acidosis was noted in one patient.[100] Persistent neurologic deficit, probably resulting from the initial insult, is common. Recurrent episodes are common and generally have been successfully treated with glucose infusion. They have been avoided by preventing fasting. Frequent feeding and replacement of dietary long chain fat with MCT have been beneficial.

The classical findings of hypoketotic hypoglycemia without dicarboxylic aciduria and with a high plasma carnitine level (both total and free) distinguish CPT I deficiency from the other known defects of the pathway. Acylcarnitines in urine have been examined in only one patient; only acetylcarnitine, the normal species, was excreted.[99] The plasma acylcarnitine profile was normal.

Recently, as with LCHAD deficiency (see below), an Inuit mother was reported to have acute fatty liver of pregnancy (AFLP) while delivering a fetus affected with the hepatic form of CPT I deficiency.[103]

Diagnosis. The definitive diagnosis of CPT I deficiency is made by measuring enzyme activity in fibroblasts, leukocytes, or solid tissues. CPT I activity is measured as palmitoyl-L-[methyl-^{14}C]-carnitine formed from L-[methyl-^{14}C]-carnitine and palmitoyl-CoA in the presence of albumin and carnitine. CPT I, unlike CPT II, is inhibited by malonyl-CoA. Among eight patients, CPT I activity in fibroblasts was 9 to 16 percent of control values. All patients had normal levels of CPT II activity in their fibroblasts. The parents of two sibs with CPT I deficiency had intermediate levels of CPT I activity in their fibroblasts, consistent with heterozygosity. CPT I deficiency has been demonstrated in liver, but not in muscle,[94] of several patients. This supports the hypothesis that CPT I is different in liver and muscle,[96] as in the rat.[28] Fibroblasts from patients with CPT I deficiency oxidize long chain fatty acids poorly, at 5 to 26 percent of control rates.[94,95,99,100,104]

Pathogenesis. CPT I is the enzyme that converts long chain acyl-CoA substrates to their respective acylcarnitines for transport into mitochondria. Deficiency of the enzyme in liver results in a failure of acylcarnitine formation and hence little or no entry of long chain substrates into mitochondria for oxidative metabolism. As is the case with the carnitine transport defect, accumulated long chain acyl-CoA compounds undergo alternative metabolism, producing medium chain intermediates that are fully oxidized by mitochondria. This also provides the rationale for treatment of CPT I-deficient patients with diets containing MCT. The lack of significant muscle findings in most patients with CPT I deficiency is consistent with the normal activity of this enzyme in muscle. It

is not easy to explain the striking CPK elevations in the two sibs with hepatic CPT I deficiency whose skeletal muscle CPT I activity was entirely normal.

Molecular Aspects. Two different isoforms of CPT I have been described with distinct tissue distributions.[105,106,109] The hepatic isoform (CPT IA) is expressed in liver, kidney, fibroblasts, and heart; the muscle form (CPT IB) is expressed in skeletal muscle, heart, brown and white adipocytes, and testes. These two forms, the CPT IA and the CPT IB, are encoded by different genes localized on chromosome 11q13.1-13.5 and 22q13.31-13.32, respectively.[107,108] The CPT IA mRNA (GenBank L39211) is approximately 4.7 kb and encodes a protein of 773 amino acids;[109] the CPT IB mRNA (GenBank Y08683) is approximately 3.1 kb and encodes a protein of 772 amino acids.[108,110,111]

Molecular analysis of CPT IA cDNA from one patient with CPT I deficiency revealed a homozygous 1361A > G nucleotide substitution resulting in the missense mutation D454G. The D454G CPT IA cDNA displayed only 2 percent of the activity of expressed wild-type CPT IA.[112] So far, no information is available regarding mutations in CPT IB.

The primary structure of CPT IA and CPT IB have also been established in rat. The nucleotide sequence of the rat CPT IA cDNA and the predicted amino acid sequence of the protein are very similar to those of the human CPT IA (82 percent and 88 percent identity, respectively).[109] The rat CPT IB gene (GenBank AF029875) has 19 exons.[113] The nucleotide sequence of the coding region show 85 percent homology to the human CPT IB cDNA. Interestingly, mutant rat CPT IA protein lacking the first 18 N-terminal amino acid residues still had activity and kinetic properties similar to wild-type of the CPT IA but was insensitive to malonyl-CoA inhibition due to a marked reduction in affinity for malonyl CoA compared to the wild-type liver isoform.[114]

Recently, Yu et al.[115] hypothesized the existence of additional isoforms in heart to account for unique kinetic characteristics of enzyme activity. Hybridization and PCR screening of a human cardiac cDNA library revealed the expression of two novel CPT I isoforms, generated by alternative splicing of the CPT IB transcript. These are present in heart, skeletal muscle, and liver, in differing relative concentrations. The novel isoforms of CPT IB could exhibit unique features with respect to outer mitochondrial membrane topology and response to physiological and pharmacologic inhibitors.

Carnitine/Acylcarnitine Translocase Deficiency

Clinical Presentation. Stanley et al. described the first patient with this defect in mitochondrial acylcarnitine transport.[116] There are two phenotypes for this disorder: severe neonatal onset with cardiomyopathy (no survivors)[117–120] and a milder phenotype with hypoglycemia but no cardiomyopathy.[121,122]

Case Reports: Neonatal Onset. A Caucasian male infant presented with acute cardiorespiratory collapse at 36 h of age associated with fasting stress and ventricular arrhythmias for several days. Over the next 2 years, he had occasional episodes of hypoglycemic coma, recurrent vomiting, gastroesophageal reflux, and mild chronic hyperammonemia. He had severe, chronic muscle weakness and mild hypertrophic cardiomyopathy. Continuous nasogastric feeding of a low-fat, high-carbohydrate diet failed to normalize his muscle strength, although his mental development was normal. At 2.5 years of age, he deteriorated rapidly, with increasing weakness and liver failure; he died following aspiration pneumonia. His total plasma carnitine was low (30 μM), most of which (22 μM) was in the long chain esterified fraction. During treatment with carnitine and a high-carbohydrate diet, his total plasma carnitine level was within normal limits, but it was almost all esterified, including not only long chain acylcarnitines (C16:0, C18:1, C18:2), but also the corresponding dicarboxylic species as acylcarnitines. An older brother died in the newborn period with a similar episode of ventricular arrhythmias and cardiorespiratory arrest; his defect was not documented.[116]

Mild Phenotype. First-cousin Pakistani parents had seven children, four of whom were healthy. One died at 3 months of age without explanation; another died at 48 h of age with undetectable blood glucose and developed ventricular tachycardia and seizures. Autopsy revealed severe steatosis involving myocardium, liver, and kidneys. The seventh child was diagnosed with carnitine acylcarnitine translocase deficiency by analysis of fibroblasts and was found to have a residual activity of 3.0 to 6.8 percent of controls. (The severe neonatal form has no detectable activity.) This child had recurrent hypoglycemia but no cardiac involvement and had normal growth and development at 3 years of age.[122]

Diagnosis. Translocase deficiency was first demonstrated in fibroblasts.[116] The patient's cells had less than 1 percent of control enzyme activity, while cells from his parents had half-normal levels, suggesting autosomal recessive inheritance. Long chain fatty acid oxidation (< 5 percent of control rates with palmitic and oleic acids) was profoundly reduced in fibroblasts. Analysis of blood acylcarnitines by tandem mass spectrometry is also a rapid and simple test to identify the presence of this disease although the profile of acylcarnitines observed is identical for translocase deficiency and CPT II deficiency requiring direct assay of fibroblasts for the definitive diagnosis.

Pathogenesis. A failure to transport long chain (C10 to C18) acylcarnitines formed by CPT I leads to their accumulation along with long chain acyl-CoA intermediates and free long chain fatty acids outside of the mitochondrion. It is not clear which of these intermediates is responsible for the pathogenesis of this deficiency. It is of interest that despite the translocase deficiency, short chain acylcarnitines are excreted in urine and are present in plasma. These include propionylcarnitine, butyryl-/isobutyryl-carnitine, and isovaleryl-/2-methylbutyryl-carnitine. These are produced in the mitochondrial matrix and are derived from the branched-chain amino acid pathways. Incubation of cells from children with the severe neonatal phenotype with $^2H_3$-L-carnitine reveals that there is a normal exchange of both labeled and unlabeled short chain acylcarnitines across the mitochondrial membrane even when the classical translocase is absent. These results suggest the presence of another translocating system serving the short chain (C3 to C5) intermediates of branched-chain amino acid degradation (Roe DS, Roe CR, et al., unpublished observations).

Treatment of translocase deficiency focuses on the need to control endogenous lipolysis due to fasting or infection as well as attempting to maintain normal glucose homeostasis through frequent feeding. Diets (formulas) containing medium chain triglycerides also form the primary treatment strategy. Aggressive intervention during the neonatal period appears to be vital to survival.[122]

Molecular Aspects. A human carnitine/acylcarnitine translocase cDNA has been cloned[123] and disease-causing mutations have been identified. A C insertion in the cytosine-rich region of bp 955 to 959, the first mutation identified in a patient with mild translocase symptoms, results in a frameshift extending the length of the protein from 301 to 322 amino acids.[123] In other patients, deletions in the coding segment of the transcript suggesting abnormal splicing have been observed.[124,125] Sequencing the intron of the translocase gene from these patients should elucidate the primary mutation that leads to aberrant splicing. Very recently, a 558C > T mutation leading to a premature stop codon has been identified.[126] This nonsense mutation results in a truncated protein of 166 amino acids in a translocase patient with the severe phenotype. The family history was notable for the death of seven siblings in the neonatal period. The parents were first cousins. In another severe translocase patient, a 241G > A substitution producing a G81R missense mutation was reported.[127] This

mutation is located in the second membrane-spanning region of the translocase. In addition, a 459C > T substitution producing the missense mutation R133W has been detected in a compound heterozygous patient with severe translocase deficiency. The second mutation has not yet been identified.[125] These results indicate significant genetic heterogeneity in translocase deficiency.

Carnitine Palmitoyltransferase II (CPT II) Deficiency (MIM 600650)

Clinical Presentations. There are three distinct clinical forms of CPT II deficiency. The most common is the "classical" muscular form of CPT II deficiency, described originally in 1973.[2] Patients with this defect generally present in adulthood with episodic myoglobinuria and muscle weakness prompted by prolonged exercise.[128] Occasionally, these episodes have been prompted by fasting, mild infections, emotional stress, or cold exposure. Most patients present with their first episode between 15 and 30 years of age. Most affected patients are males, although the disorder is inherited in an autosomal recessive manner. Between episodes, serum CPK levels are usually normal. Fasting ketogenesis is decreased in some patients, although they rarely show the acute decompensation common in other defects of β-oxidation. Carnitine levels are usually normal in plasma and in tissues. Cardiac dysfunction is rarely seen. Renal failure, as a result of episodes of myoglobinuria, is found in 25 percent of patients. Permanent muscle weakness is rare. Lipid storage in muscle is found in 20 percent of patients; hepatic lipid storage is rare.

An often fatal infantile form of CPT II deficiency has been recognized in six patients.[129–135] The first complete report[129] described a 3-month-old boy with coma, seizures, hypoketotic hypoglycemia without dicarboxylic aciduria, hepatomegaly, cardiomegaly, cardiac arrhythmia, and low plasma and tissue carnitine levels associated with an increase in the long chain acylcarnitine fraction. He died at 17 months of age. Another patient presented at 2 years with fasting hypoglycemia and cardiomyopathy and is still alive.[132] One diagnosis was made prenatally.[133]

The remaining patients reported with CPT II deficiency have presented in the newborn period and died.[130,131,134,135] Hypoketotic hypoglycemia and cardiomyopathy, as well as skeletal muscle involvement point to the defect in these patients affecting multiple tissues. Renal dysgenesis was noted in three patients,[131,133] a feature not observed in other fatty acid oxidation defects although it has been described in some patients with glutaric acidemia type II (see Chap. 103).

Diagnosis. The specific enzyme defect, CPT II deficiency, has been demonstrated in skeletal muscle mitochondria from patients with the adult-onset disease. In spite of the primarily muscular presentation, their enzyme defect is not restricted to muscle, but is expressed in other tissues, such as liver, fibroblasts, and leukocytes. At least some of the phenotypic heterogeneity can be explained by the degree of enzyme deficit. Generally, patients with the late onset form have a partial deficiency of CPT II in fibroblasts, approximately 25 percent of control levels, while those with the more severe neonatal and infantile forms generally have < 10 percent of control activity.[135] Normal rates of long chain fatty acid oxidation have been found in fibroblasts from most, but not all, patients with adult onset CPT II deficiency,[94,135] while cells from patients with the severe infantile form[129,135] have had < 15 percent of control rates. Although not distinguishable from the profile of carnitine-acylcarnitine translocase deficiency, the blood acylcarnitine profile is consistently abnormal in both of these defects with elevations of palmitoylcarnitine, oleoylcarnitine and linoleoylcarnitine reflecting the absence or reduced levels (adult onset) of either translocase or CPT II.

Pathogenesis. In this disorder, long-chain acylcarnitines are translocated across the inner mitochondrial membrane, but are not efficiently converted to their corresponding acyl-CoAs. Hence, there is accumulation of long chain acylcarnitines in the mitochondrial matrix. These must be transported out of mitochondria, as suggested by the prominent long chain acylcarnitine species seen in plasma. It has been speculated[129,130,132] that increased concentrations of long chain acylcarnitines in patients with the severe form of CPT II deficiency, as in the case of translocase deficiency,[116] may promote cardiac arrhythmia. This has been described in a cat heart model,[136] but has not been proven in humans. The absence of arrhythmia in children with the cardiomyopathic form of VLCAD deficiency while receiving oral or intravenous carnitine during illness makes this suggestion seem unlikely in humans (Slonim A, unpublished results).

Molecular Aspects. The human *CPT II* gene, spanning about 20 kb is located at 1p32.[24,137] Its five exons encode a 658 amino acid protein including a 25-residue N-terminal leader peptide.[24,138] The cloning of the *CPT II* gene has enabled the identification of mutations in CPT II patients, and the correlation of mutant genotype to clinical phenotype. In 1992, the first mutation, R631C, was identified in an infant presenting with hypoketotic hypoglycemia and cardiomyopathy.[139] Several *CPT II* mutations have recently been detected (Fig. 101-4). The common mutation in adult CPT II deficiency is S113L,[140,141] while most other mutations have been described only in a single patient.[142–146] In addition, three apparently normal variants, F352C, V368I and M647V, have been identified.[139,146,147] Some of the functionally significant missense mutations have occurred on one of these polymorphic variants. For example, the disease-producing mutation, P227L, occurs on an allele with V368L. The association of P227L with V368L in several patients. In contrast, one patient carrying the S113L mutation presented with the polymorphism M647V, while in another patient, the S113L allele had no such polymorphism. The divergent haplotypes probably reflect the occurrence of independent mutations.

Defects of the β-Oxidation Spiral

Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) Deficiency (MIM 201475)

Clinical Presentations. Until the discovery of VLCFAD, these patients were originally thought to have a deficiency of mitochondrial matrix LCAD.[148–152] The two cases described

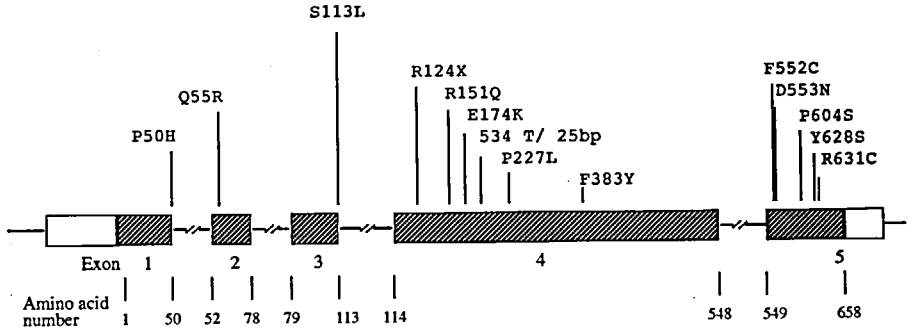


Fig. 101-4 The human CPT II gene and mutations. Boxes indicate exons. The hatched areas are coding regions with mutation positions; open boxes indicate either 5′ or 3′ UTRs. Below the exons, each vertical line indicates the amino acid number in the CPT II protein.

below reflect the distinct clinical phenotypes for this disease: hypertrophic cardiomyopathy with hypoglycemia and skeletal myopathy; or hypoketotic hypoglycemia without cardiac manifestations.

Case 1. Of four children from unrelated parents, two were affected.[153] The first male infant was the 3.3 kg product of a full-term uncomplicated pregnancy and delivery. During the first 24 h, he became tachypneic and intermittently lethargic, which was associated with metabolic acidosis (pH 7.23) and hypoglycemia (0.78 mM or 14 mg%). Urine organic acid analysis revealed dicarboxylic aciduria along with increased blood uric acid (13.3 mg/dl). Negative studies included plasma amino acids, very -long chain fatty acids, ammonia, lactate, pyruvate, and urine acylglycines. He responded to intravenous fluid and glucose therapy and was discharged at 2 weeks of age. At 10 weeks of age, he was readmitted due to poor feeding, irritability, and lethargy associated with metabolic acidosis, hypoglycemia, hepatomegaly, and hyperammonemia (1176 μM). He died following cardiorespiratory arrest. Autopsy revealed cardiac biventricular hypertrophy (weight 83 g; controls 26 g) and hepatomegaly (weight 510 g; controls 160 g) with extensive steatosis and cholestasis.

The fourth child, a female, was the 4.0 kg product of a full-term uncomplicated pregnancy and delivery. At 29 h of age, she became tachypneic, hypotonic, and increasingly lethargic with blood glucose of 0.6 mM (11 mg%). Urine revealed persistent proteinuria and hematuria in the absence of red cells. Liver enzymes were significantly elevated, but CPK was not measured. She responded to intravenous glucose for this event and a similar one at 5 days of age. At 4 months of age, she was admitted for lethargy following 4 days of diarrhea. Abnormal findings included marked hepatomegaly and hypertrophic cardiomyopathy with pericardial effusion by echocardiogram. She had hypoglycemia with an elevated CPK (2255 U/L) and uric acid (13.1 mg/dl), metabolic acidosis, and increased AST and ALT. Blood acylcarnitine analysis revealed dominance of the profile by *cis*-5-tetradecenoylcarnitine (C14:1). Assay of VLCAD activity in fibroblasts revealed an activity of 0.02 nM ETF red/min/mg protein (controls: 1.57 ± 0.31). Unlike most children with the "cardiomyopathic" form of VLCAD deficiency, she recovered and, at 5 years of age, still survives following treatment with a medium chain triglyceride-containing formula (Portagen) and carnitine supplementation.

Case 2. A 32-year-old white female (patient HC in Hale et al.[148] and Naylor et al.[151]) had several unexplained episodes of lethargy and coma associated with fasting, hypoketotic hypoglycemia, and dicarboxylic aciduria early in life. The oldest of six siblings born to healthy unrelated parents, she began at 18 months to experience frequent episodes of lethargy and unresponsiveness, invariably precipitated by an upper respiratory infection. While hospitalized at 2 years for such an episode, hepatomegaly was noted, and liver biopsy showed marked fatty infiltration, moderate lymphocytic infiltration, and perilobular fibrosis. At 5 years, she was hospitalized when she became lethargic, then comatose and unresponsive. Blood glucose was 1.1 mM (20 mg%), blood ammonia was 62 μM, blood urea nitrogen was 31 mg/dl, total CO_2 was 14.8 mM, pH was 7.37, and urine was negative for ketones. She recovered promptly after receiving IV glucose. Glucose infusion was discontinued and the next morning she again became lethargic, although her blood glucose was 3.8 mM (68 mg%). On a high-fat ketogenic diet, she became hypoglycemic and vomited, but no ketones were found in her urine. She had a normal electroencephalogram. She had episodes of hypoglycemia and lethargy over the next few years, which were aggravated by a low-carbohydrate diet. Further investigation was prompted by recurrent episodes of fatigue and muscle soreness, beginning in the second decade. At 20 years, when clinically well, her blood glucose and electrolytes were normal while organic acids revealed dicarboxylic aciduria. Total plasma carnitine was 10.6 μM, of which 4.4 μM was free carnitine, 0.4 μM was short chain acylcarnitine, and 5.8 μM was long chain acylcarnitine. The plasma acylcarnitine profile by tandem MS was dominated by tetradecenoylcarnitine (C14:1). Urinary carnitine excretion (mM/kg/day) was 1.2 total, 0.1 free, and 1.1 acyl (controls 5.4, 3.0, and 2.4, respectively). Unlike plasma, the major urinary species was acetylcarnitine. Her serum CPK was 374 IU/L (controls, < 130). ECG and chest x-ray films were normal. Echocardiography was normal, with no evidence of left ventricular dysfunction. Radionuclide angiogram showed a normal ejection fraction, with normal wall motion at rest or on exercise. She experienced further episodes of fatigue and muscle soreness, usually following either emotional stress or periods of decreased dietary intake, during which CPK levels ranged from 6400 to 32,200 IU/L; only the highest level was associated with frank myoglobinuria. While none of these episodes was associated with obvious hypoglycemia, treatment with 10 percent IV glucose and pain medication has led to a reduction in the CPK level, resolution of dicarboxylic aciduria, and cessation of muscle discomfort. At 25 years of age, she became pregnant. Although the first 16 weeks were uneventful, she had five hospitalizations from 17 to 36 weeks of gestation, all of which were precipitated by muscle tenderness and pain; two were associated with pharyngitis. Peak CPK levels during these admissions ranged from 5600 to 20,580 IU/L, but there was no hypoglycemia. Long-term treatment included a low-fat diet and supplementation with cornstarch at bedtime, fructose, and low-dose carnitine (330 mg four times daily) to normalize plasma carnitine levels, because the implications of her profound carnitine deficiency relative to the fetus were unknown. Short-term treatment included oral starch supplementation, IV glucose, and IV pain medication. She underwent cesarean section at 37 weeks and delivered a normal male infant who was unaffected and was developmentally normal at 8 years of age.

Diagnosis. Most patients with VLCAD deficiency, regardless of phenotype, are at initial risk in the neonatal period often presenting with hypoglycemia, irritability, and lethargy precipitating evaluation for sepsis. Because they often respond rapidly to glucose infusion, they can be discharged without knowledge of the diagnosis until a more severe event occurs. Children with the "cardiomyopathic" form of this disease may also have had transient neonatal hypoglycemia, but then present with hypertrophic cardiomyopathy and pericardial effusion between 2 and 5 months of age, and usually die. Initial studies should include glucose, lactate, ammonia, CPK, electrolytes, and urine organic acid analysis. Urine organic acid analysis, which reveals both saturated and unsaturated dicarboxylic aciduria, is direct evidence for some problem with mitochondrial fat oxidation. Simultaneously, blood acylcarnitine profile analysis should be obtained (usually < 72 h for result).The abnormal acylcarnitine profile is identical for both phenotypes of VLCAD deficiency and is dominated by the oleate metabolite, 5-*cis*-tetradecenoylcarnitine (C14:1).[153] Acylglycine analysis will not reveal any diagnostic abnormalities. Skin biopsy should be obtained for direct enzyme assay and in vitro studies using deuterated palmitate to determine which clinical course can be anticipated.[154] Figure 101-5 illustrates the distinctive profiles of acylcarnitines observed with the two phenotypes of VLCAD deficiency when fibroblasts are incubated with 16-$^2H_3$-palmitate. Sixteen cases of VLCAD deficiency (10 cardiomyopathic cases and 6 hypoglycemic forms), documented by direct enzyme assay, were compared to 6 controls. The ratio of $^2H_3$-palmitoylcarnitine (δ3-C16) to $^2H_3$-dodecanoylcarnitine (δ3-C12) was 4.0 in the cardiomyopathic cases, as compared to 1.0 in the children with the milder hypoglycemic phenotype ($P < 0.0005$). Unfortunately, unlike MCAD and LCHAD deficiencies, there is no mutation, sufficiently common, to assist in the acute diagnostic work-up. Similarly, measuring the oxidation rates with various substrates in

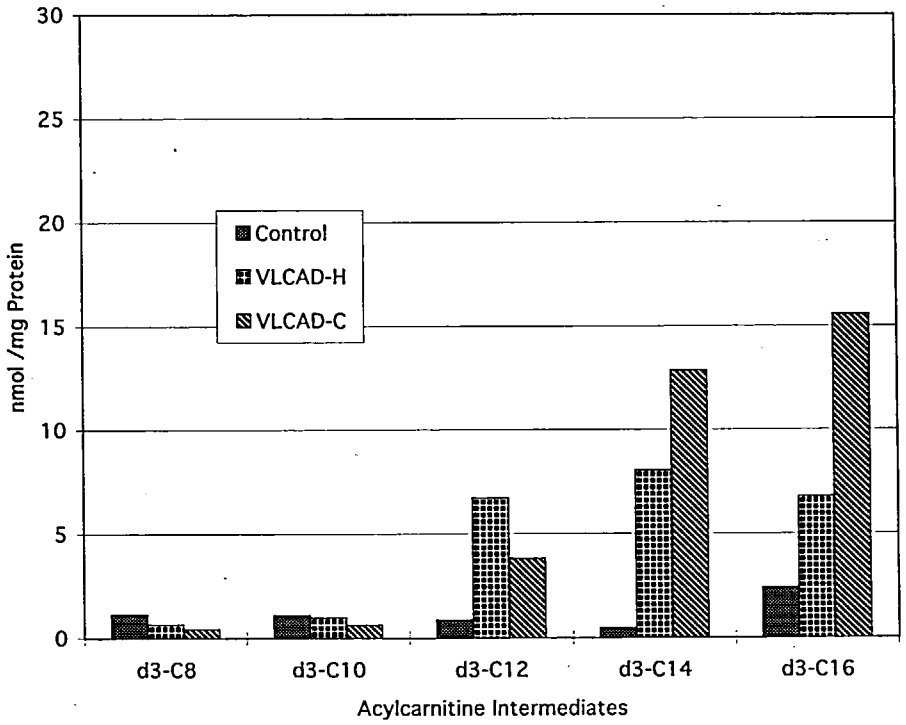


Fig. 101-5 VLCAD phenotypes. Quantitative acylcarnitine intermediates following incubation of fibroblasts from the two clinical phenotypes of VLCAD deficiency with 16-$^2H_3$-palmitate. VLCAD-C = cardiomyopathic phenotype; VLCAD-H = hypoglycemic phenotype. (The differences between these two phenotypes for d3-C16, d3-C14, and d3-C12 were significant: P-values of < 0.002, < 0.02, and < 0.02 respectively.)

fibroblasts will only indicate a problem might be present but will not be definitive as is direct enzyme assay or other more specific in vitro studies.

Families affected by VLCAD deficiency or other mitochondrial fat oxidation defects may have a history of a previous child who died without a specific diagnosis. Often an original newborn screening card or blood obtained by the medical examiner for toxicology studies may still be available for investigation. The diagnosis can often be obtained from these materials by acylcarnitine analysis. When a subsequent pregnancy occurs, direct enzyme assay and in vitro studies with labeled palmitate can provide the specific diagnosis and the anticipated phenotype for an affected fetus.[155]

Management includes avoiding fasting, maintaining high-carbohydrate intake, frequent feeding, maintaining a diet with both high-carbohydrate and medium chain triglyceride, and treating episodes of illness with IV glucose and carnitine. Intravenous and oral carnitine have been used in both phenotypes of this disease without any apparent cardiac side effects despite high plasma levels (45 μM) of long chain acylcarnitines (Slonim A, Roe CR, unpublished observations). MCT replacement of long-chain fatty acids in the diet even during severe episodes involving hypertrophic cardiomyopathy with pericardial effusion appears to be effective for management of these patients.[153,156] Clearly, this approach should only be used after ruling out MCAD deficiency or glutaric acidemia type II.

Pathogenesis. A defect in mitochondrial long chain fatty acid oxidation such as VLCAD deficiency would have far-reaching effects on other metabolic pathways, including impaired energy production during periods of fasting stress, and toxic effects of the long chain acyl-CoA intermediates, which accumulate within mitochondria. The adenine nucleotide translocase, for example, an important site for regulation of oxidative phosphorylation, is sensitive to inhibition by long chain acyl-CoA esters.[157] That peroxisomal β-oxidation and microsomal ω-oxidation play a role in disposing of accumulated acyl-CoA intermediates in VLCAD deficiency is reflected by the significant medium chain dicarboxylic aciduria.

Hepatic light microscopic changes include panlobular steatosis, with both macrovesicular and microvesicular droplets, portal fibrosis, and inflammation usually without necrosis or cholestasis. Electron microscopic findings can reveal increased mitochondrial matrix density and widening of intracristal spaces, giving the mitochondria a condensed appearance.[158] Steatosis is also observed in skeletal and cardiac muscle. In the cardiomyopathic phenotype, heart weight is increased and there is obvious hypertrophy of the ventricular walls.

Molecular Aspects. Sequence analysis of the cloned human VLCAD cDNA (GenBank L46590) isolated from a human heart cDNA library using a rat VLCAD cDNA[159] as a probe revealed a 5′ untranslated sequence of 88 bp, an open reading frame of 1965 bp encoding the entire protein of 655 amino acids, and a 3′ untranslated sequence of 171 bp followed by a short poly A tract.[160] Human heart and liver VLCAD cDNAs have identical coding regions.[161] The human *VLCAD* gene is comprised of 20 exons and is located at 17p13.[160,162,163,166]

The human VLCAD protein has an N-terminal 40 amino acid mitochondrial leader peptide 68 percent identical to the rat VLCAD. This cleavable leader peptide directing the precursor to mitochondria contains basic residues which are similar to other mitochondrial signal peptides. Mature VLCAD is a homodimer of a 70-kDa protein associated with the mitochondrial membrane. MCAD and SCAD, the other acyl-CoA dehydrogenases, are mitochondrial matrix proteins and are homotetramers of approximately 40-kDa polypeptides. The N-terminal region of mature VLCAD shows significant sequence homology to MCAD and SCAD, but the C-terminal 180 amino acid sequence, is not homologous to the other acyl-CoA dehydrogenases.[159,161]

A total of 19 VLCAD mutations have been characterized. These are summarized in Table 101-1. Interestingly, for dimer formation of mature VLCAD, the association of VLCAD protein with the mitochondrial inner membrane is necessary. An S583W mutation identified from a patient with VLCAD deficiency prevents association with the inner membrane after import into the mitochondria. The mutant protein mostly remains in the mitochondrial matrix. However, for VLCHA mutations altering

Table 101-1 Summary of Mutations Identified in the Human VLCAD Gene

Mutation	Exon	Amino acid change	References
1-bp g deletion (intron-exon 6 boundary)	6	Frameshift	160
135-bp deletion (343–477)	6	Loss of 45 amino acids	164
3-bp deletion 388–390	6	E130del	164
C 779 T	9	T220M	166
C842A	9	A241D	166
T848C	9	V243A	166
3-bp deletion 895–897	10	K299del	164
T950C	10	V277A	166
105-bp deletion (g + 1 to a) 1078–1182 (exon 11 skipping)	11	Loss of 35 amino acids	160,161
C1096T	11	R326C	166
3-bp deletion 1141–1143	11	E341del	166
A1144C	11	K382Q	164
G1322A	13	G401D	166
G1349A	14	R410H	167
T1372C	14	F458L	156
4-bp insertion (ACAG1679)	18	Frameshift	156,166
C1748G	18	S583W	165
C 1804 A	19	L562I	166,167
C 1837 T	20	R613W	164

the N-terminal region, such as E130del, K299del, and K382Q, the mutant proteins showed some degree of dimer formation.[164,165] The correlation of mutant genotypes to clinical phenotypes is still unclear.

Defects Affecting the Mitochondrial Trifunctional Protein

The mitochondrial trifunctional protein is a heterooctomeric ($\alpha_4\beta_4$) enzyme complex associated with the inner mitochondrial membrane. The complex has been purified and characterized in both rat and human liver.[168,169] It has L-3-hydroxyacyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, and 3-oxoacyl-CoA thiolase activities for the degradation of long chain acyl-CoA thioesters. From a molecular point of view, disorders of this complex can be divided into those patients whose molecular defect is in the gene encoding the α-subunit (*HADH-A*) and those with defects in the gene encoding the β-subunit of the protein (*HADH-B*). However, from a functional viewpoint, these disorders can be considered according to the specific enzyme activities that are affected, for example, L-3-hydroxyacyl-CoA dehydrogenase (LCHAD), 2-enoyl-CoA hydratase, and 3-oxoacyl-CoA thiolase (thiolase). Functional considerations are necessary for the formulation of dietary treatment strategies for these patients. Thus, there are two inherited disorders of this complex that have been described: isolated long chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) and a deficiency affecting the activities of all three enzymatic components — mitochondrial trifunctional protein (MTP) deficiency. MTP deficiency can result from mutations that affect the assembly of and/or degradation of the heterooctomeric holoenzyme. In either case, both the amount of enzyme protein is reduced as is all three enzymatic activities. Unfortunately, the cases reported in the literature often have only enzymatic information related to the impact on LCHAD (α subunit) and thiolase (β subunit) activities but, rarely, information on the status of the long chain enoyl-CoA hydratase.

Long Chain L-3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) Deficiency

Clinical Presentations. This disorder has been documented in many patients,[170–184] and is common among the fatty acid oxidation disorders. There is considerable clinical heterogeneity in LCHAD deficiency, with phenotypes including fulminant hepatic disease, hypertrophic cardiomyopathy, rhabdomyolysis, and in some patients, unusual features such as sensorimotor neuropathy and pigmentary retinopathy.

Case Reports. The first patient in whom LCHAD deficiency was confirmed was initially described clinically by Glasgow et al. in 1983.[170] A boy, one of fraternal twins, presented at 9 months of age with the first of many episodes of fasting-induced vomiting and hypoketotic hypoglycemia resembling Reye syndrome. He had significant hypotonia, cardiomyopathy, and liver dysfunction with low plasma and tissue carnitine levels and dicarboxylic and hydroxydicarboxylic aciduria. Liver biopsy showed mild fibrosis and severe fatty infiltration. He died in cardiorespiratory arrest at 19 months. At autopsy, the liver showed extensive fibrosis, massive necrosis, and steatosis.

His twin sister had a series of similar but less dramatic episodes, also beginning at 9 months. She had cardiomegaly and myopathy early in life, and her episodes were characterized by elevated blood CPK. Treatment with an MCT supplement to her diet appeared to be protective; despite several clinical episodes early in life, her cardiac function and muscle strength normalized.

Of the reported LCHAD-deficient patients, the age of onset of first symptoms has ranged from 1 day to 39 months. Most present with signs of fasting-induced hypoketotic hypoglycemia, although a few have presented with cardiomyopathy (usually hypertrophic) and muscle weakness. Episodes of illness are sometimes associated with striking elevations of serum CPK, occasionally accompanied by myoglobinuria. In a few cases, sensorimotor neuropathy or pigmentary retinopathy has been described. About half the patients died, either from the first episode or with progressive disease ending in cardiorespiratory failure.

Excretion of large quantities of 3-hydroxydicarboxylic acids of 6 to 14 carbons in length, as well as medium chain (C6 to C10) dicarboxylic acids, in urine has most often been the clue to the diagnosis of LCHAD deficiency. Acylcarnitine species corresponding to the 3-hydroxy C16:0, C18:1, and C18:2 monocarboxylic acids have been observed routinely by tandem-MS of blood or plasma from patients with LCHAD deficiency. There are a number of clinical and biochemical features of this disorder that

distinguish it from other fatty acid oxidation defects. The degree of liver damage found in some patients with this defect is remarkable. The peripheral neuropathy[172,175] and the pigmentary retinopathy[172,177,178] found in many patients are not characteristic of mitochondrial fatty acid oxidation defects; the latter has been observed in some of the disorders of peroxisomal β-oxidation.

Tyni et al.[185] recently reviewed the most homogeneous population of LCHAD-deficient patients, all 13 of whom were homozygous for the 1528G > C mutation, which affects the L-3-hyroxyacyl-CoA dehydrogenase coding part of the α-subunit of the trifunctional protein producing isolated LCHAD deficiency.[186] The age of onset of symptoms ranged from 2 days to 21 months. The primary symptoms included hypoglycemia, hypotonia, hepatomegaly, and cardiomyopathy. Six of 11 patients examined had pigmentary retinopathy, first detected between 4 and 17 months of age. Jaundice was observed in only two patients who died of hepatic failure within a month of that observation. Most patients were categorized as having hepatopathy but without jaundice. Hypotonia was consistently observed in all patients who were examined. CPK elevation was reported in 6 of 10 patients examined. Neurologically, early psychomotor development was normal but they tended to lose skills with episodes of metabolic illness. Carnitine deficiency was usually observed in these patients. Interestingly, 6 of 10 examined had anemia and thrombocytopenia. Although hypertension was not reported, elevated liver enzymes and low platelets were frequent (see "HELLP Syndrome" below). The cause of death was usually cardiac or respiratory failure, but two died from hepatic failure.

Treatment of patients with LCHAD deficiency has usually involved reducing the long chain fatty acid content of the diet, with frequent carbohydrate-enriched feedings or supplementation with uncooked cornstarch. MCT supplementation has also been used with some success and probably should be a part of the nutritional therapy. Carnitine and riboflavin have also been tried, although without obvious benefit.

Pathologic Findings. Fat accumulation has been found in liver, skeletal muscle, and heart. In the few cases in which electron microscopic study of liver was performed, there were alterations of mitochondrial ultrastructure similar to those seen in MCAD and VLCAD deficiencies, including condensed mitochondrial matrixes and widened cristal spaces.[173,182] In a few cases, the liver was reported to be fibrotic or frankly cirrhotic, which is not a typical feature of fatty acid oxidation disorders.

Diagnosis. Because neonatal hypoglycemia is often the earliest symptom and may respond to therapy, it should not be ignored because the major symptoms of LCHAD deficiency appear shortly thereafter. Blood acylcarnitine analysis at the time of hypoglycemia can often reveal the presence of this disorder permitting early dietary intervention. Similarly, analysis of a blood spot for the 1528G > C mutation can also be helpful. This mutation is present in homozygous form in about 70 percent of affected individuals. For that reason, the combination of acylcarnitine analysis and mutation analysis from blood spots will identify those affected compound heterozygotes compared to true heterozygotes for that mutation.

LCHAD activity in fibroblasts is usually assayed in the reverse of the physiological direction, using 3-ketoacyl-CoA compounds such as 3-ketopalmitoyl-CoA as substrate. Because of the overlapping substrate specificities of LCHAD and SCHAD, however, most patients with LCHAD deficiency have demonstrated 15 to 35 percent of control levels of activity with 3-ketopalmitoyl-CoA as substrate. The specific LCHAD defect is more clearly revealed using an antibody directed against SCHAD to remove this activity. Under these assay conditions, parents' cells have intermediate levels of LCHAD activity, consistent with heterozygosity. As illustrated below, it is best to assay for all three enzyme activities to identify the trifunctional protein deficiency.[185] Prenatal diagnosis can also be accomplished by direct enzyme assay of amniocytes or by probing the β oxidative pathway as previously described.[155]

Obstetrical Complications and LCHAD Deficiency. The association between acute fatty liver of pregnancy and LCHAD deficiency, was first pointed out in 1991 by Wilcken's group.[187] That same year, in an editorial, several kindred were reviewed, which illustrated that this complication of pregnancy, as well as the HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelets), and/or hyperemesis was observed, and was indeed frequent. Of six pregnancies in which the fetus was affected with LCHAD deficiency, these complications were observed.[188] Subsequently, the evidence for this association with LCHAD deficiency has increased such that it is now prudent to consider determining if the mother with a history of such pregnancy complications or with the initial onset of these problems is heterozygous for 1528G > C.[189–192] With prior history of AFLP or HELLP syndrome and demonstration of this common LCHAD mutation in the mother, amniocentesis should be considered to determine whether the fetus is affected with LCHAD deficiency in order to preserve mother and child and provide opportunity for early therapy of the affected infant.

The study by Tyni et al.[185] revealed that many of the LCHAD-deficient cases they reviewed had anemia (hemolysis), elevated liver enzymes, and low platelets raising the question of HELLP syndrome in the affected children. More detailed investigation of affected children may clarify this issue and might relate to the pathogenesis of the obstetrical complications in mothers of affected fetuses. To date, there has been no clear correlation of obstetrical complications in mothers whose fetus is not affected with LCHAD deficiency.

There has also been a report of acute fatty liver of pregnancy in a mother at risk for having a fetus with carnitine palmitoyltransferase I deficiency (hepatic form).[193] These obstetrical complications have, as yet, not been associated with other disorders of fat oxidation.

The demonstration that both rat[41] and human[90] liver LCHAD activities reside in a trifunctional protein bound to the inner mitochondrial membrane, along with 2-enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities, has a bearing on the interpretation of this enzyme defect. Jackson et al.[22] were the first to report a combined defect of these three activities. Most patients with LCHAD deficiency have not been evaluated for the other enzyme activities of the trifunctional protein, but in a few patients in whom they have been measured, long-chain 2-enoyl-CoA hydratase[22,140,147] and long chain 3-ketoacyl-CoA thiolase[22,140,146,147] activities also were reduced. Residual thiolase and hydratase activities were not zero, suggesting that either more than one enzyme contributes to each reaction or that the mutation(s) may have more severe effects on LCHAD than on the other activities of the trifunctional protein.

Mitochondrial Trifunctional Protein Deficiency. Using HPLC techniques and enzyme assays of activities associated with the purified trifunctional enzyme, a combined deficiency of LCHAD long chain oxoacyl-CoA thiolase and long chain 2-enoyl-CoA hydratase has been documented.[181,194] Even though these studies have shown reduction of all three activities, some patients excrete 3-hydroxy monocarboxylic and 3-hydroxy dicarboxylic acids. This observation suggests trifunctional enzyme deficiency in some of those patients previously identified as long chain 3-hydroxyacyl-CoA dehydrogenase deficiency.

Clinical Presentations: Case 1. Of three children in the family, one was normal and two died. The first, a boy, presented at 6 months of age after a period of refusing feeds. He was lethargic, severely hypotonic, areflexic and unresponsive to pain. Serum CPK was 3859 U/liter. He recovered but 6 weeks later was still areflexic and hypotonic. Muscle biopsy when well revealed type 1

fiber predominance and an excess of type 2c fibers. Muscle and serum carnitine concentrations were low. He remained areflexic and hypotonic and by 2 years he had developed plantiflexion contractures and was not walking. Following a 2-week respiratory illness at age 2.5 years, he presented with cardiac failure, an enlarged dilated heart, and generalized weakness. He died and autopsy was refused.

His sister was noted to be areflexic at 3 months of age. A mild equinus deformity and toe walking were noted at 20 months. She had two episodes of anorexia, hypotonia, and weakness by age 3 years without serious consequence. Following a 3-day illness, she was admitted with generalized muscle weakness and areflexia. The weakness progressed rapidly and she died on the third day with severe hyponatremia. Her serum CPK was 33,000 U/liter, glucose levels were normal, lactate was 4.92 mM, and urine organic acids showed small amounts of ethylmalonate, adipic, and suberic acids. A rapid postmortem was performed. In muscle, there was necrosis of 5 to 10 percent of fibers, the 95 percent of which were type II. There was very little lipid storage in muscle fibers, none in heart, and marked steatosis (microvesicular and macrovesicular) in the liver. Direct enzyme assay in isolated mitochondria was consistent with trifunctional protein deficiency with decreased levels of enoyl-CoA hydratase, LCHAD, and thiolase in muscle. Liver and heart mitochondria were assayed for the LCHAD and thiolase (but not the hydratase), and these activities were significantly reduced.[181]

Case 2. This child was the product of a normal uncomplicated pregnancy and delivery. The parents were consanguineous. At 48 h of age, the baby became hypoglycemic and developed hypotonia, which persisted despite normalization of blood glucose. Respiratory failure occurred on the eighth day requiring assisted ventilation. Cardiac failure occurred on the twenty-eighth day due to a hypokinetic-dilated cardiomyopathy. Death occurred on day 30 and autopsy was refused. Serum CPK was not determined. Urine organic acids showed saturated and unsaturated dicarboxylic and 3-hydroxydicarboxylic acids with the longest chain length being 12 carbons. Direct enzyme assay of the trifunctional protein in fibroblasts revealed significant decrease in all three activities.[194]

Pathologic Findings of LCHAD Deficiency. The information on the pathology of LCHAD deficiency is relatively limited. Skeletal muscle seems altered in that there is a predominance of type II fibers (slow oxidative) and necrosis associated with elevated CPK levels consistent with a rhabdomyolytic process. Dilated cardiomyopathy and hepatic steatosis are also prominent, but interestingly, lipid storage in skeletal and cardiac muscle was not impressive.

Diagnosis. Deficiency of the mitochondrial trifunctional protein is difficult to recognize and identify by the usual methods for diagnosis of fatty acid oxidation disorders. Urine organic acid analysis may or may not show dicarboxylic and 3-hydroxydicarboxylic aciduria. Unlike isolated LCHAD deficiency, there is no common mutant allele. The α subunit 1528G > C mutation is not observed in complete trifunctional protein deficiency. The disorder should be considered whenever there is hypoglycemia associated with hypotonia with or without dilated cardiomyopathy, especially when serum CPK and blood lactate levels are increased and hydroxydicarboxylic aciduria is present without the 1528G > C mutation and with a normal blood acylcarnitine profile. The trifunctional protein deficiency seems to be the only fatty acid oxidation disorder in which blood lactate is consistently elevated even when the patient is asymptomatic. Currently, there is no simple direct means to rapidly validate the presence of this defect. Skin biopsy and fibroblast studies are required. Oxidation rates in fibroblasts are reduced with palmitate, but that is not specific for the defect. Direct assay of the three enzyme activities is required to make the diagnosis.

Molecular Aspects. The human mitochondrial trifunctional protein (MTP) is a heteromer of four α and four β subunits. The α and β subunits are encoded by two nuclear genes,[168,195] and in humans, the MTP-α and MTP-β genes are located in the same region of chromosome 2p23.[196,197] The α-subunit (GenBank D16480) has two different activities: long chain enoyl-CoA hydratase and LCHAD; while the β-subunit (GenBank D16481) has the long chain 3-ketoacyl-CoA thiolase activity.

Recently, a G to C mutation at position 1528 (1528G > C) causing the missense mutation E510Q in the MTP α-subunit was reported.[186,190,198] This mutation is frequent in patients with LCHAD deficiency[186] and normal or near normal activities of long chain enoyl-CoA hydratase and long chain 3-ketoacyl-CoA thiolase.

Interestingly, exon skipping or point mutations that result in frameshifts or nonsense mutations in the α subunit cDNA apparently cause complete trifunctional protein deficiency with decreased activity of the three enzyme activities. For example, a patient with neonatal MTP deficiency was a compound heterozygote with two alleles each with mutations in the intron 3 donor splice site: a G to A transversion at the invariant position +1; and, an A to G transversion at position +3. Both alleles caused exon 3 (71 bp) skipping with a resulting frameshift, premature translational termination and undetectable levels of α-subunit protein and complete loss of MTP activity.[199]

Mutations in the β-subunit apparently cause MTP deficiency instead of LCHAD deficiency. Ushikubo et al. first reported disease-causing mutations in the MTP β gene.[200] Two nucleotide substitutions, 182G > A and 740G > A, were identified in a Japanese patient with MTP deficiency and an 788A > G mutation was found in the MTP β-subunit cDNA in another MTP deficiency patient. Aoyama et al. identified two Japanese patients with MTP deficiency.[201] One was homozygous for a 1331G > A transition (R411K) in MTP-β while a second was a compound heterozygote with an exonic insertion of a T which activates a new cryptic 5′ splice site and a 1331G > A transition (R411K). All three enzyme activities of MTP were undetectable in fibroblasts from these patients.[200,201] Mutation and cDNA expression data suggested that MTP activity depends on forming the $\alpha_4\beta_4$ heterooctamer.

Two patients with the unique and infrequent presentation of chronic progressive polyneuropathy and myopathy (with recurrent rhabdomyolysis) but no hepatic or cardiac symptoms were recently described. The mutations were identified only in exon 9 of the MTP α-gene. One child was homozygous for 845T > A while the other was a compound heterozygote for 914T > A (I-N) and 871C > T causing premature termination at residue 255.[202] It is not clear how these mutations produced this specific phenotype. Another study of 46 patients with LCHAD deficiency revealed 12 who were compound heterozygotes for the common mutation (1528G > C). In two of these, the second mutation was a 2129C insertion that changed the α-subunit reading frame and created a premature stop. In a third, a 1025T > C mutation was identified in the α-subunit, resulting in an L342P substitution.[203]

Currently, there are no obvious explanations correlating genotype with the multiple phenotypes associated with defects involving the MTP. Only a few laboratories provide reliable assays of all three enzymatic activities involved in this complex protein. This has impeded the development of a clear picture of the functional impact of the observed mutations on mitochondrial fatty acid oxidation.

2,4-Dienoyl-CoA Reductase Deficiency. ***Clinical Presentation.*** A single patient[204] has been described with this enzyme deficiency affecting the degradation of unsaturated fatty acids with even-numbered double bonds, such as linoleate (9-*cis*,12-*cis*-C18:2).

Case Report. This 2.2-kg black girl was the product of an uncomplicated pregnancy, born to unrelated parents. Hypotonia, intact deep tendon reflexes, microcephaly, a small ventricular septal defect, short trunk, arms and fingers, small feet, and a large

face were noted. Her karyotype was 46XX. Although she was discharged from the hospital at 2 days, she was readmitted a few hours later with sepsis. Despite therapy, she remained hypotonic, with poor feeding, inadequate weight gain, and intermittent vomiting. Gastrostomy and fundoplication were required. Unresponsive respiratory acidosis developed, and she died at 4 months of age. Organic acid analysis of many urine samples was normal, with no evidence of dicarboxylic acids or acylglycines. Plasma amino acid analysis revealed an unexplained hyperlysinemia. Plasma carnitine was low (total, 16 μM; esterified, 6 μM).

Clarification of the phenotype of this disorder must await the identification of other patients. Their recognition appears possible only if hypotonic infants are evaluated for the presence of decadienoyl (C10:2)-carnitine in plasma (see below). Preliminary management of this disorder probably should include avoidance of fasting, reduction of long chain unsaturated fat in the diet and nutritional supplementation with MCT and perhaps carnitine.

Diagnosis. Analysis of acylcarnitines in plasma and urine by tandem mass spectrometry demonstrated a C10:2-acylcarnitine species identified as 2-*trans*,4-*cis*-C10:2, an intermediate in the degradation of linoleic acid and a substrate for 2,4-dienoyl-CoA reductase. Assay of this enzyme in postmortem muscle and liver revealed 17 percent and 40 percent of control activities, respectively. Fibroblasts were not available for analysis. Using a more sensitive assay, enzyme activity in lymphoblastoid cells from the father was 52 percent of controls. It is assumed, therefore, that the defect is inherited as an autosomal recessive trait. A sib born subsequently appears normal, without hypotonia, and with no evidence for the defect, either by enzyme assay or acylcarnitine analysis.

Pathologic Findings. Autopsy revealed only pulmonary vascular congestion and bilateral ventricular hypertrophy, in addition to the congenital abnormalities described above. There was no evidence of steatosis; no ultrastructural studies were performed.

Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

Clinical Presentations. Since the first children with MCAD deficiency were described in 1982-1983,[4-7] it has become recognized as one of the most common inherited disorders of fatty acid oxidation mainly affecting Caucasians of northern European origin. The family in the following case report illustrates the marked phenotypic heterogeneity associated with MCAD deficiency even in the same family.

Case Report. The parents were unrelated and of English-Irish origin. They had three sons, the youngest of whom had an apparently minor respiratory illness at 18 months of age and was found dead in bed the following morning. Despite his age, the autopsy findings were considered consistent with SIDS. Subsequently, his 23-month-old brother had gastroenteritis and vomiting, which progressed to lethargy and seizures. He was hospitalized and noted to be hypoglycemic and hyperammonemic. He became comatose and died 4 days later. Autopsy revealed marked hepatic steatosis and cerebral edema, and it was concluded that he died of Reye syndrome. An older brother was asymptomatic. At the time of the second child's death, the mother gave birth to her first daughter. A combination of studies—organic acids, enzyme assays, acylcarnitine profiling, and molecular analysis—confirmed that all four siblings had MCAD deficiency; in the two autopsy cases, molecular analysis was performed on paraffin-embedded liver. The oldest male, now 10 years old, has never had an episode of illness related to his MCAD deficiency. He and his sister have been treated with frequent feeding, avoidance of fasting, and long-term carnitine supplementation. His sister had multiple episodes of illness requiring hospitalization during the first 2 years of life, for which she was treated in the short-term with IV glucose and oral carnitine supplementation. Now aged 15 years, she has been free of episodes for over 10 years. Both sibs had varicella with no significant metabolic imbalance during that time.[205] This family exemplifies the considerable phenotypic heterogeneity of MCAD deficiency: sudden death in infancy, Reye syndrome, and asymptomatic and presymptomatic detection with subsequent illness treated with appropriate intervention. It also emphasizes the absolute necessity for evaluating sibs of affected children.

While there is no typical presentation of MCAD deficiency, some common features of the disease should be noted.[8,9] The child with MCAD deficiency often presents with an episode of vomiting and lethargy following a period of fasting. There may have been a prior viral infection (gastrointestinal, upper respiratory), associated with decreased oral intake. There is occasionally a history of previous similar episodes. On presentation, the child may be comatose; blood glucose may be low and often there are no or only low to moderate levels of ketones in the urine. Blood ammonia levels and liver function tests may be abnormally high. Intravenous infusion with 10 percent glucose results in some improvement but even after correction of hypoglycemia, the child may remain obtunded for several hours. These patients are asymptomatic between episodes and can be prevented from having additional episodes by avoidance of fasting and provision of carbohydrate calories as intravenous glucose during intercurrent infections.

With the exception of a few reports in which a parent and several children all had MCAD deficiency,[206] the parents in all other families tested have half-normal levels of MCAD activity and normal plasma carnitine levels; they do not excrete unusual organic acids in urine and are clinically asymptomatic. These data are consistent with heterozygosity for an autosomal recessive disease.

The case report above documents that there is significant phenotypic heterogeneity in MCAD deficiency, even within the same family. There is little doubt that the first episode can be devastating, resulting in sudden death. The children may have only one episode of illness or multiple recurrences; in some cases, they appear to be asymptomatic.

One clinical review[207] showed that of 94 families with MCAD deficiency, 19 (20 percent) had one or more unexplained childhood deaths. In all of these, the diagnosis of MCAD deficiency was made postmortem. Of the 104 affected children in these families, there were 25 deaths. The earliest onset of symptoms and sudden death was in the neonatal period, although this is rare. The latest onset of the first episode, to our knowledge, was at 14 years of age in a girl who enrolled in a weight loss program and became comatose after losing 20 kg of weight (Marsden D, personal communication). To date, there have been a few known MCAD-deficient women who have tolerated pregnancy and delivery without significant problems.

Most children present with acute illness between 3 and 15 months of age. There are few reports of first symptoms after age 4 years, there are fewer recurrent episodes after 4 years, and symptoms requiring repeated hospitalization in the second decade are unusual. In the first year of life, 12 of 63 children (19 percent) died with their first episode. After 12 months of age, 9 of 41 children (22 percent) died with their first episode. Four children died with a later episode. Children with MCAD deficiency clearly are at significant risk of death with either the initial or a later episode. At the time of death, the diagnosis of MCAD deficiency had not been established in 25 children.[207] In contrast, the diagnosis had been established in all 79 survivors. There were no deaths due to MCAD deficiency following diagnosis, suggesting that early diagnosis, possibly by neonatal screening, before onset of symptoms, may be associated with a striking reduction in mortality.

The risk of death in these children is not the only consequence of MCAD deficiency. It was previously assumed, erroneously, that survivors were normal and had few long-term sequelae. A follow-up survey of 78 MCAD-deficient survivors (all > 2 years of age) revealed a number of unexpected problems[207,208] (Table 101-2).

Table 101-2 Consequences of Illness in 78 Surviving MCAD-Deficient Patients

Clinical Finding	No. (%) of Patients*
Developmental disability	16 (21)
Speech and language delay	16 (21)
Behavioral problems	12 (15)
Attention deficit disorder	9 (12)
Proximal muscle weakness	13 (17)
Chronic seizure disorder	13 (17)
Cerebral palsy	8 (10)
Failure to thrive	10 (13)

*These total more than 100 percent because some patients had more than one finding.

Routine developmental assessment was abnormal in 29 patients (37 percent) and showed global developmental disability in 16 (21 percent), speech and language delay in 16 (21 percent), behavioral problems in 12 (15 percent), and attention deficit disorder in 9 (12 percent), 8 of whom were females, in contrast to the usual male preponderance of this disorder in the general population. Complete aphasia followed an episode of illness in four (5 percent). Chronic somatic complaints included proximal muscle weakness in 13 (17 percent), seizure disorder in 13 (17 percent), cerebral palsy in 8 (10 percent), and failure to thrive in 10 (13 percent). The development of muscle weakness was strongly correlated with the length of time between symptomatic presentation and the institution of appropriate measures to prevent further episodes of illness. Therefore, seemingly minor delays in recognizing this disease and initiating appropriate therapy may place these patients at high risk of long-term disability.

Information was also gathered on the sibs of 55 probands with MCAD deficiency.[206] Of 109 sibs, 15 died, 8 of whom were shown to have MCAD deficiency by DNA and acylcarnitine analysis. In all, 64 surviving sibs were investigated: 15 (24 percent) were MCAD-deficient, 29 (46 percent) were carriers, and 20 (32 percent) were normal, close to the expected numbers for an autosomal recessive disorder. These data emphasize the importance of investigating any apparently normal sibs following identification of a new proband with MCAD deficiency. The younger sibs, in particular, are at risk for a fatal first episode. The parents also should be investigated; it is possible, given the frequency of the mutant gene in the population, that an apparently unaffected parent is MCAD-deficient.[206] When unexplained deaths occur in families known to be segregating MCAD deficiency, an effort should be made to establish a postmortem diagnosis, by analysis of postmortem blood or the original newborn screening card. Techniques for these studies are described below.

Diagnosis. The clinical findings of recurrent vomiting leading to obtundation or coma in a child or neonate should immediately raise the possibility of this and other diseases of fatty acid oxidation. Failure to consider the diagnosis of MCAD deficiency in children with this phenotype and to initiate appropriate therapy immediately can have fatal consequences. Even the interval between presentation and receipt of results of diagnosis laboratory results is critical. During the interval of 1 to 2 h required to receive results of such tests, many children with MCAD deficiency have died. In some instances intravenous therapy was instituted but with normal saline rather than a high carbohydrate solution. Although the reflex of administering normal saline may be valid for adults with diabetes mellitus, it is not appropriate for children with these symptoms. Once the blood has been drawn and a blood glucose has been determined at the bedside, a solution of 10 percent glucose, $\frac{1}{4}$ normal saline should be given intravenously at a rate of 1.5 to 2 times maintenance while waiting for the results of early diagnostic tests. It is critical to be aware that the hypoglycemia in this disease follows obtundation. A child with MCAD deficiency may be obtunded due to toxic effects of accumulated metabolites (medium chain fats, ammonium) before hypoglycemia becomes apparent. Correction of hypoglycemia and provision of adequate amounts of fluids and electrolytes is the initial step towards correction of metabolic imbalance and elimination of toxic metabolites.

The diagnosis of MCAD deficiency can be determined in 24 to 48 h by any specialized laboratory using tandem MS of blood spots for acylcarnitine species. The profile of acylcarnitines is unique and specific for MCAD deficiency.[209,210] It includes C6:0-, 4-*cis*- and 5-*cis*-C8:1-, C8:0, and 4-*cis*-C10:1 acylcarnitine species. These can be quantitated by stable isotope dilution mass spectrometry, but the plasma acylcarnitine profile is so specific for MCAD deficiency that quantitation is generally not necessary. With this technique of plasma analysis, there is no longer any requirement for oral carnitine loading as a diagnostic test. Unlike the urinary organic acid profile, the plasma acylcarnitine profile is diagnostic in both sick and well children with MCAD deficiency and can be analyzed with high sensitivity using blood spotted onto a newborn screening (Guthrie) card.[211] Affected infants can therefore be detected prior to the onset of symptoms. Postmortem blood obtained for other reasons (e.g., toxicologic analysis) in cases of sudden death can be analyzed in the same manner.[210–212] Analysis of the same blood spot for the 985G mutation commonly found in MCAD deficiency is also helpful with the caveat that ≈10 percent of patients are compound heterozygotes. Urine organic acids during acute illness will show the characteristic acylglycine elevations (hexanoyl- and suberyl-glycine), which are also elevated in children with multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II).

In view of the specificity and sensitivity of acylcarnitine analysis, it is not necessary to wait for establishment of a skin fibroblast culture to analyze fatty acid oxidation or to measure MCAD activity directly. The 8- to 10-week delay incurred by these tests is a deterrent to appropriate intervention for the affected child and further delays assessment of potentially affected sibs. In many children with MCAD deficiency, absolute carnitine excretion (μM/kg day) is decreased, paralleling the reduced concentration of carnitine in tissues. Six untreated cases of MCAD deficiency had tissue carnitine levels 16 to 42 percent of normal in liver and 16 to 25 percent of normal in muscle.[7,213,214] Prior to onset of symptoms an affected, breast-fed infant may have normal plasma carnitine levels. However, plasma carnitine levels are not diagnostic and should not precede the analysis of the acylcarnitines by tandem MS for a rapid and specific diagnosis.

The preferred enzymatic method for measuring MCAD and other acyl-CoA dehydrogenase activities is the ETF-based assay.[215,216] The specific enzyme defect has been demonstrated in fibroblasts, peripheral mononuclear leukocytes, liver, heart, skeletal muscle, and amniocytes.[8] There has been no obvious association between the residual MCAD activity and the severity of clinical disease in these patients. Nor has there been any association between measured enzyme activity and genotype, as determined by molecular analysis (described below).

Prenatal diagnosis of MCAD deficiency is an option for early detection of an affected fetus. It is likely, however, that prenatal diagnosis will rarely be sought for the purpose of pregnancy termination, because appropriate management and intervention seem to be effective. At the very least, prenatal diagnosis offers the earliest possible detection of an affected child, so that treatment can be instituted. An alternative to direct enzyme assay for prenatal diagnosis, which is especially useful in pregnancies following the death of a proband not specifically diagnosed, is the incubation of amniocytes with radiolabeled palmitate followed by acylcarnitine analysis of the culture media. This assay permits identification of most fatty acid oxidation defects including MCAD deficiency.[155] It is not reliable for prenatal diagnosis of CPT I and SCAD deficiencies.

Treatment of MCAD deficiency continues to emphasize avoidance of fasting, mild reduction of dietary fat intake (≈20 percent of total calories), and carnitine supplementation which is especially helpful during illness for conjugation of the toxic acyl-CoA intermediates which are then readily excreted as non-toxic acylcarnitines. Considering the high occurrence of sudden death in children under 2 years of age, and our inability to predict episodes, it is wise to provide this supplement from early in life. The family and the personal physician should be educated to understand that an intercurrent viral illness well tolerated by a normal child can produce a fatal episode in a child with MCAD deficiency. Anticipatory treatment with intravenous $D_{10}W$ plus $\frac{1}{4}$ normal saline at 1.5 to 2 times maintenance can prevent these episodes.

Pathologic Findings. The primary pathologic findings in MCAD-deficient patients include light microscopic and ultrastructural changes mainly in liver, but alterations have been observed in other tissues. Cerebral edema is noted postmortem in most cases.[7] Hepatic light microscopic alterations in specimens taken during acute illness are usually limited to steatosis,[158,214] although this is not a universal finding,[217] and may be either macrovesicular or microvesicular in nature.[158] Generally, the steatosis disappears on recovery. In some cases, the microvesicular fat accumulation has been similar to that seen in Reye syndrome. In those instances, however, ultrastructural studies[158,218] clearly demonstrate that the generalized mitochondrial changes characteristic of Reye syndrome are not present. Specifically, the matrix swelling and rarefaction commonly seen in hepatic mitochondria of patients with Reye syndrome have not been observed in MCAD deficiency.[158] Instead, increased matrix density and intracristal widening give the mitochondria a condensed appearance. In a few patients, there are so-called crystalloids in the matrix, associated with an increased number of cristae and with enlargement and abnormal shape of the mitochondria, although some have normal mitochondrial ultrastructure. Condensed mitochondria have been described in other disease states, and crystalloids have been noted in mitochondria from patients with other fatty acid oxidation defects (see above), carnitine-deficient myopathy, and other myopathies,[219] so these findings are not diagnostic.

Molecular Aspects. Within the past few years, a single, highly prevalent mutation within the coding region of MCAD cDNA has been identified among patients with documented MCAD deficiency.[220–230] This nucleotide substitution (985A > G) results in missense mutation (K329E) which alters the amino acid sequence in an α-helical region in the C-terminal half of the peptide.

Figure 101-6 illustrates the structure of the MCAD gene and the location of mutations associated with MCAD deficiency. Among 172 patients with confirmed MCAD deficiency,[229] 138 (80.2 percent) were homozygous for 985A > G, a further 30 (17.4 percent) were compound heterozygotes for this allele and 4 (2.3 percent) did not have the 985A > G change on either allele. Of the 344 mutant MCAD genes, 306 (88.9 percent) were 985A > G. Among the remaining 38, the mutations in 11 were identified: 3 were a 13-bp repeat from nt 999;[227,231,232] 2 were a 4-bp deletion from nt 1100;[233,234] 2 were a 799G > A transition;[227] and 4 were nucleotide substitutions, 157C > T,[232] 447G > A, 730T > C, and 1124T > C,[227] identified in single alleles. Hence, none of the mutations other than 985A > G accounted for more than 1 percent of variant alleles. A total of 27 MCAD mutations remained unidentified in this series of patients, but in many cases, the above-mentioned mutations were excluded. The 985A > G allele, therefore, accounts for most of the mutant MCAD genes characterized in this large series of MCAD deficient patients. The approximate frequency of MCAD deficiency among Caucasians, based on several studies,[235,236] is between 1 in 6400 and 1 in 46,000, with a heterozygote frequency of 1 to 2 percent. That a single mutation accounts for approximately 90 percent of the disease-causing mutant genes in an outbred population is unusual. Furthermore, it is striking that MCAD deficiency is found almost exclusively among Caucasians, particularly those of northwestern European origin.[225,227,232,237] The few patients identified in other populations include one Pakistani patient (homozygous for 985A > G,[223] one African-American (Roe CR, unpublished observation), and isolated cases of southern European and North African origin. No patients have been identified in the Far East, nor were any carriers found by newborn screening in Japan.[236]

The human MCAD gene is highly polymorphic,[238–240] and with the use of three restriction enzymes, *Sac*I (or *Ban*II), *Taq*I and *Pst*I, several restriction fragment-polymorphism (RFLP) haplotypes have been identified.[231,241,242] One haplotype, resistant to all three enzymes (designated ———), occurred in 23 percent[241] and 35 percent[227] of control alleles, but was found in virtually all of the 985A > G alleles in all three studies. This result suggests that the 985A > G mutation arose on the background of a ——— allele and gradually spread in the population. Two studies have demonstrated the particularly high frequency of the allele in patients of northwestern European origin, particularly among MCAD-deficient patients in the United Kingdom and Germany. These observations suggest that the 985A > G mutation occurred in a historical population occupying one of these regions and spread throughout northwestern Europe.[227,232]

Even before the discovery of the 985A > G allele (K329E), studies aimed at understanding the molecular basis of MCAD deficiency were performed.[243] By pulse-labeling of nascent MCAD in cultured fibroblasts with [^{35}S] methionine, the synthesis of the precursor MCAD subunit and its proteolytic processing to a mature subunit within the mitochondrial matrix were shown to be normal in cells from patients with MCAD deficiency. All but one of these patients were later found to be homozygous for the 985A > G mutation; the exception was a compound heterozygote[231] for 985A > G and the 13-bp insertion beginning at base 999. Immunoblot analysis of MCAD in fibroblasts from all of these patients,[244] as well as from other homozygotes for 985A > G,[245,246] have shown little or no immunoreactive MCAD. These results demonstrate that, despite normal translation and immediate posttranslational processing of the K329E-MCAD subunit, virtually none of the variant protein is detectable in the steady state. This suggests that the K329E-MCAD protein is unstable in the mitochondria. This conclusion is supported by

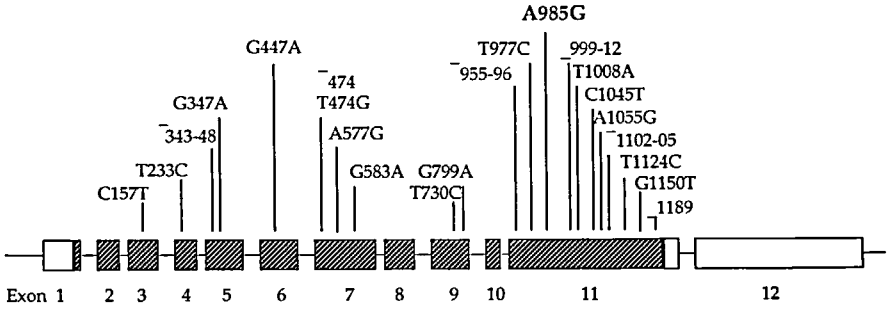


Fig. 101-6 The human MCAD gene and mutations. Boxes indicate exons; hatched areas are coding regions with mutation positions; open boxes indicate either 5′ or 3′ UTRs.

pulse-chase metabolic-labeling experiments using [^{35}S]-methionine, showing that the K329E MCAD protein, in contrast to wild-type MCAD, disappears almost completely over 24 h of incubation in unlabeled methionine.[244] However, these data do not agree with those of Kelly et al.[224] whose immunoblot studies demonstrated that fibroblasts from 985A > G homozygotes had detectable MCAD. There is, as yet, no obvious explanation for the discrepancy between these findings.

Several mechanisms could explain the apparent instability of the K329E MCAD subunit. These mechanisms include inherent instability of the mutant protein, inhibition of tetramer formation or disruption of normal tetramer structure. Computer analysis predicts that the K329E substitution would not cause drastic changes in MCAD secondary structure.[221] K329E is not involved in FAD binding or in substrate binding,[247] but, instead, contributes to the interface between subunits in the tetramer structure.[56,248] Replacement of a basic residue in this region by an acidic one may hinder tetramer formation or disrupt normal tetramer structure. Site-directed mutagenesis showed that a basic residue at position 329 is important for tetramer stability and/or assembly.[249,250]

Transfection of an 985A > G MCAD cDNA into bacteria,[225,251,252] green monkey kidney (COS-7) cells,[251] and Chinese hamster ovary cells[253] demonstrated that the K329E substitution significantly impairs MCAD activity. A normal amount of mRNA was produced but most of the variant protein recovered was in insoluble aggregates (bacteria) or partially degraded (COS-7 cells). By contrast, normal MCAD was soluble.[251,252]

Curiously, the 985A > G mutation, which occurs in exon 11, appears to be associated with a high degree of missplicing of MCAD mRNA; 30 to 40 percent of the mature 985A > G transcripts have exon deletions (particularly exon 2 or 5) and/or intron retention.[54,224,225] In some transcripts, the splicing apparatus utilizes a cryptic acceptor splice site in exon 11 downstream from the site of the nucleotide substitution, with the result that the sequence encoded in the 5′ half of exon 11 is deleted from the mature transcript. The molecular explanation(s) for the association of the aberrant splicing with the nucleotide substitution at position 985 is not known; however, even the transcripts derived from the normal MCAD gene exhibit a high degree of aberrant splicing (5 to 10 percent of the total cDNA clones isolated from various tissues).[54]

Studies of the molecular pathology of some of the other MCAD mutations have been reported. The 4-bp deletion (nt 1100 to 1103) described in two compound heterozygous patients[233,234] predicts a frameshift beginning at codon 369, leading to an MCAD precursor with 16 altered residues and truncated at residue 385, 36 residues shorter than normal. By immunoblot analysis, a variant MCAD precursor 4 to 5 kDa smaller than normal was identified in liver from a patient with this allele[234] and in *E. coli* transfected with mutant cDNA.[233] The 13-bp repeat insertion described in heterozygous form in three patients[231,232] predicts a truncated protein, although none was found in cell labeling[243] or immunoblot[244] experiments. This result suggests that the RNA produced from this allele or its protein product is unstable.[227]

Implications for Diagnosis and Screening. The molecular characterization of MCAD deficiency has made it possible to assess the various methods employed in the diagnosis of this disease. For this purpose, data were gathered on acylcarnitine profiles and DNA analysis in samples from members of 25 families with MCAD deficiency. Of the 62 children analyzed, 36 had MCAD deficiency, 28 were homozygous for 985A > G, and 8 were compound heterozygotes for this mutation and a rarer allele (4-bp deletion from nt 1100, 799A > G, 157C > T). Blood acylcarnitine profiles were diagnostic of MCAD deficiency in all cases. A further 18 children were heterozygotes for A985G and a normal allele, 1 was a carrier for a rare mutation (799A > G), and 5 were normal; acylcarnitine profiles were normal in all 62 of these children. They were also normal in blood from all 46 of their parents. Thus, blood acylcarnitine profile appears to identify all patients with MCAD deficiency and is normal in heterozygotes. Mutation analysis clearly distinguishes 985A > G homozygotes and carriers from normals but cannot distinguish compound heterozygotes for 985A > G and some other rare alleles. The combination of molecular analysis plus acylcarnitine profile provides the most information.

Direct assay of MCAD activity does not always discriminate between affected, carrier, and normal individuals. It can occasionally miss affected individuals, and is not absolutely reliable for carrier detection. Given that other biochemical and molecular methods have been developed which are reliable and rapid, enzyme assay would not have a role in screening.

MCAD deficiency satisfies all the major criteria for newborn screening.[228] It is a common inherited disease, with a frequency approaching that of phenylketonuria. The disorder can result in significant clinical disease that may be fatal. Relatively simple dietary means and anticipatory management can avert the clinical phenotype of MCAD deficiency. Affected individuals are for the most part asymptomatic in the newborn period, but the molecular defect which underlies most mutant alleles, as well as some of the key abnormal metabolites, can be detected with high accuracy and specificity in blood samples spotted onto newborn screening cards.[210,232,235,236,254,255] For these reasons, the screening of all newborns for MCAD deficiency appears to be justified. Similarly, screening of at-risk populations, either retrospectively (e.g., by analysis of postmortem specimens[222,256,257] from patients dying with SIDS) or prospectively (e.g., sibs of patients with confirmed MCAD deficiency) can be done.

Management and Treatment. The primary goals for management of MCAD-deficient patients include provision of adequate caloric intake, avoidance of fasting, and aggressive support during infectious episodes. The anorexia that often accompanies infection and fever predisposes to mobilization of endogenous lipid stores, producing toxic intermediates (see "Pathogenesis of MCAD Deficiency" below) which can lead to vomiting, lethargy, coma, and even death. Although some children with MCAD deficiency appear to have good fasting tolerance in the absence of infection, they usually do not do well with infection and may require IV glucose to halt the process. The risk of death and the frequency of other residual abnormalities (see Table 101-2) leave little doubt about the urgency of early diagnosis and appropriate treatment.

L-Carnitine supplementation has been advocated in the management of MCAD deficiency, as well as other causes of secondary carnitine deficiency. Its use in this setting represents a useful conjugation pathway for the removal of potentially toxic intermediates that accumulate under conditions of fasting stress or infection in these patients.[204] For example,[258] an asymptomatic MCAD-deficient patient receiving oral carnitine supplementation (100 mg/kg day) excreted 7 mM total carnitine per milligram of creatinine, of which only 0.4 mM was octanoylcarnitine. The same patient when acutely ill and treated with IV carnitine (30 mg/kg day) excreted 13 mM octanoylcarnitine/mg creatinine; when she had recovered and was receiving the same IV dose of carnitine, she excreted only 0.6 mM octanoylcarnitine/mg creatinine. Thus, on IV therapy octanoylcarnitine excretion during illness was 21 times greater than that observed when well. The enhanced production and excretion of octanoylcarnitine in the sick child suggests that carnitine supplementation is useful for conjugation and excretion of toxic metabolites during illness, but may serve little purpose during periods of good health.

Carnitine supplementation in MCAD deficiency is analogous to the provision of glycine to patients with isovaleric acidemia (see Chap. 87). Glycine supplementation augments the excretion of toxic intermediates (isovalerate) from tissues while restoring CoA levels. Carnitine supplementation does not correct the underlying defect in MCAD deficiency,[259,260] but neither is it associated with enhanced turnover of fat in this disorder,[261] a theoretical concern that arises because carnitine mediates the mitochondrial uptake of fatty acids. There are apparently no toxic effects from carnitine

supplementation other than occasional loose stools and a dose-dependent fishlike body odor in children receiving very high doses. This odor results from bacterial degradation of L-carnitine in the gastrointestinal tract producing trimethylamine, and is not apparent with the usual doses utilized in therapy.

Pathogenesis of MCAD Deficiency. There is substantial information available from controlled fasting studies[9,262] and from the analysis of urinary metabolite excretion profiles[204,263–265] to allow speculation on the sequence of metabolic events in MCAD deficiency and their potential consequences. While the following discussion refers to MCAD deficiency, much of it is relevant to the pathogenesis of all fatty acid oxidation defects.

One major consequence of MCAD deficiency is the failure to make ketone bodies in quantities sufficient to meet tissue energy demands under conditions of fasting stress. During the initial stages of fasting, patients remain well and their glucose levels are normal. As plasma free fatty acids rise with continued fasting, there is no attendant increase in plasma ketones. Hypoglycemia develops presumably as a result of the exhaustion of glucose production, because ketones and fatty acids are unavailable to substitute for glucose as metabolic fuels.

A further consequence is the accumulation of medium chain (C8-C12) acyl-CoA intermediates in mitochondria at the expense of acetyl-CoA. Free CoA, which does not exchange between the mitochondrial and cytosolic compartments, is also compromised. Thus, increased acyl-CoA production results in decreased CoA availability for other mitochondrial reactions. The acyl-CoA:CoA ratio in mitochondria exerts regulatory control over pyruvate dehydrogenase[266] and α-ketoglutarate dehydrogenase.[267] When this ratio is elevated, both enzymes are inhibited, resulting in reduced conversion of pyruvate to acetyl-CoA and reduced flux through the tricarboxylic acid cycle, because citrate synthesis and flux from α-ketoglutarate to succinyl-CoA are impeded. Succinyl-CoA ligase is also inhibited by octanoate,[268] as well as by other acyl-CoA intermediates. When these intermediates accumulate, mitochondrial β-oxidation is inhibited;[11] the expected result is fatty acid incorporation into triglycerides, consistent with the marked accumulation of fat in liver during acute episodes.[158]

Inadequate acetyl-CoA production has significant secondary effects on flux through the tricarboxylic acid cycle, on regulation of fatty acid oxidation in mitochondria, and on the efficiency of gluconeogenesis. Acetyl-CoA is a substrate, along with oxaloacetate, for the citrate synthase reaction. Inadequate amounts of either substrate result in diminished citrate synthesis. Inhibition of α-ketoglutarate dehydrogenase by the elevated acyl-CoA:CoA ratio reduces flux through the tricarboxylic acid cycle and impairs oxaloacetate synthesis. Furthermore, because citrate serves as a means to transport acetyl-CoA to the cytosol, decreased availability of citrate influences the regulation of both fatty acid oxidation and gluconeogenesis. When there is a sufficient quantity of citrate, it is transported to the cytosol and converted by citrate lyase into acetyl-CoA and oxaloacetate. The latter may be converted to malate or to phosphoenolpyruvate and ultimately glucose. Thus, both the redox state and gluconeogenesis are affected. Acetyl-CoA produced from citrate by citrate lyase in the cytoplasm is a substrate for acetyl-CoA carboxylase and synthesis of malonyl-CoA, the primary regulator of CPT I. Citrate not only is the source of substrate for acetyl-CoA carboxylase, but also is its primary activator. Decreased mitochondrial acetyl-CoA limits the amount of cytoplasmic citrate available to both activate and provide substrate for acetyl-CoA carboxylase. Reduced malonyl-CoA levels allow unregulated entry of fatty acids into mitochondria and, in MCAD deficiency, increased production of medium chain acyl-CoA intermediates.

Inadequate mitochondrial acetyl-CoA also affects pyruvate carboxylase. Acetyl-CoA is the primary activator of this biotin-dependent enzyme, which converts pyruvate to oxaloacetate and is critical for gluconeogenesis. Propionyl-CoA also activates pyruvate carboxylase, but longer chain compounds such as octanoyl-CoA do not. Compromise of this pathway in MCAD deficiency may account for the hypoglycemia.

The clinical presentation and many of the routine laboratory observations in MCAD-deficient patients are indistinguishable from those in Reye syndrome.[269] Octanoate infused into rabbits produces many of the pathologic findings of Reye syndrome.[270] The encephalopathy and cerebral edema observed in MCAD deficiency may result from similar mechanisms.[270,271] Acyl compounds with three or more carbons have significant encephalopathic properties;[272] the greater the chain length of the compounds, the more rapidly coma occurs. Furthermore, acyl compounds such as propionate, octanoate, and palmitate enter the central nervous system at rates increasing with longer carbon-chain length. There is potential, therefore, for rapid accumulation of fatty acids within the central nervous system. This may be exacerbated by octanoate-induced inhibition of the choroid plexus organic anion uptake system largely responsible for egress of these compounds from the central nervous system.[273] Experimentally induced octanoic acidemia damages neuronal mitochondria with distension and separation of mitochondrial cristae, and loss of matrix integrity.[271] Cultured astrocytes exposed to octanoate fail to maintain volume control.[274] These structural abnormalities together with depression of energy metabolism and the resultant decreased availability of high-energy phosphate compounds,[275] may lead to cerebral edema. Although hypoglycemia is a common finding in MCAD deficiency, coma in these patients is not due entirely to low blood glucose levels, since they may be encephalopathic despite correction of hypoglycemia.[4,7] Coma is more likely the result of toxic effect of fatty acids or their metabolites.

Short Chain Acyl CoA Dehydrogenase (SCAD) Deficiency

Clinical Presentations. SCAD deficiency has been identified in only a few patients[276–278] with highly variable clinical and laboratory findings, as demonstrated by the following cases.

Case 1. A female infant of unrelated parents (neonate II[277]) was delivered normally and began cow's milk formula on day 2. On day 3, she fed poorly, began to vomit, and became lethargic and hypertonic. She was hypoglycemic, acidotic (pH 7.28), and hyperammonemic (399 μM). In spite of IV glucose therapy, she became more lethargic, unresponsive, and hypotonic, with increasing respiratory effort. Organic acid analysis showed lactic acidosis, ketosis, and increased excretion of butyrate, ethylmalonate, and adipate. She died on day 6. Postmortem examination revealed cerebral edema, hepatosplenomegaly with fatty changes, cholestasis, and focal hepatocellular necrosis.

Case 2. A female infant[278] of unrelated parents presented in early postnatal life with poor feeding and frequent emesis. She exhibited poor weight gain, developmental delay, progressive skeletal muscle weakness, hypotonia, and microcephaly. Skeletal muscle biopsy showed minor generalized lipid accumulation in type I fibers. She never had episodes of hypoglycemia, rarely had organic aciduria, and had low-normal plasma carnitine levels. Her muscle carnitine level was 50 percent of control with 75 percent esterified. She responded poorly to a fat-restricted diet supplemented with L-carnitine. At 21 months, she had significant developmental delay. Increasing difficulty with poor oral intake required a gastrostomy tube at 23 months. At 32 months, she showed significant weight gain and overall improvement in strength.

Case 3. A 46-year-old woman[276] with no previous neuromuscular disorder presented with persistent weakness in one arm and both legs, exacerbated by mild exertion. Neurologic examination revealed a proximal myopathy, which was confirmed by electromyography. Serum CPK was normal. There was excess neutral lipid in type I skeletal muscle fibers, with no other abnormalities. Muscle carnitine levels were low (25 percent of control) with an

increased proportion of acyl to free carnitine. Plasma carnitine levels were low-normal. The major urinary metabolite was ethylmalonate, fasting was not associated with hypoglycemia, and blood ketone body levels were elevated. Her weakness did not respond either to carnitine or prednisolone.

Case 4. A female infant born to related Hispanic parents was severely developmentally delayed and extremely hypotonic. She excreted large quantities of ethylmalonate along with methylsuccinate. Her plasma butyrylcarnitine was consistently elevated. Her plasma lactate was normal. When these same parents had another pregnancy, an amniocentesis with enzyme assay in amniocytes yielded equivocal results suggesting partial decrease in SCAD activity. The family elected to terminate the pregnancy. Direct enzyme assay for SCAD with and without anti-MCAD antibody revealed normal activity in fetal liver but severe deficiency in fetal muscle (Kobori J, Vianey-Saban C, Roe CR, unpublished data).

Pathologic Findings. Pathologic findings in Case 3 were limited to muscle and included lipid vacuolization, especially in type I fibers.[276] Despite Case 2's clinical phenotype being primarily muscular,[278] her liver showed ultrastructural evidence of both microvesicular and macrovesicular steatosis, and mitochondrial changes reminiscent of those seen in MCAD deficiency, namely, increased matrix density and crystalloids (Douglas SD, Coates PM, unpublished observation). Case 1 had hepatic steatosis at autopsy.

Organic Acid Analysis. Patients with generalized SCAD deficiency excrete short chain organic acids (ethylmalonate, methylsuccinate, butyrylglycine) and butyrylcarnitine in urine. Although these metabolites are also excreted by patients with multiple acyl-CoA dehydrogenation defects,[279–281] the latter may be recognized by the presence of acylcarnitines[210] and other metabolites[279] derived from defective amino acid oxidation.

Diagnosis. The diagnosis of SCAD deficiency is made by measuring acyl-CoA dehydrogenase activities in available tissues. Because MCAD has broad substrate specificity with some activity towards short chain acyl-CoAs, the most direct measurements of SCAD are made in extracts in which MCAD activity has been eliminated by incubation with anti-MCAD antibody. With butyryl-CoA as substrate, fibroblasts from Cases 1 and 2 and another patient (neonate I)[277] had 50 percent of control SCAD activity, all of which was inhibited by incubation of the cells with anti-MCAD antibody.[277,278] Activity toward palmitoyl-CoA and octanoyl-CoA was normal. Cells from the parents of Case 2 had intermediate SCAD activity, consistent with heterozygosity for an autosomal trait.

Skeletal muscle SCAD activity in Case 3, measured with butyryl-CoA as substrate,[276] was 25 percent of control levels, and was associated with reduced immunoreactive SCAD;[282] dehydrogenase activities toward longer-chain acyl-CoA substrates (C8 or longer) were well within normal limits. SCAD activity in fibroblasts was normal.[277,278] While careful study of SCAD in other tissues from Case 3 could not be performed, the data suggested that this patient had an isolated deficiency of muscle SCAD, although a variant multiple acyl-CoA dehydrogenation defect could not be ruled out. The results with case 4, a severely affected infant, and analysis of an at risk pregnancy in the same family, suggest that there is a form of SCAD deficiency which is limited to muscle, without involvement of liver, which may manifest severe symptoms in infancy. Unfortunately, no direct assays have been performed on muscle or liver from the affected child, so this hypothesis cannot be tested directly. If the hypothesis is correct, prenatal diagnosis of such cases by enzyme assay of amniocyte extracts could be misleading.

In this context, there are a few patients with lipid storage myopathy in whom muscle SCAD (and sometimes MCAD) activity and antigen are substantially reduced,[283,284] who show clinical and biochemical improvement on treatment with pharmacologic doses of riboflavin; furthermore, SCAD activity and antigen are restored. These data are similar to those found in the riboflavin-deficient rat,[285–287] and suggest that patients such as these may have a form of riboflavin-responsive multiple acyl-CoA dehydrogenation defect.[288–295]

Butyryl-CoA dehydrogenase (SCAD) is known to be very labile and easily affected secondarily. Because some patients thought to have SCAD deficiency due to increased ethylmalonate excretion have also had persistent elevations of lactate, the possibility of a primary lesion in the respiratory chain should also be considered.

Pathogenesis. The pathogenesis of disease associated with SCAD deficiency presents some puzzling aspects, because there is no common thread among the patients who have been reported with this defect. Cases 3 and 4 appear to have an isolated muscle SCAD deficiency appearing either in infancy or adulthood, while other patients[277,278] have an enzyme defect expressed in fibroblasts. Because of its position in the β-oxidation pathway, it is unlikely that a defect in SCAD would have a major effect on the yield of energy from fatty acid oxidation, because at least three-fourths of a long chain fatty acid can be oxidized before SCAD activity is required. This prediction is supported by the finding that under conditions of fasting stress, SCAD-deficient patients were capable of mounting a ketogenic response. That some SCAD-deficient patients are profoundly affected suggests that the pathophysiology involves more than simple energy deficit.

Molecular Aspects. SCAD is a homotetrameric mitochondrial flavoenzyme that catalyzes the initial reaction in short chain fatty acid β-oxidation. The human *SCAD* structural gene is approximately 13 kb in length and is located at 12q22-qter.[296,297] Its 10 exons encode the entire 412 amino acid precursor SCAD (44.3 kDa) (GenBank M26393) including the 24-amino acid mitochondrial leader peptide and a 388-amino-acid mature protein (41.7 kDa).[52,297] Comparison of SCAD and MCAD sequences reveals a high degree of similarity, suggesting that these enzymes evolved from a common ancestral gene.[52]

Four *SCAD* gene polymorphisms have been reported. Three are synonymous variants (321T > C, 990C > T, 1260G > C), while one (625G > A) results in a glycine to serine substitution (G209S) that has not been associated with SCAD deficiency but that has been associated with ethylmalonic aciduria.[297]

136C > T (R22W) and 319C > T (R83C) mutations were first reported in a compound heterozygous patient with SCAD deficiency.[298] Four additional mutations, 274G > T, 529T > C, 1147C > T, and 511C > T, have also been identified.[299]

Elevated urinary excretion of ethylmalonic acid (EMA) is a common biochemical finding in patients with SCAD deficiency. When butyryl-CoA oxidation is reduced, as in SCAD deficiency, it is alternatively metabolized by propionyl-CoA carboxylase to EMA. Among 135 individuals with abnormal EMA excretion ranging from 18 to 1185 μmol/mol of creatinine (controls < 18 μmol/mol of creatinine) a significant overrepresentation of a variant allele was found.[300] Eighty-one individuals (60 percent) were homozygous for the 625G > A allele, 40 (30 percent) were heterozygous, and only 14 (10 percent) were homozygous for the wild-type allele. Corydon et al. believe that the 625G > A allele, as well as a recently identified 511C > T mutation,[299] are associated with the development of ethylmalonic aciduria.[297,300] The extent to which these mutations reduce SCAD activity, as determined by direct enzyme assay, has not yet been determined.

The BALB/cBy mouse strain is a model for SCAD deficiency. In this inbred line, a 278-bp deletion was identified in the 3′ end of the structural SCAD gene. This deletion results in missplicing of mRNA. The abnormal transcripts have aberrant stop codons and reduced steady state levels of SCAD mRNA.[301] Although the biochemical markers of the disease are present, there are no comparable symptoms as seen in affected humans.

Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase (SCHAD) Deficiency

Clinical presentations: Case 1. A 16-year-old girl presented with recurrent episodes of myoglobinuria, hypoketotic hypoglycemia, encephalopathy, and hypertrophic cardiomyopathy. Enzyme assays of a skeletal muscle homogenate had normal LCHAD activity with 3-ketopalmitoyl-CoA as substrate, but markedly reduced SCHAD activity with acetoacetyl-CoA as substrate; the defect was not expressed in fibroblasts. SCHAD activity was not measured in liver.[302]

Case 2. This boy was normal at birth and developed normally for the first year of life. At age 13 months, he had an episode of vomiting, lethargy, and dehydration. He also had three seizures associated with hyponatremia. A high-carbohydrate diet was not effective and he had continued to have episodes up to 3.5 years of age. Laboratory studies including glucose, electrolytes, bicarbonate, calcium, bilirubin, liver enzymes, ammonia, lactate/pyruvate, plasma amino acids, carnitine, and acylcarnitine analyses were normal. Urine organic acids revealed to reveal massive ketosis (β-hydroxybutyrate and acetoacetate) associated with long chain saturated and unsaturated dicarboxylic and monocarboxylic acids. Enzyme assays for LCHAD, SCHAD, and short chain 3-ketoacyl-CoA thiolase (SKAT) were performed on fibroblast extracts and on an isolated mitochondrial fraction. These enzyme activities were decreased from 34 to 41 percent in fibroblast extracts, and in mitochondrial fraction, LCHAD was reduced by 29 percent and SKAT by 43 percent, while there was a 95 percent decrease in SCHAD activity.[303]

Case 3. This female was diagnosed with DiGeorge syndrome (MIM 188400) with a microdeletion in 22q11. At 3 months of age, 1 month following cytomegalovirus septicemia, hepatomegaly was noted and electron microscopy of a liver biopsy showed lipid droplets and enlarged mitochondria. At 15 months, she had an episode of mild hypoglycemia associated with ketonuria. MRI of her head revealed bilateral demyelinating lesions of the periventricular white matter. Plasma carnitine was reduced at 22 mM and only C4 (butyryl-isobutyryl-) acylcarnitine was elevated on tandem MS analysis. Skin fibroblasts enzyme assays for LCHAD, SCHAD, and SKAT were not performed on disrupted cells but isolated mitochondria were assayed for SCHAD which was reduced to 6.6 percent of mean values and for SKAT, which was also reduced by 43 percent.[303]

These three cases reflect three very different phenotypes. Many more well-documented cases will have to be studied to determine optimal diagnostic tests, treatment, and pathogenesis.

Molecular Aspects. The human short chain L-3-hydroxyacyl-CoA dehydrogenase (*HADHSC*; EC1.1.1.35) structural gene (GenBank AF026853) is comprised of at least eight exons and is located at 4q22-26.[304,305] A cloned human liver SCHAD cDNA (GenBank X96752) has a 5′ untranslated sequence of 87 bases, an open reading frame of 942 nucleotides encoding a precursor protein of 314 amino acids and a 3′ untranslated sequence of 845 bases including the poly (A) tail.[305] The precursor protein (34.3 kDa) has a mitochondrial import signal peptide of 12 amino acids and a 302-amino-acid mature protein, which has 92 percent identity with the porcine enzyme. The human gene is expressed in skeletal and cardiac muscle, liver, kidney, and pancreas,[305] but not in fibroblasts, which raises a conflict with one of the case reports.[204,303]

A putative SCHAD pseudogene has also been identified and linked to marker D15S1324, located at Chr 15q17-21.[304]

Interestingly, a novel L-3-hydroxyacyl-CoA dehydrogenase from human brain has also been cloned, which is identical to an endoplasmic reticulum amyloid β-peptide-binding protein (ERAB) involved in Alzheimer's disease.[306] This human short chain L-3-hydroxyacyl-CoA dehydrogenase gene is organized into six exons and five introns and maps to chromosome Xp11.2.

No information is available about the mutations in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency.

GENERAL APPROACH TO THE PATIENT WITH A SUSPECTED FATTY ACID OXIDATION DISORDER

Individual defects and their various (often multiple) clinical phenotypes have been described above. This concluding section provides a summary of clinical and laboratory considerations for patients in whom a disorder of fatty acid oxidation is suspected.

When the possibility of a mitochondrial fat oxidation disorder is considered, certain routine and specialized tests should be obtained. These include (especially during the acute illness) blood glucose, electrolytes, urea, ammonia, uric acid, CPK, lactate, transaminases (AST/ALT), and complete blood count, including platelets. From routine urinalysis, examine results for "blood" from the dipstick and compare with the presence or absence of red blood cells detected in the microscopic analysis to see if myoglobinuria is present. More specialized tests include plasma carnitine levels (increase suggests CPT I) and an acylcarnitine profile (useful for the diagnosis of translocase, CPT II, VLCAD, LCHAD, MCAD, and possibly SCAD and trifunctional protein deficiencies) along with quantitative urinary organic acid analysis by GC-MS (includes acylglycine quantification-MCAD and MADD only). Skin biopsy to establish a fibroblast culture should be done for direct enzyme assay or for probing the metabolic pathway with 16-$^2H_3$-palmitate as a substrate, in vitro. It is important to recognize that fibroblast fatty acid oxidation studies with labeled myristate or palmitate can only suggest a problem exists without necessarily providing specific information unlike direct enzyme assay or probing the β oxidation pathway using 16-$^2H_3$-palmitate as substrate and tandem-MS analysis of the acylcarnitines as the assay. If deficiency of the muscle-kidney plasma membrane carnitine transporter or hepatic CPT I is prominent on the differential list, then specific direct assays should be sought.

Although there is overlap of presenting symptoms and organ involvement, these disorders generally fall into three major groups dominated by the organ system most involved. These include the hepatic-, cardiac-, and muscle-dominant phenotypes.[307,308]

The hepatic group is mainly characterized by recurrent Reye-like illness involving hypoketotic hypoglycemia leading to obtundation and coma. Metabolic acidosis is unusual unless terminal lactic acidosis has developed. Hyperammonemia, hyperuricemia, and increased levels of serum transaminases are usually observed. Plasma carnitine levels are usually decreased secondarily although they are characteristically increased in the hepatic form of CPT I deficiency. Liver biopsy, acutely or at postmortem, will reveal micro- and/or macrovesicular steatosis. These observations have been reported commonly in MCAD deficiency, and in the hypoglycemic form of VLCAD deficiency, LCHAD, infantile CPT II, mild translocase, and CPT I deficiencies. The cardiac form of VLCAD deficiency often presents with neonatal hypoketotic hypoglycemia responsive to fluid therapy in the neonatal period, but returns between 2 and 5 months of age with severe hypertrophic cardiomyopathy that is usually fatal. Any neonatal hypoglycemia should be evaluated with an acylcarnitine profile to identify this disorder.

When cardiac abnormalities dominate the presentation, there is often an acute or chronic hypertrophic or dilated cardiomyopathy. Pericardial effusion often accompanies the hypertrophic cardiomyopathy and is often responsible for death in the cardiac form of VLCAD deficiency. Isolated transient arrhythmia should not be considered as a major cardiac finding in these diseases. The first case of VLCAD reported had a transient arrhythmia but the real problem was hypoglycemia and the patient never had any other cardiac manifestations. Cardiac abnormalities have been prominent with the muscle-kidney carnitine transporter defect, LCHAD, trifunctional protein deficiency, neonatal CPT II deficiency, as well as the cardiac phenotype of VLCAD deficiency.

A muscle phenotype usually includes moderate to severe hypotonia or recurrent rhabdomyolysis. Hypotonia, developmental delay, with or without seizures, should suggest the muscle phenotype of SCAD deficiency. Hypotonia can be observed during illness in many of these disorders but other organ system involvement often leads to consideration of the other categories. Recurrent rhabdomyolysis is characteristic of the adult form of CPT II deficiency and is often associated with deficiency of LCHAD, trifunctional protein, VLCAD, and in at least one case of SCHAD. It is important to realize that gross myoglobinuria ("burgundy"-colored urine) is often not observed until the serum CPK level is in excess of 30,000 IU/liter. Therefore dark or concentrated-appearing urine can represent myoglobinuria, which is confirmed by a positive urine dipstick test for blood in the absence of red cells in the urinary sediment. Serum CPK should routinely be measured when considering these diseases.

Family history is also important to determine if prior sibs have died—often categorized as SIDS or sudden unexplained death. Many families with these disorders of fat oxidation have already suffered the loss of at least one child. Similarly, prior pregnancies may have been associated with life-threatening acute fatty liver of pregnancy (AFLP) or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets). These third trimester maternal syndromes have been associated both with fetuses with either LCHAD or CPT I deficiency.

Investigation of sibling deaths is also often extremely helpful. These children are frequently medical examiner cases and extensive information may be obtained from review of the postmortem examination. Steatosis and/or cerebral edema are often observed but the absence of these abnormalities does not, by itself, rule out fatty acid oxidation disorders. The original neonatal screening card may still be available and can be utilized for acylcarnitine assay or molecular analysis. Similarly, blood samples taken for toxicology analysis are frequently stored for up to 1 year and have proved very useful in detecting MCAD, VLCAD, CPT II, translocase, and LCHAD deficiencies. Molecular analysis of these samples, as well as from amplified DNA obtained from either formalin-fixed or paraffin embedded tissues, has been useful in screening for MCAD and LCHAD mutations. Molecular screening for other disorders of fat oxidation has been less useful due to the lack of a frequent disease-causing mutation.

In the past, prenatal diagnosis was traditionally done by direct enzyme assay of amniocytes or chorionic villous samples (CVS). Analysis of cell-free amniotic fluid for metabolites is not a useful alternative for the diagnosis of fat oxidation disorders as diagnostic metabolites are usually undetectable, even in MCAD deficiency. Because the various disorders have overlapping phenotypes, direct enzyme assays are only useful if the disorder segregation in a family has already been identified. An alternative for simultaneous analysis for many of these disorders is the incubation of amniocytes with stable isotope-labeled precursors for the β oxidative pathway such as 16-$^2H_3$-palmitate followed by acylcarnitine analysis of the labeled intermediates by tandem-MS. These quantified, labeled intermediate profiles have been successfully utilized for prenatal diagnosis of deficiency of translocase, CPT II, VLCAD, LCHAD, and MCAD. Complete trifunctional protein deficiency has not yet been studied with this method. Because there are no characteristic acylcarnitines for CPT I deficiency and the muscle-kidney carnitine transporter defect, other methods are needed to diagnosis these disorders. Although SCAD deficiency should be easily detected, in the muscle form of this disease, neither incubation of amniocytes with 16-$^2H_3$-palmitate nor direct enzyme assay with butyryl-CoA and ETF were able to identify an affected fetus (later proven by direct enzyme assay of fetal muscle tissue). Despite these limitations, when the specific defect is not known, the incubation with 16-$^2H_3$-palmitate has proven to be very useful. Given the complexity of the β-oxidation spiral, the fact that all the enzymes in the pathway-acyl-CoA dehydrogenases, 2-enoyl-CoA hydratases, 3-hydroxyacyl-CoA dehydrogenases, and 3-ketoacyl-CoA thiolases have been found to exist in multiple forms with overlapping chain-length specificity, and the fact that the complete oxidation of unsaturated fatty acids requires additional enzyme-mediated steps, it is likely that additional defects in fatty acid oxidation will be identified. They represent ongoing challenges for the study of inherited disorders of mitochondrial fatty acid oxidation.

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