Original Article

Role of Carnitine and Fatty Acid Oxidation and Its Defects in Infantile Epilepsy

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ABSTRACT

Defects in fatty acid oxidation are a source of major morbidity and are potentially rapidly fatal. Fatty acid oxidation defects encompass a spectrum of clinical disorders, including recurrent hypoglycemic, hypoketotic encephalopathy or Reye-like syndrome in infancy with secondary seizures and potential developmental delay, progressive lipid storage myopathy, recurrent myoglobinuria, neuropathy, and progressive cardiomyopathy. As all of the known conditions are inherited as autosomal recessive diseases, there is often a family history of sudden infant death syndrome in siblings. Early recognition and prompt initiation of therapy and the institution of preventive measures may be life saving and significantly decrease long-term morbidity, particularly with respect to central nervous system sequelae. Seizures may be the result of cerebral bioenergetic failure associated with acute episodes of hypoglycemic, hypoketotic encephalopathy, or hypoxic-ischemic encephalopathy in the context of cardiac arrhythmias and/or cardiomyopathy. This review provides an overview of the fatty acid oxidation pathway and the central role of carnitine, as well as a discussion of normal fasting adaptation and the critical metabolic adaptations that occur at birth. The increased vulnerability of infants and young children to fasting and defective fatty acid oxidation is discussed in the context of the heightened bioenergetic demands of the developing brain. Clinical and laboratory features of specific genetic defects in fatty acid oxidation, approaches to diagnosis, and current treatment methodologies are described. Indications for carnitine supplementation in childhood epilepsy are also discussed. (*J Child Neurol* 2002;17(Suppl 3):3S57–3S83).

CARNITINE AND FATTY ACID OXIDATION AND ITS ASSOCIATED DEFECTS: ROLE IN EARLY BRAIN DEVELOPMENT

Defects in fatty acid oxidation are a source of major morbidity and may be rapidly fatal. Fatty acid oxidation defects encompass a spectrum of clinical disorders, including recurrent hypoglycemic, hypoketotic encephalopathy or Reye-like syndrome in infancy with secondary seizures and potential developmental delay, progressive lipid storage myopathy, recurrent myoglobinuria, neuropathy, and progressive cardiomyopathy.[1–18]

Received May 15, 2002. Received revised Oct 9, 2002. Accepted for publication Oct 9, 2002.

This work was supported in part by an Operating Grant from the Heart and Stroke Foundation of Ontario (NA 4964) and from the physicians of Ontario through the Physicians' Services Incorporated Foundation. Dr Tein is a recipient of a Medical Research Council of Canada Scholarship.

Presented in part at the symposium "Metabolic Determinants of Infantile Epilepsy," a Continuing Medical Education Program supported by an unrestricted educational grant from Sigma-Tau Pharmaceuticals, Inc., in Amalfi, Italy, April 27, 2001.

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There are at least 16 recognized enzyme defects in fatty acid oxidation, most of which have been diagnosed in the last 15 years. With significant biomedical advances, there has been a rapid increase in the number of diagnosed cases. In a recent survey from the Pennsylvania newborn screening program using tandem mass spectrometry, followed by confirmation through molecular analysis for several common mutations, the incidence of medium-chain acyl-coenzyme A (CoA) dehydrogenase deficiency was found to be as high as 1 in 8930 live births.[19]

Delay in recognition of these disorders can be attributed to three major factors. First, fatty acid oxidation does not play a major role in energy production until relatively late in fasting. Thus, affected individuals may remain clinically silent until exposed to periods of fasting beyond 12 hours or to prolonged exercise. Acute decompensation may be precipitated by an intercurrent infection with fasting, vomiting, and shivering thermogenesis. Second, routine laboratory tests, such as urinary ketones, may not demonstrate a defect in fatty acid oxidation unless blood and urine samples are obtained at the time of the acute episode. If samples are obtained after the child has received intravenous glucose therapy or has recovered from the acute illness, the defect may be missed. Third, there has

been a delay in the development of methods to identify abnormal fatty acid metabolites in urine using gas chromatography-mass spectrometry, which has been available only since the mid-1970s.

Normal Pathway of Fatty Acid Oxidation

The pathway of mitochondrial fatty acid oxidation is outlined in Figure 1. During fasting, free fatty acids are liberated from adipocytes and are transported to other tissues either as triglyceride-rich lipoproteins or bound to serum albumin.[20] Triacylglycerols are hydrolyzed outside the cells by lipoprotein lipase to yield free fatty acids. The mechanism of free fatty acid entry into cells is not well understood; kinetic evidence suggests both a saturable and a nonsaturable uptake. Once across the plasma membrane, short- (eg, 4-carbon) and medium-chain (eg, 8-carbon) fatty acids of less than 10 carbons are able to cross both outer and inner mitochondrial membranes as free acids to enter the mitochondrial matrix. There they are activated by their respective short- and medium-chain acyl-CoA synthetases into CoA esters for ensuant intramitochondrial β-oxidation.

The mitochondrial membrane is impermeable to long-chain fatty acids (eg, 16 carbon). Therefore, long-chain fatty acids diffuse or are transported to the outer mitochondrial membrane and the endoplasmic reticulum. At both locations, they are activated by conversion to their CoA thioesters. Long-chain acyl-CoA synthetase is a membrane-bound enzyme located in the endoplasmic reticulum and in the outer mitochondrial membrane. This enzyme acts on saturated fatty acids containing 10- to 18-carbon atoms and on unsaturated fatty acids containing 16- to 20-carbon atoms.[21] Most of the activated fatty acids are directed toward mitochondrial β-oxidation.[22] The inner mitochondrial membrane is impermeable to CoA and its derivatives; thus, fatty acyl-CoA thioesters cannot directly enter the mitochondrial matrix. The long-chain acyl-CoA must first be converted to its acylcarnitine form (eg, palmitoylcarnitine), with release of free CoA. This is accomplished by the reversible enzyme carnitine palmitoyltransferase I, which is located on the inner side of the outer mitochondrial membrane and uses carnitine as a cofactor.[23] Palmitoylcarnitine is then translocated across the inner mitochondrial membrane by carnitine-acylcarnitine translocase,[24]

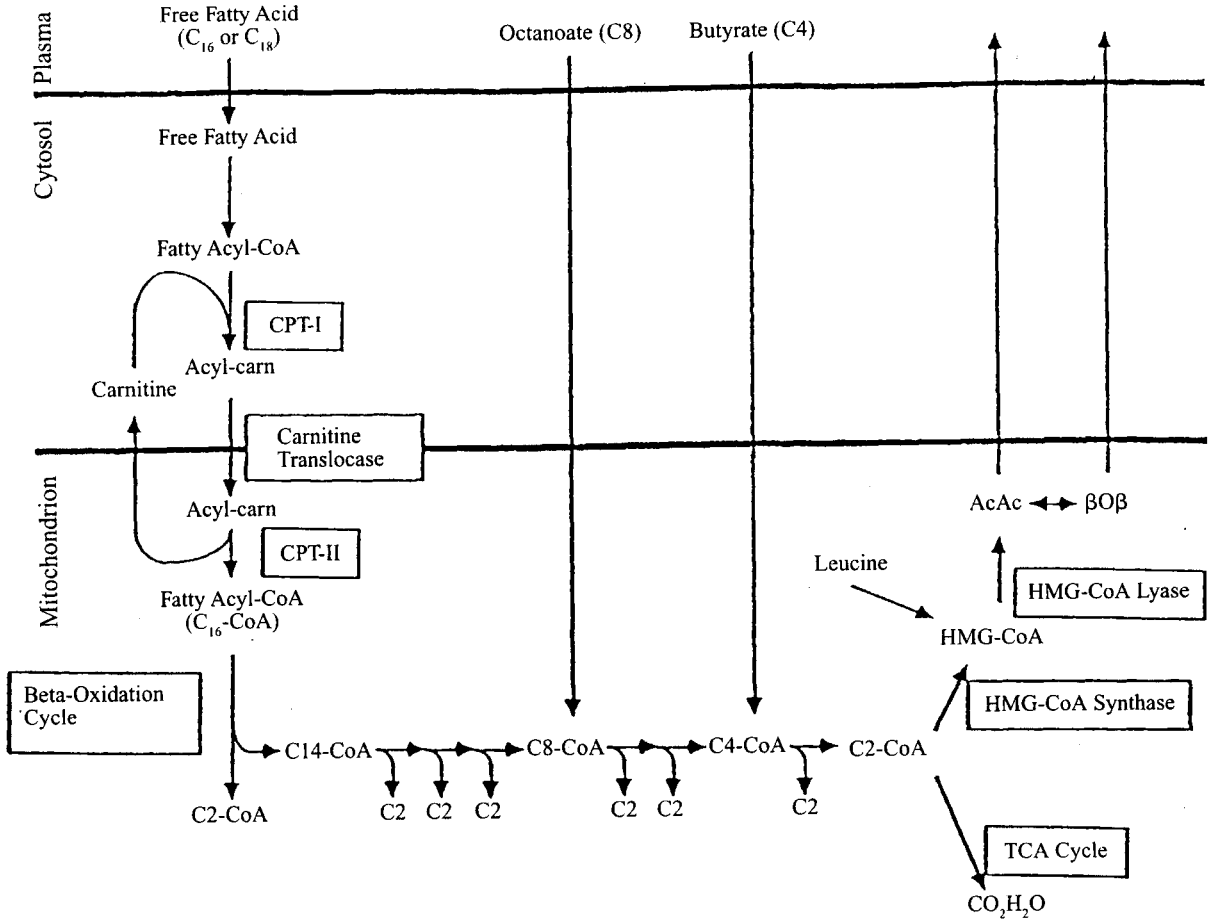


Figure 1. Pathway of mitochondrial fatty acid oxidation, showing the sequential degradation of a typical, 16-carbon long-chain fatty acid (palmitate) to 2-carbon acetyl-coenzyme A (CoA) units, which are then used for ketone synthesis (in liver) or oxidized in the tricarboxylic acid cycle (in muscle). CPT = carnitine palmitoyltransferase; Carn = carnitine; AcAc = acetoacetate; βOβ = β-hydroxybutyrate; HMG = β-hydroxy-β-methylglutaryl; CoA = coenzyme A; TCA = tricarboxylic acid; CO_2 = carbon dioxide. Reprinted with permission from Stanley.[6]

which catalyzes a slow unidirectional diffusion of carnitine both in and out of the mitochondrial matrix, in addition to a much faster mole-to-mole exchange of acylcarnitine for carnitine, carnitine for carnitine, and acylcarnitine for acylcarnitine. In the mitochondrial matrix, carnitine palmitoyltransferase II, situated on the inner side of the inner mitochondrial membrane, converts palmitoylcarnitine in the presence of free CoA to palmitoyl-CoA and carnitine.

There are four sequential steps in mitochondrial β-oxidation, composed of chain-length specific enzymes (Figure 2). Until recently, it was held that the complete intramitochondrial β-oxidation of long-chain fatty acids required a minimum of nine enzymes, including three genetically distinct acyl-CoA dehydrogenases (short-, medium-, and long-chain),[25] at least two enoyl-CoA hydratases (crotonase and long-chain enoyl-CoA hydratase),[26] two L-3-hydroxyacyl-CoA dehydrogenases (short and long chain),[27] and two 3-ketoacyl-CoA thiolases (acetoacetyl-CoA thiolase and generalized 3-ketoacyl-CoA thiolase).[28] However, further enzymatic and protein characterization has revealed the presence of an additional very-long-chain acyl-CoA dehydrogenase enzyme[29,30] and a trifunctional protein that combines the activities of long-chain enoyl-CoA hydratase, long-chain L-3-hydroxyacyl-CoA dehydrogenase, and long-chain thiolase enzymes.[31–33] These enzymes are chain length specific. For example, the long-chain acyl-CoA dehydrogenase has specificity for 12- to 18-carbon fatty acids, and the medium-chain acyl-CoA dehydrogenase has specificity for 4- to 12-carbon fatty acids.[18] With each complete cycle, a 2-carbon fragment is cleaved, and an acetyl-CoA moiety is released. In most tissues, such as muscle and heart, the acetyl-CoA is oxidized for energy production via the tricarboxylic acid cycle. In liver, about 90% of the hepatic acetyl-CoA is converted into ketones via acetyl-CoA acetyltransferase, β-hydroxy-β-methylglutaryl-CoA lyase, and β-hydroxybutyrate dehydrogenase.[34] These ketones are then exported for final oxidation by other tissues, such as brain.

Regulation of Fatty Acid Oxidation

Fatty acid oxidation is regulated at several levels.[21] The rate of fatty acid oxidation is determined by the availability of fatty acids and the rate of use of β-oxidation products.

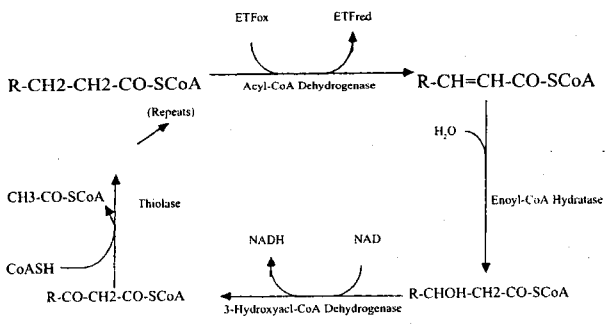


Figure 2. Four steps of the β-oxidation cycle that shorten a fatty acid chain by two carbons with the release of a two-carbon acetyl-CoA moiety. ETF = electron-transferring flavoprotein; ox = oxidized; red = reduced; CoA = coenzyme A; NADH = reduced nicotinamide adenine dinucleotide; NAD = nicotinamide adenine dinucleotide. Reprinted with permission from Stanley.[6]

The concentration of nonesterified fatty acids in plasma is regulated by the hormones glucagon, which stimulates, and insulin, which inhibits the breakdown of triacylglycerols in adipose tissue. Glucagon activates adenylate cyclase, leading to an increase in the concentration of cellular cyclic adenosine monophosphate, which, in turn, activates protein kinase. One of the substrates of protein kinase in adipose tissue is the hormone-sensitive lipase. This lipase is activated by phosphorylation and inactivated by dephosphorylation. When the glucose concentration is low, as in fasting, there is a high glucagon-to-insulin ratio that results in an increase in plasma nonesterified free fatty acids. These fatty acids enter cells, where they are degraded to acetyl-CoA or incorporated into other lipids. The use of fatty acids for either oxidation or lipid synthesis depends both on the availability of carbohydrates and on the nutritional state.

Because of its role in ketogenesis, the regulation of fatty acid oxidation in liver differs from and is more complex than its regulation in heart and muscle. Liver breaks down carbohydrates to synthesize fatty acids in the fed state. In the fasted animal, fatty acid oxidation, ketogenesis, and gluconeogenesis are more active. McGarry and Foster[35] suggested that the concentration of malonyl-CoA, the specific reversible inhibitor of carnitine palmitoyltransferase I, determines the rate of fatty acid oxidation. In the fed state, where glucose is converted to fatty acids, the concentration of malonyl-CoA is elevated, thereby leading to an inhibition of carnitine palmitoyltransferase I activity. This inhibits the transfer of long-chain fatty acyl residues from CoA to carnitine; thus, long-chain acylcarnitines cannot be translocated into the mitochondria, and β-oxidation is depressed.

When there is a change from the fed to the fasted state, hepatic metabolism shifts from glucose breakdown to gluconeogenesis, leading to a decrease in fatty acid synthesis. The concentration of malonyl-CoA decreases and the inhibition of carnitine palmitoyltransferase I is reversed, whereby acylcarnitines are then formed and translocated into mitochondria for β-oxidation and ketogenesis. The cellular concentration of malonyl-CoA is directly related to the activity of acetyl-CoA carboxylase, which is hormonally regulated. In fasting, an increase in the glucagon-to-insulin ratio causes an increase in cellular cyclic adenosine monophosphate, which is responsible for the phosphorylation and inactivation of acetyl-CoA carboxylase. As a consequence, the concentration of malonyl-CoA and the rate of fatty acid synthesis decrease, whereas β-oxidation increases. A decrease in the glucagon-to-insulin ratio reverses this process. Thus, both fatty acid synthesis and fatty acid oxidation are regulated by the glucagon-to-insulin ratio.

Another area that may play an important role in regulating fatty acid oxidation is within the mitochondria, where end-product inhibition of proximal steps of β-oxidation by more distal acyl-CoA intermediates prevents excessive accumulation of acyl-CoAs even under conditions of very rapid fatty acid oxidation.[6]

Alternative Pathways for Fatty Acid Metabolism

Mitochondrial β-oxidation accounts for the majority of fatty acid metabolism; however, there are other available mech-

anisms within the cell for the oxidation or disposal of fatty acid intermediates. The peroxisomal oxidation pathway is composed of enzymes that are genetically distinct from the mitochondrial enzymes[36] and may contribute up to 20% of total cellular fatty acid oxidation under conditions of prolonged fasting.[37] When there is an excessive accumulation of acyl-CoAs, the excess acyl-CoAs are shunted to microsomes, where they undergo ω-oxidation. This places a carboxyl group on the methyl-terminal end of the fatty acid, resulting in the formation of dicarboxylic acid.[38] Dicarboxylic acids are found in most conditions in which the capacity for β-oxidation is exceeded, including normal fasting, feeding with medium-chain triglycerides, diabetic ketoacidosis, and genetic defects of intramitochondrial β-oxidation. The specific pattern of dicarboxylic acids found in serum or urine may be diagnostically useful.

Three additional mechanisms may be important when there is an impairment in mitochondrial β-oxidation: the conjugation of acyl groups to glycine and to carnitine and the deacylation of CoA by thioesterases.[39,40] These glycine and carnitine conjugates cross cellular membranes more readily than the respective CoA ester.[41] This is important because acyl-CoA esters inhibit specific enzymes and transporters. The removal of intracellular acyl groups also preserves free CoA for other enzymes that require CoA as a cofactor. Measurement of both acylcarnitines and acylglycines is diagnostically useful in the identification of genetic defects in fatty acid oxidation.[42,43]

Central Role of Carnitine Metabolism

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid), a water-soluble quaternary amine, has several important intracellular functions.[44,45] Carnitine (1) modulates the intramitochondrial acyl-CoA-to-CoA sulfhydryl ratio in mammalian cells; (2) serves as an essential cofactor for mitochondrial fatty acid oxidation by transferring long-chain fatty acids as acylcarnitine esters across the inner mitochondrial membrane; (3) facilitates branched-chain α-keto acid oxidation; (4) shuttles acyl moities that have been chain-shortened by β-oxidation out of peroxisomes in the liver; and (5) traps potentially toxic acyl-CoA metabolites that may be in excess during acute metabolic crises through esterification to carnitine. These metabolites may otherwise secondarily impair the citric acid cycle, gluconeogenesis, urea cycle, and fatty acid oxidation.

In omnivores, approximately 75% of carnitine comes from the diet and 25% from endogenous biosynthesis.[46] Meat, poultry, fish, and dairy products are the principal dietary sources of carnitine.[47] Approximately 70 to 80% of dietary carnitine is absorbed in omnivores,[48] whereas in strict vegetarians, endogenous carnitine synthesis provides > 90% of total available carnitine.[46] There are adequate carnitine concentrations in human milk and most supplemented milk-based formulas to sustain early growth and development. Plasma carnitine concentrations were found to be markedly reduced in term infants fed unsupplemented, soy protein-based formulas compared with those receiving supplemented formulas.[49] Interestingly, no deficits were noted in growth and development in term infants consuming carnitine-free formulas for the first 4 months of life.[50]

Skeletal muscle is the major tissue reservoir of carnitine, containing over 90% of total body carnitine stores.[46] Under normal conditions, carnitine concentration in tissues (other than brain) is 20- to 50-fold higher than in plasma and parallels the capacity of the tissue to metabolize fatty acids; human tissue carnitine concentrations (nmol/g) are heart (3500 to 6000) > muscle (2000 to 4600) > liver (1000 to 1900) > brain (200 to 500).[6] Thus, plasmalemmal carnitine uptake occurs across a large concentration gradient, maintained by a transport system that is generally held to be sodium-gradient and energy dependent.[44,45,51] The normal serum carnitine concentration is tightly maintained by the renal threshold of 40 μmol/L. The kidney is capable of adjusting to wide variations in dietary carnitine as carnitine is not significantly degraded in the body.[52] More than 90% of filtered carnitine is reabsorbed by the kidney at normal physiologic plasma carnitine concentrations.[51] Human skeletal muscle, heart, liver, kidney, and brain are capable of carnitine biosynthesis from methionine and lysine to its immediate precursor γ-butyrobetaine.[53] However, the final conversion of γ-butyrobetaine to L-carnitine by γ-butyrobetaine hydroxylase can occur only in liver, kidney, and brain.[53,54] Gamma-butyrobetaine is thus exported to these tissues for final conversion to L-carnitine. Hepatic γ-butyrobetaine hydroxylase is developmentally regulated, being approximately 25% of adult activity at birth.[55]

Many patients with carnitine deficiency have been described since 1973. Originally, patients were categorized into two groups: (1) having a systemic form characterized by recurrent coma with low carnitine concentrations in serum, liver, and muscle or (2) having a muscular form characterized by progressive lipid storage myopathy in which serum concentrations were normal and carnitine deficiency was confined to skeletal muscle. However, with recent advances, many of these cases were found to be attributable to intramitochondrial β-oxidation defects with secondary carnitine deficiency (Figure 3). For example, many cases of the systemic form were identified as medium-chain acyl-CoA dehydrogenase deficiency. The secondary carnitine deficiency disorders can be divided into genetic, acquired, and iatrogenic forms (Table 1).

Critical Metabolic Adaptations at Birth

Birth marks a critical turning point in the capacity for fatty acid oxidation in many species, including man, and carnitine plays a pivotal role. The fetus is primarily dependent on a continuous, intravenous high-carbohydrate/low-fat diet from the mother.[56,57] When the mother is correctly fed, gluconeogenesis[58] and ketogenesis[59,60] are absent or minimal in fetal liver. During birth, cold exposure, catecholamine secretion, hypoxia, and cord cutting markedly stimulate glucagon secretion and inhibit insulin secretion,[61] leading to a rapid increase in serum free fatty acids from adipose tissue.[62] Following birth, infants are exposed to a brief period of starvation before receiving milk. During this time, they are entirely dependent on the mobilization of glycogen

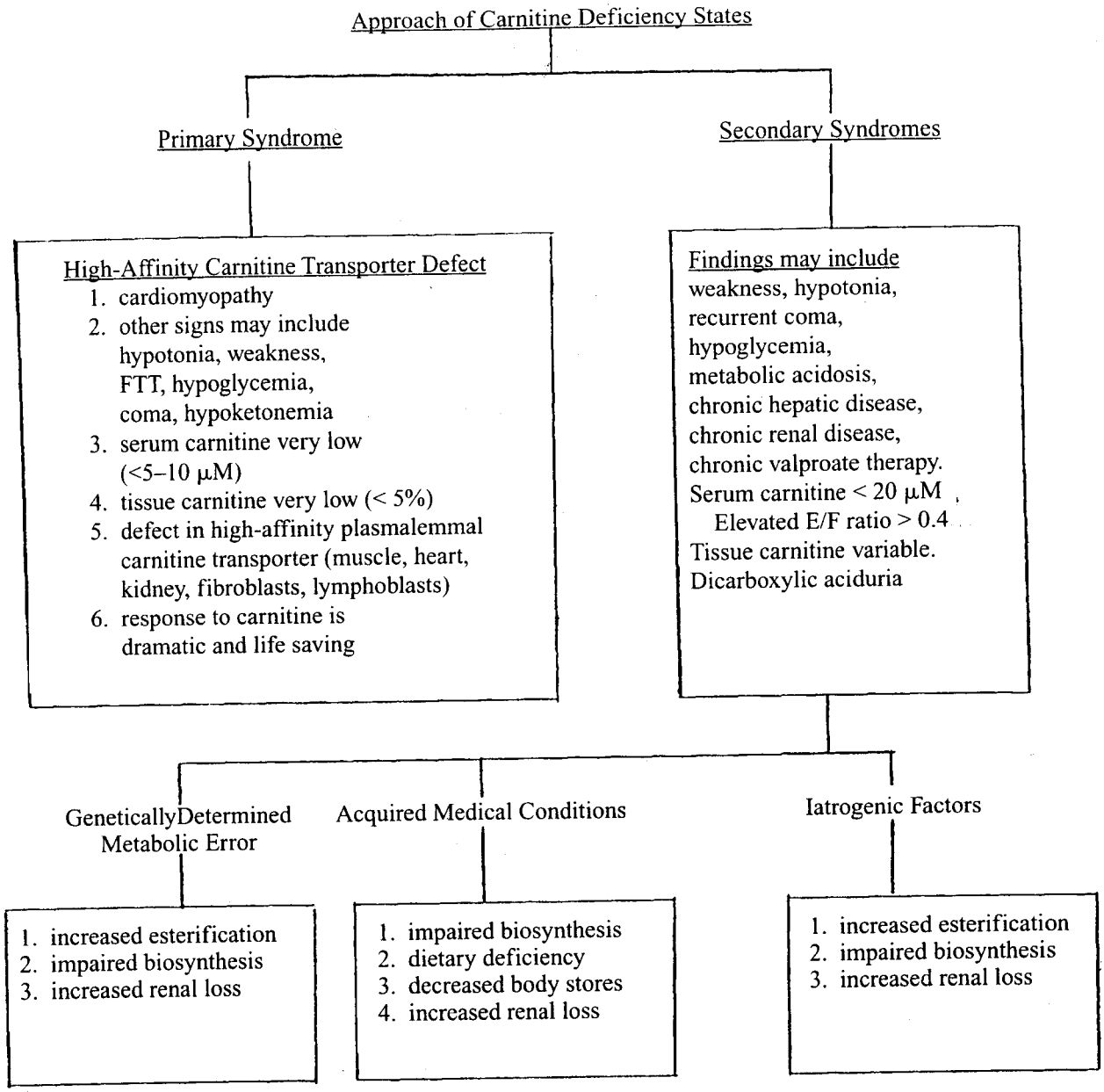


Figure 3. An approach to the recognition of the primary and secondary disorders of carnitine metabolism. Certain clinical features are distinctive. The serum carnitine concentrations are normal in the myopathic form, markedly decreased in the systemic form, and more variable in the secondary forms. Increased esterification and dicarboxylicaciduria are notable in the secondary forms. FTT = failure to thrive; E/F = esterified/free. Adapted from De Vivo and Tein.[15]

and fat stores for survival. The large liver glycogen stores accumulated during late fetal life are rapidly mobilized to provide an immediate source of glucose to cells and tissues (including erythrocytes, brain, and kidney medulla), which depend on glucose to maintain their functional activity.[63] However, liver glycogen stores are limited and exhausted within 12 hours after delivery. The newborn is then dependent on ketogenesis and gluconeogenesis and is exposed to an intermittent, enteral high-fat/low-carbohydrate diet and fasting. More than 90% of milk fat is in the form of triglycerides containing mainly long-chain fatty acids.[64]

The capacity for long-chain fatty acid oxidation is very low in fetal liver and heart,[59,60,65–68] but markedly increases during the first 24 hours after birth, with a rapid increase in carnitine palmitoyltransferase activity in liver[65,69] and in heart.[67] The increase in the glucagon-to-insulin ratio at birth leads to an increase in hepatic fatty acid oxidation by three mechanisms: (1) decreasing lipogenesis and decreasing malonyl-CoA concentration,[70,71] in which malonyl-CoA is a strong inhibitor of carnitine palmitoyltransferase I (the rate-limiting enzyme of long-chain fatty acid oxidation),[35] for which carnitine is a critical substrate; (2) decreasing the sensitiv-

Table 1. Secondary Carnitine Deficiency States and Possible Mechanisms

Genetically determined metabolic errors
Increased esterification due to acyl-CoA accumulation
Carnitine-acylcarnitine translocase deficiency
Carnitine palmitoyltransferase II deficiency
Very-long-chain acyl-CoA dehydrogenase deficiency
Long-chain acyl-CoA dehydrogenase deficiency
Trifunctional protein deficiency
Medium-chain acyl-CoA dehydrogenase deficiency
Short-chain acyl-CoA dehydrogenase deficiency
Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency
Multiple acyl-CoA dehydrogenase (ETF and ETF-Qo) deficiency
β-hydroxy- β-methylglutaryl-CoA lyase deficiency
Isovaleric acidemia
Propionic acidemia
Methylmalonic aciduria
Glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I)
β-ketothiolase deficiency
Decreased biosynthesis
Homocystinuria
5-Methylene tetrahydrofolate reductase deficiency
Adenosine deaminase deficiency
Ornithine transcarbamylase heterozygote state
Increased urinary loss
Cystinosis
Cytochrome oxidase deficiency
Acquired medical conditions
Decreased biosynthesis*
Cirrhosis
Chronic renal disease
Extreme prematurity
Dietary deficiency*
Chronic TPN
Malabsorption (cystic fibrosis, short-gut syndrome)
Soybean protein–derived infant formula
Decreased body stores
Extreme prematurity
Intrauterine growth retardation
Infant of carnitine-deficient mother
Increased urinary loss
Fanconi's syndrome
Renal tubular acidosis
Iatrogenic factors—drug therapy
Increased esterification and competitive inhibition of carnitine uptake by valproylcarnitine
Chronic valproic acid administration
Impaired hepatic biosynthesis
Chronic valproic acid administration
Increased loss
Chronic hemodialysis

*Often combined factors. CoA = coenzyme A; ETF = electron transfer flavoprotein; Qo = coenzyme Q oxidoreductase; TPN = total parenteral nutrition.
Adapted from De Vivo and Tein.[15]

ity of carnitine palmitoyltransferase I to the inhibitory influence of malonyl-CoA[60,71,72]; and (3) activating β-hydroxy-β-methylglutaryl-CoA synthase by desuccinylation of the enzyme in response to a decrease in mitochondrial succinyl-CoA concentration,[73] promoting ketogenesis. In rats, plasma ketone concentrations are low in the fetus and increase 10-fold during the first 24 hours postpartum, thereafter gradually declining such that normal values are found at the end of the suckling period.[74] There is a concomitant rapid increase in hepatic gluconeogenesis with the appearance of the rate-limiting phosphoenolpyruvate carboxykinase.[58,75] Hepatic fatty acid oxidation provides essential cofactors to support gluconeogenesis, for example, acetyl-CoA, reduced nicotinamide adenine dinucleotide (NADH), and adenosine triphosphate (ATP). Thus, within the first 24 hours after birth, there is a dramatic induction of fatty acid oxidation, hepatic ketogenesis, and gluconeogenesis in response to the changing diet and extrauterine metabolic demands.[15] Cerebral ketone body use spares body glucose turnover and thereby provides a metabolic margin of safety during the critical period of brain growth and maturation.[76] Thus, carnitine is considered to be essential in the newborn diet.[77] Furthermore, efficient fatty acid oxidation is critical for newborn thermogenesis.[78]

Fasting Adaptation

Fats comprise the most important and efficient fuel for oxidative metabolism. The largest reserve of fuel in the body consists of fatty acids stored as adipose tissue triglyceride. Because liver glycogen stores are depleted within a few hours of a meal and there are no reserve stores of protein in the body, fatty acids become the predominant substrate for oxidation quite early in fasting.[6] In adults, approximately 80% of caloric requirements after a 24-hour period of fasting is supplied by fatty acids, which increases to 94% during more prolonged fasting.

Fatty acids serve three major functions. First, the partial oxidation of fatty acids by the liver produces ketones (acetoacetate, β-hydroxybutyrate) that serve as an important auxiliary fuel for almost all tissues and particularly brain as the blood-brain barrier prevents the direct use of long-chain fatty acids by the brain. This provides an important mechanism to spare glucose oxidation and proteolysis during prolonged fasting.[79] Second, fatty acids serve as a major fuel for cardiac and skeletal muscle. Energy demands of resting muscle depend mostly on fatty acid oxidation.[80] Energy in working muscle is derived from either the combination of triglyceride and stored glycogen or the combination of glucose and free fatty acids, depending on the type, intensity, and duration of exercise.[81,82] After 90 minutes, glucose and free fatty acids are the major sources of fuel. During 1 to 4 hours of mild-to-moderate prolonged aerobic exercise, free fatty acid uptake by muscle increases by 70%; after 4 hours, free fatty acid use is double that of carbohydrate sources. Third, the high rates of hepatic gluconeogenesis and ureagenesis needed to maintain fasting homeostasis are sustained by the production of energy (ATP), reducing equivalents ($NADH^+$, H^+) and metabolic intermediates (acetyl-CoA) derived from fatty acid oxidation.[18]

Infants and young children have an increased risk of difficulties with fasting adaptation that may be attributed to several factors.[83–85] First, the infant brain is larger relative to body size, is highly dependent on glucose, and has a high rate of metabolism.[18] Thus, infants and children demonstrate an earlier activation of fatty acid oxidation, with hyperketonemia within 12 to 24 hours of fasting. Second, basal energy needs in the infant are high in order to maintain body temperature, given the large ratio of surface area to mass. Body temperature is maintained by shivering thermogenesis, which is highly dependent on efficient fatty acid oxidation. Third, there is lower activity of several key enzymes involved in

energy production in the infant compared with the older child and adult, which leads to further impairment of the infant's ability to maintain glucose homeostasis.[86]

Clinical and Biochemical Features of Identified Defects

Defects in fatty acid oxidation share a number of clinical features. The common features of these defects are presented, followed by a discussion of differentiating clinical and laboratory features.

Common Features of Fatty Acid Oxidation Disorders

It has been suggested that there are at least four clinical and laboratory features that should lead the clinician to suspect a genetic defect in fatty acid metabolism (Table 2).[18] These common features include the following:

1. *Acute metabolic decompensation in association with fasting.* Affected individuals suffer episodes of metabolic decompensation during conditions that place stress on the fatty acid oxidation pathway for fuel generation, in the context of depleted glycogen and glucose reserves. These stressors include fasting, prolonged exercise (≥ 1 hour of mild-to-moderate aerobic exercise), infection with vomiting, and cold-induced shivering thermogenesis. Shivering is an involuntary form of muscle activity that depends on long-chain fatty acid oxidation.[87] Thus, ketogenesis is stimulated in normal individuals during cold exposure.[88] Children are most likely to be found comatose in the early-morning hours after an overnight fast. Infections such as a viral illness are frequent precipitating factors because of the combination of vomiting and decreased oral intake with shivering. If not recognized, children may progress to a Reye-like syndrome, as seen in medium-chain acyl-CoA dehydrogenase deficiency.[8] Infants and younger children are at greater risk during fasting than older children and adults because of their limited fasting capabilities. Prolonged fasting is 6 to 10 hours for an infant less than 1 year of age versus 12 hours for a child between 1 and 4 years of age.[18]
2. *Recurrent episodes of hypoketotic hypoglycemia.* The accelerated rate of glucose use that occurs when fatty acids cannot be used as fuel and when ketone bodies are not generated to spare glucose/glycogen stores results in hypoketotic hypoglycemia. An increase in the serum free fatty acid-to-ketone ratio is another clue to a block in β-oxidation. The normal ratio is 1:1. A ratio greater than 2:1 suggests a block in β-oxidation. Furthermore, disorders of fat mobilization, such as hyperinsulinism, are excluded by the finding of increased serum free fatty acids.
3. *Chronic involvement of tissues highly dependent on efficient fatty acid oxidation.* Tissues with high energy demands such as skeletal muscle, heart, and liver are dependent on efficient fatty acid oxidation. With deficient hepatic ketogenesis, glucose becomes the only available fuel and is rate limiting under conditions of fatty acid oxidation stress when glycogen and glucose stores have been depleted. As a result, free fatty acids liberated during fasting that cannot be metabolized owing to the block may be stored in the cytosol as triglycerides. This may lead to progressive lipid storage myopathy with weakness, as well as hypertrophic and/or dilatative cardiomyopathy and fatty liver, which are pathologic signatures of genetic defects in fatty acid oxidation. Increased concentrations of short- or medium-chain fatty acids and their dicarboxylic metabolites from compensatory ω-oxidation may cause secondary metabolic abnormalities, including impairment of gluconeogenesis, β-oxidation, and the citric acid cycle.[41,89,90] This results in a further decrease in cellular ATP production.

Table 2. Common Features of Fatty Acid Oxidation Disorders

Metabolic decompensation during fasting, infection, prolonged exercise, cold exposure, and stress
Decreased oral intake, increased energy expenditure
Progressive obtundation leading to coma
Reye-like syndrome
SIDS or "near-miss" SIDS
Recurrence, familial occurrence
Involvement of fatty acid–dependent tissues
Myalgia, exercise intolerance, myoglobinuria, hypotonia, weakness
Cardiac hypertrophy/dilatation, endocardial fibroelastosis, arrhythmias
Hepatomegaly and hepatic dysfunction
Fatty infiltration of tissues on biopsy or autopsy
Hypoketotic hypoglycemia
Elevated serum free fatty acids with serum free fatty acid to ketone body ratio > 2:1
Hyperinsulinism and hypopituitarism ruled out (mandatory)
Alterations in plasma or tissue carnitine concentration
Decreased total carnitine concentration (10–50% of normal) with increased esterified fraction in intramitochondrial fatty acid oxidation defects
Marked decrease in total carnitine concentration (< 5% of normal) with normal esterified fraction in plasmalemmal carnitine transporter defect
CPT I deficiency is exception with high to normal carnitine concentration and low esterified fraction
Clinical laboratory abnormal findings
Dicarboxylic aciduria
Hyperammonemia
Acidosis
During myoglobinuria, increased serum creatine kinase, hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia, and possible increased creatinine (with renal failure)
Increased alanine and aspartate aminotransferase

CPT = carnitine palmitoyltransferase; SIDS = sudden infant death syndrome.
Adapted from Hale and Bennett.[18]

In the long-chain fatty acid oxidation disorders, which may present with recurrent episodes of acute muscle breakdown or myoglobinuria (eg, carnitine palmitoyltransferase II, long-chain acyl-CoA dehydrogenase/very-long-chain acyl-CoA dehydrogenase, trifunctional enzyme deficiencies), the accumulation of long-chain fatty acids and long-chain acylcarnitines may have detergent-like actions on muscle membranes. Excessive amounts of palmitoyl-CoA and palmitoylcarnitine have been shown to have detergent properties on isolated

canine myocytic sarcolemmal membranes and to potentiate free radical-induced lipid membrane peroxidative injury in ischemia.[91] Long-chain acylcarnitines have been shown to activate calcium channels in cardiac[92,93] and smooth muscle myocytes[93,94] and thus may potentiate the increase in cytosolic calcium associated with arrhythmogenesis observed in ischemic myocardium.[95]

4. *Alterations in the total carnitine concentrations or in the percentage of esterified carnitine in plasma and tissue.* In most cases of intramitochondrial β-oxidation defects, total carnitine concentration is decreased (< 50% of normal) and acylcarnitine fraction is increased (> 50% esterified; normal range, 10 to 25% in the fed state, 30 to 40% in the fasted state).[18] Excessive acyl-CoAs that accumulate proximal to the block in intramitochondrial β-oxidation defects may be converted to acylcarnitines by chain-length–specific carnitine acyltransferases.[44] When filtered through the kidney, acylcarnitines compete with free carnitine at the renal tubular reabsorptive site. As demonstrated by Stanley et al[96] in a cultured skin fibroblast model, free carnitine will be excreted when there is an accumulation of longer-chain acylcarnitines because of their higher affinity for the carnitine transporter than free carnitine. With time, total body and muscle stores of free carnitine are depleted. In the case of the plasmalemmal carnitine transporter defect, total carnitine is markedly reduced (eg, < 5% of normal), and the esterified fraction is normal as the transport defect in kidney leads to a decreased renal reabsorption of carnitine.[14]
5. *Additional laboratory findings.* A number of other biochemical abnormalities may be noted during acute catabolic crises. In the Reye-like syndrome presentation, modest hyperammonemia (100 to 200 μmol/L) may be documented, accompanied by three- to fivefold elevations of liver transaminases.[18] In acute myoglobinuria, there are marked increases in the concentrations of sarcoplasmic enzymes such as serum creatine kinase, which may exeed 100,000 U/L (normal, < 250 U/L). There may be increased serum concentrations of creatinine, potassium, phosphate, urate, and amino acids (especially taurine).[97] These changes may have deleterious effects on the kidneys and heart, increasing the damage.[98,99] Hyperphosphatemia may lead to secondary hypocalcemia; hypercalcemia may follow owing to increased secretion of parathormone and vitamin D with deposition of calcium in injured muscle, which is subsequently released.[100] It is urgent to prevent renal failure. Lactic acidosis may be noted during the acute catabolic episode, reflecting poor perfusion or inhibition of critical enzymes (eg, pyruvate carboxylase) by accumulated metabolites.[41] Urine organic acid screening may demonstrate unusual compounds or excessive amounts of organic acids, which may be diagnostic of a specific block in β-oxidation.

Specific Features of Individual Genetic Defects

With the exception of medium-chain acyl-CoA dehydrogenase, carnitine palmitoyltransferase II, and β-hydroxy-β-methylglutaryl-CoA lyase deficiencies, there are fewer than 50 published cases of each of the other fatty acid oxidation defects. The clinical and laboratory characteristics presented here are based on a summary of these cases; the phenotypes may be broadened further as additional cases are identified. Key clinical features are summarized in Table 3.

There are several differentiating features. In the defects involving the transport of fatty acids into mitochondria (plasmalemmal long-chain fatty acid uptake defect, plasmalemmal carnitine transporter defect, carnitine palmitoyltransferase I deficiency, carnitine-acylcarnitine translocase deficiency, and carnitine palmityoltransferase II deficiency), there is generally not an associated abnormal dicarboxylic aciduria. However, this has been reported in one case of severe infantile carnitine palmitoyltransferase II deficiency[101] and on one occasion during an acute metabolic decompensation in a case of infantile carnitine palmitoyltransferase I deficiency presenting with recurrent Reye-like syndrome.[102] The carnitine transporter, severe infantile carnitine palmitoyltransferase II, and carnitine acylcarnitine translocase defects present in infancy or early childhood and involve both cardiac and skeletal muscle. The carnitine transporter defect is characterized by progressive hypertrophic and/or dilatative cardiomyopathy and is pathologically similar to endocardial fibroelastosis.[103] It is exquisitely responsive to high-dose oral carnitine supplementation, which reverses the cardiomyopathy.[14,96] Renal tubular acidosis has been documented in one case of carnitine palmitoyltransferase I deficiency.[104]

Classic carnitine palmitoyltransferase II deficiency presents with adolescent-onset recurrent episodes of acute myoglobinuria precipitated by prolonged exercise or fasting, in which power between episodes is normal and lipid accumulation in muscle is noted only under conditions of fasting and prolonged exercise.[105] Fasting ketogenesis is generally normal in this condition, although it may be delayed. There is no fixed cardiomyopathy; however, arrhythmias may occur secondary to hyperkalemia and hypocalcemia during acute myoglobinuric crises. However, several cases of severe infantile carnitine palmitoyltransferase II deficiency have been described that presented with recurrent Reye-like syndrome, hepatomegaly with hypoketotic hypoglycemia, elevated liver aminotransferase, cardiomegaly with cardiac arrhythmias, and elevated serum creatine kinase, as well as evidence of lipid storage in heart, skeletal muscle, liver, and kidney.[101,106,107] In these severe cases, carnitine palmitoyltransferase II activity in cultured skin fibroblasts was less than 10% of control values compared with 25% residual activity documented in the classical form of carnitine palmitoyltransferase II deficiency.[106] The clinical presentation of carnitine palmitoyltransferase II deficiency therefore may depend in part on the degree of residual carnitine palmitoyltransferase II activity.

Most recently, a new defect in the active transport of long-chain free fatty acids across the plasma membrane has been described in two young boys who presented with acute liver failure, hyperammonemia, hyperbilirubinemia, coagulopathy, and mild encephalopathy.[108] There was no evidence of cardiac or skeletal myopathy. One boy had documented

Table 3. Clinical Features Associated With Specific Genetic Defects of Fatty Acid Oxidation

Enzyme Defect	*Approximate No. of Cases*	*Fasting Disorder*	*Tissue Involved*	*Hypoketotic Hypoglycemia*	*Altered Carnitine*	*Dicarboxylic Acids*	*Reye-like Syndrome*	*SIDS*
LCFAUD	2	+	L	+	+	NR	NR	NR
CUD	45	+	H, M	+	+	NR	+	NR
CPT I	10	+	K	+	+	NR	+	NR
TRANS	7	±	H, M, (Mg)	+	+	NR	+	+
CPT II (mild)	50	±	M, Mg, P	NR	+	NR	NR	NR
CPT II (severe)	5	+	H, M, Mg, L	+	+	NR	+	+
VLCAD/LCAD	20	+	H, M, Mg, L	+	+	+	+	+
TFP/LCHAD	100	+	H, M, Mg, L, N, R	+	+	+	+	+
Dienoyl-CoA reductase	1	NR	M, D, B, (H)	NR	+	NR	NR	NR
MCAD	> 150	+	NR	+	+	+	+	+
SCAD	9	NR	M, B, D, H	NR	+	+	NR	+
SCHAD	5	+	H, M, Mg, L	+	+	+	NR	+
ETF and ETF-Qo	25	+	M, H, K, B, D	+	+	+	NR	+
HMG-CoA lyase	50	+	B, P	+	+	+	+	+ ?

LCFAUD = long-chain fatty acid uptake defect; CUD = carnitine transporter defect; CPT = carnitine palmitoyltransferase; TRANS = carnitine acylcarnitine translocase; VLCAD = very-long-chain acyl-CoA dehydrogenase; LCAD = long-chain acyl-CoA dehydrogenase; TFP = trifunctional protein; LCHAD = long-chain L-3-hydroxyacyl-CoA dehydrogenase; MCAD = medium-chain acyl-CoA dehydrogenase; SCAD = short-chain acyl-CoA dehydrogenase; SCHAD = short-chain L-3-hydroxyacyl-CoA dehydrogenase; ETF = electron transfer flavoprotein; Qo = coenzyme Q oxidoreductase; HMG = β-hydroxy-β-methylglutaryl; B = brain; D = dysmorphic features; H = heart; K = kidney; L = liver; M = muscle; Mg = myoglobinuria; N = neuropathy; P = pancreatitis; R = retinopathy; NR = no case yet reported; SIDS = sudden infant death syndrome.
Adapted from Hale and Bennett.[18]

hypoketotic hypoglycemia. Both required liver transplantation. The uptake of oleic acid ($C_{18:1}$) in skin fibroblasts from these patients was lower than in controls. Atypical features for a fatty acid oxidation defect included the absence of prominent hepatic microvesicular steatosis and low concentrations of long-chain free fatty acids and elevated carnitine concentrations in liver, which were attributable to the defective transport of long-chain free fatty acids.

There are many common features shared by the intramitochondrial β-oxidation defects (short-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, very-long-chain acyl-CoA dehydrogenase, electron-transferring flavoprotein, electron-transferring flavoprotein-CoQ, short-chain L-3-hydroxyacyl-CoA dehydrogenase, long-chain L-3-hydroxyacyl-CoA dehydrogenase, trifunctional protein, and β-hydroxy-β-methylglutaryl-CoA lyase deficiencies); however, there are also differentiating characteristics. It should be noted that certain cases previously attributed to long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency are now known to be owing to a defect in the trifunctional protein, which combines the activities of the long-chain enoyl-CoA hydratase, long-chain L-3-hydroxyacyl-CoA dehydrogenase, and long-chain 3-ketoacyl-CoA thiolase enzymes.[31] All intramitochondrial enzyme defects demonstrate hepatic dysfunction at the time of acute catabolic decompensation, but persistent dysfunction has been documented only in trifunctional protein/long-chain L-3-hydroxyacyl-CoA dehydrogenase[109] and long-chain acyl-CoA dehydrogenase/very-long-chain acyl-CoA dehydrogenase[3] deficiencies. Intramitochondrial β-oxidation defects result in significant skeletal muscle weakness, with the general exceptions of medium-chain acyl-CoA dehydrogenase[16] and β-hydroxy-β-methylglutaryl-CoA lyase deficiencies.[9] Recently, a unique case of short-chain acyl-CoA dehydrogenase deficiency was reported in a 13-year-old girl with progressive external ophthalmoplegia, ptosis, cardiomyopathy, and multicore myopathy.[110] Recurrent episodes of myoglobinuria have been reported in association with long-chain acyl-CoA dehydrogenase,[111] very-long-chain acyl-CoA dehydrogenase,[112,113] trifunctional protein,[114,115] and short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiencies.[17]

Cardiac involvement (eg, cardiomegaly, cardiomyopathy) is more commonly observed in the long-chain defects (eg, long-chain acyl-CoA dehydrogenase, very-long-chain acyl-CoA dehydrogenase, trifunctional protein)[3,111,116] but has been documented in one case of short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency.[17] Cardiomyopathy has been reported in cases of trifunctional protein deficiency with presumed or proven deficiency of the long-chain L-3-hydroxyacyl-CoA dehydrogenase component.[109,114,116–123] Of note, there is increasing evidence that a significant subgroup of women with acute fatty liver of pregnancy or hemolysis, elevated liver enzymes, and low platelets syndrome are heterozygous for long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency and carry affected long-chain L-3-hydroxyacyl-CoA dehydrogenase-deficient fetuses with a common mutation (G1528C, E474Q) in one or both alleles.[124–127] There appear to be three major phenotypes of trifunctional protein/long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency with overlap: a hepatic form with Reye-like syndrome and associated with acute fatty liver of pregnancy, a neonatal cardiomyopathic form, and a less common, milder myoneuropathic form. These phenotypes appear to correlate with specific genotypes.[128–131] One patient with medium-chain 3-ketoacyl-CoA thiolase deficiency has been described, a male neonate who died at 13 days of age after presenting at 2 days of age with vomiting, dehydration, metabolic acidosis, liver dysfunction, and myoglobinuria.[132]

Short-Chain Acyl-CoA Dehydrogenase Deficiency

In short-chain acyl-CoA dehydrogenase deficiency, there is a particularly high incidence of neurologic abnormalities, including infantile-onset developmental delay, central hypo-

tonia or hypertonia, and seizures.[110,133] Furthermore, in this disorder, there tends to be an absence of episodic hypoglycemic, hypoketotic encephalopathy because of the intact oxidation of medium- and long-chain fatty acids for adequate ketogenesis. The mechanism for the neurologic abnormalities may therefore be related to the neurotoxicity of short-chain fatty acid metabolites.

Case Report

A female infant was born to a Caucasian mother at 39 weeks' gestation.[7] The pregnancy was uneventful, with the exception of a history of nausea, vomiting, and diarrhea of viral etiology in the mother and her family 1 month prior to delivery. The mother was of Italian and Portugese origin and the father of French Canadian origin. There were five normal male siblings. The proband was born by spontaneous vaginal delivery under local anesthesia, with Apgar scores of 9 and 9 at 1 and 5 minutes. The neonatal examination was normal, and cow's milk formula was started on day 2. On the third day of life, the infant fed poorly, began to vomit, and became lethargic and hypertonic. The respiratory rate was 60 and the liver extended 3 cm below the right costal margin. No other findings of the examination were remarkable.

Biochemical investigations at this time included blood glucose 38 mg/100 mL (normal, 65 to 110 mg/100 mL), subsequently normal on intravenous glucose; bilirubin 8/0.9 mg/100 mL (normal, 0.2 to 1.0/0 to 0.2 mg/100 mL); serum glutamic oxaloacetic transaminase 93 IU/L (normal, 22 to 100 IU/L); lactic dehydrogenase 1050 IU/L (normal, 88 to 196 IU/L); serum glutamic pyruvic transaminase 50 IU/L (5 to 35 IU/L); blood urea nitrogen normal, complete blood count normal, arterial pH 7.28 (normal, 7.35 to 7.45 pH); carbon dioxide 26 mEq/L (normal, 22 to 26 mEq/L); ammonia (NH_3) 399 μmol/L (normal, 7 to 42 μmol/L); prothrombin time 16.4/12.2 seconds (normal, 10 to 14 seconds); and partial thromoboplastin time normal. After 12 hours of intravenous glucose nutrition alone, serum glutamic oxaloacetic transaminase was 211 IU/L, serum glutamic pyruvic transaminase was 77 IU/L, and NH_3 was 298 μmol/L, and the metabolic acidosis was unchanged. Bacterial and viral cultures of blood, urine, and spinal fluid were negative, and viral cultures from the mother's cervix and rectum were negative.

The infant was treated with penicillin and gentamicin and received only high-dose intravenous glucose for nutrition. Despite stable blood glucose, NH_3, and arterial pH, the infant became progressively more lethargic, unresponsive to noxious stimuli, and hypotonic and had worsening respiratory effort. An electroencephalogram (EEG) demonstrated multifocal seizures with poor background activity. The infant was treated with phenytoin and respiratory support. There was no clinical or laboratory response to intravenous riboflavin. Metabolic studies performed on day 3 revealed elevated concentrations (listed in decreasing order) of lactate, 3-hydroxybutyrate, butyrate, ethylmalonate, adipate, 2-hydroxybutyrate, and 3-hydroxyisovalerate on urinary organic acid screening by gas chromatography. Glutarate and methylsuccinate levels were not increased. Amino acid screening of blood and urine revealed nonspecific generalized increases. Urine orotic acid excretion was normal. On day 5, the EEG worsened, and a brain scan demonstrated reduced blood flow. Clinically, the infant had fixed dilated pupils and no spontaneous activity or response to stimuli. Biopsies were taken of the liver and skin, and support was discontinued on the sixth day of life. Autopsy examination showed cerebral edema, hepatomegaly with fatty changes (microvesicular fat, intracanalicular cholestasis, and focal hepatocellular necrosis), thymic involution, and early bronchopneumonia. Bacterial and viral cultures were negative.

The cultured skin fibroblasts catabolized [1-^{14}C]butyrate poorly but oxidized radiolabeled octanoate, palmitate, and/or succinate normally. The mitochondrial acyl-CoA dehydrogenase activities with butyryl- and octanoyl-CoAs were 47% and 81% of controls, respectively. Monospecific medium-chain acyl-CoA dehydrogenase antisera inhibitied medium-chain acyl-CoA dehydrogenase activity to both butyryl- and octanoyl-CoAs, revealing a markedly reduced short-chain acyl-CoA dehydrogenase activity of 11% of controls.

Differentiating Laboratory Features

The site of biochemical defect may be suggested by the chain-length specificity and species type of fatty acid intermediates detected in serum or urine of affected children. Identification of these intermediates requires biomedical technology that may be available only in specialized metabolic laboratories. Assessment of plasma total and free carnitine and serum acylcarnitines and urine acylcarnitines, acylglycines, and organic acids is particularly useful and best measured during acute catabolic crises or fasting. These intermediates may be absent when the child is metabolically stable and receiving adequate supplies of glucose, thereby eliminating stress on the fatty acid oxidation pathway. The intermediates characteristic of the specific known defects are presented in Table 4.

1. *Carnitine.* Fatty acid oxidation disorders are generally associated with a decrease in total plasma carnitine concentration (< 30 μmol/L; normal range, 40 to 60 μmol/L) The lowest concentrations are found in the carnitine transporter defect, where carnitine values are usually less than 5% of control values.[96] In intramitochondrial β-oxidation defects, total plasma carnitine concentrations vary between 10 and 50% of normal. In carnitine palmitoyltransferase I deficiency, in which the esterification of palmitate to carnitine is defective, total plasma carnitine may be normal or increased.[134]

   In most fatty acid oxidation defects, with the exception of the carnitine transporter and carnitine palmitoyl transferase I defects, there is an increase in the ratio of esterified to total carnitine. This reflects excessive acyl-CoAs, accumulating proximal to the block in β-oxidation, which become esterified to carnitine. Estimate of acylcarnitine concentrations is based on the difference between free and total carnitine measurements. Under normal conditions, the esterified carnitine fraction is 10 to 25% of total in the fed state and 30 to 40% of total in the fasted state. When filtered through the kidney, increased serum acylcarnitines compete with free carnitine at the renal tubular reabsorptive site. Because of the higher affinity of longer-chain acylcarnitines for the carnitine transporter,[96] free carnitine is excreted, leading to a decrease both in serum free and total carnitine concentrations and an increase in the esterified carnitine fraction.

Table 4. Laboratory Features Associated With Genetic Defects of Fatty Acid Oxidation

		Carnitine		*Unique or Specific Metabolites*		
Enzyme Defect	*No. of Cases*	*Total*	*Free*	*Acylcarnitine*	*Acylglycine*	*Organic Acids*
LCFAUD	2	Low	Low	NR	NR	NR
CUD	45	Very low	Low	NR	NR	NR
CPT I	10	Normal/high	High	NR	NR	NR (+)
TRANS	7	Low	Very low	Long chain	NR	NR
CPT II (mild)	50	Low	Very low	Long chain	NR	NR
CPT II (severe)	5	Low	Very low	Long chain	NR	+
VLCAD/LCAD	20	Low	Low	Long chain	NR	A, Su, Se
TFP/LCHAD	100	Low	Low	Long chain	NR	A, Su, Se, 3-0H intermediates
Dienoyl CoA reductase	1	Low	Low	Decadienoyl	NR	NR
MCAD	> 150	Low	Low	Octanoyl	Suberyl, hexanoyl, phenprop	A, Su, Se
SCAD	9	Low	Low	Butyryl	Butyryl	A, Su, Se; ethylmalonic; methylsuccinate
SCHAD	5	Low	Low	NR	NR	A, Su, Se; 3-0H intermediates
ETF and ETF-Qo	25	Low	Low	Octanoyl, glutaryl, butyryl, isovaleryl	Suberyl, hexanoyl, butyryl, isovaleryl	A, Su, Se; glutaric; ethylmalonic
HMG-CoA lyase	50	Low	Low	3-Methylglutaryl	?	3-Hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric, A, 3-hydroxy-isovaleric

LCFAUD = long-chain fatty acid uptake defect; CUD = carnitine transporter defect; CPT = carnitine palmitoyltransferase; TRANS = carnitine acylcarnitine translocase; VLCAD = very-long-chain acyl-CoA dehydrogenase; LCAD = long-chain acyl-CoA dehydrogenase; TFP = trifunctional protein; LCHAD = long-chain L-3-hydroxyacyl-CoA dehydrogenase; MCAD = medium-chain acyl-CoA dehydrogenase; SCAD = short-chain acyl-CoA dehydrogenase; SCHAD = short-chain L-3-hydroxyacyl-CoA dehydrogenase; ETF = electron transfer flavoprotein; Qo = coenzyme Q oxidoreductase; HMG = β-hydroxy-β-methylglutaryl; NR = no case yet reported; A, Su, Se = adipic, suberic, sebacic acids; phenprop = phenylpropionyl.
Adapted from Hale and Bennett.[18]

Carnitine esters can be separated further on the basis of the acid insolubility of long-chain acylcarnitine esters.[18] Separation and identification of the individual acylcarnitine esters have been facilitated by advances in biomedical technology, including the application of fast atom bombardment-tandem mass spectrometry and isotopic exchange high-performance liquid chromatography.[135,136] The identification of specific carnitine esters aids in the diagnosis of certain fatty acid oxidation defects, such as octanoyl-carnitine in medium-chain acyl-CoA dehydrogenase deficiency.[43] Thus, analysis of serum acylcarnitines may be useful in screening patients for fatty acid oxidation defects. Serum acylcarnitine analysis is particularly helpful in the diagnosis of long-chain fatty acid oxidation disorders because it overcomes the problem of the renal threshold effect whereby long-chain acylcarnitines are selectively reabsorbed at the renal carnitine transporter site, leading to a loss of free carnitine in urine. This approach also overcomes the problem of the poor solubility of long-chain fatty acids in urine.

2. *Dicarboxylic acids.* Dicarboxylic acids (adipic, suberic, sebacic acids) are observed in all identified intramitochondrial β-oxidation defects.[137] Hale and Bennett[18] point out several limitations to the value of these compounds in the recognition of fatty acid oxidation defects: (i) Dicarboxylic acids may be seen in children receiving formulas containing medium-chain triglycerides, in those who are seriously ill (eg, diabetic ketoacidosis),[138] or in those receiving medications that interfere with fatty acid oxidation (eg, valproic acid).[139] However, in each of the above conditions, ketone levels exceed dicarboxylic acid levels, whereas in the intramitochondrial fatty acid oxidation defects, dicarboxylic acid levels equal or exceed ketone levels during fasting. Furthermore, the pattern of dicarboxylic acids is easily distinguished in experienced metabolic laboratories from the pattern of pathologic dicarboxylic acids associated with fatty acid oxidation defects. (ii) Dicarboxylic acids are not present when children are not catabolic and are well and eating regularly or receiving intravenous glucose at rates in excess of normal hepatic glucose production rates, thus decreasing the dependence on fatty acid oxidation and the production of fatty acid metabolites. (iii) Increased concentrations of dicarboxylic acids in urine are generally not seen in disorders involving the transport of fats into the mitochondria. A fatty acid oxidation defect can be suspected in the presence of an excess of dicarboxylic acids relative to ketones; however, absence of dicarboxylic acids does not exclude a defect.

The organic acid pattern may suggest the site of defect. For example, children with long-chain acyl-CoA dehydrogenase/very-long-chain acyl-CoA dehydrogenase deficiency excrete primarily medium- and long-chain saturated dicarboxylic acids, in contrast to children with trifunctional protein deficiency who excrete almost equimolar amounts of saturated and 3-hydroxydicarboxylic acids.[140] However, the presence of 3-hydroxy compounds is not specific for trifunctional protein deficiency as these compounds may be seen in intrinsic liver disease and toxic reactions to acetaminophen.[141]

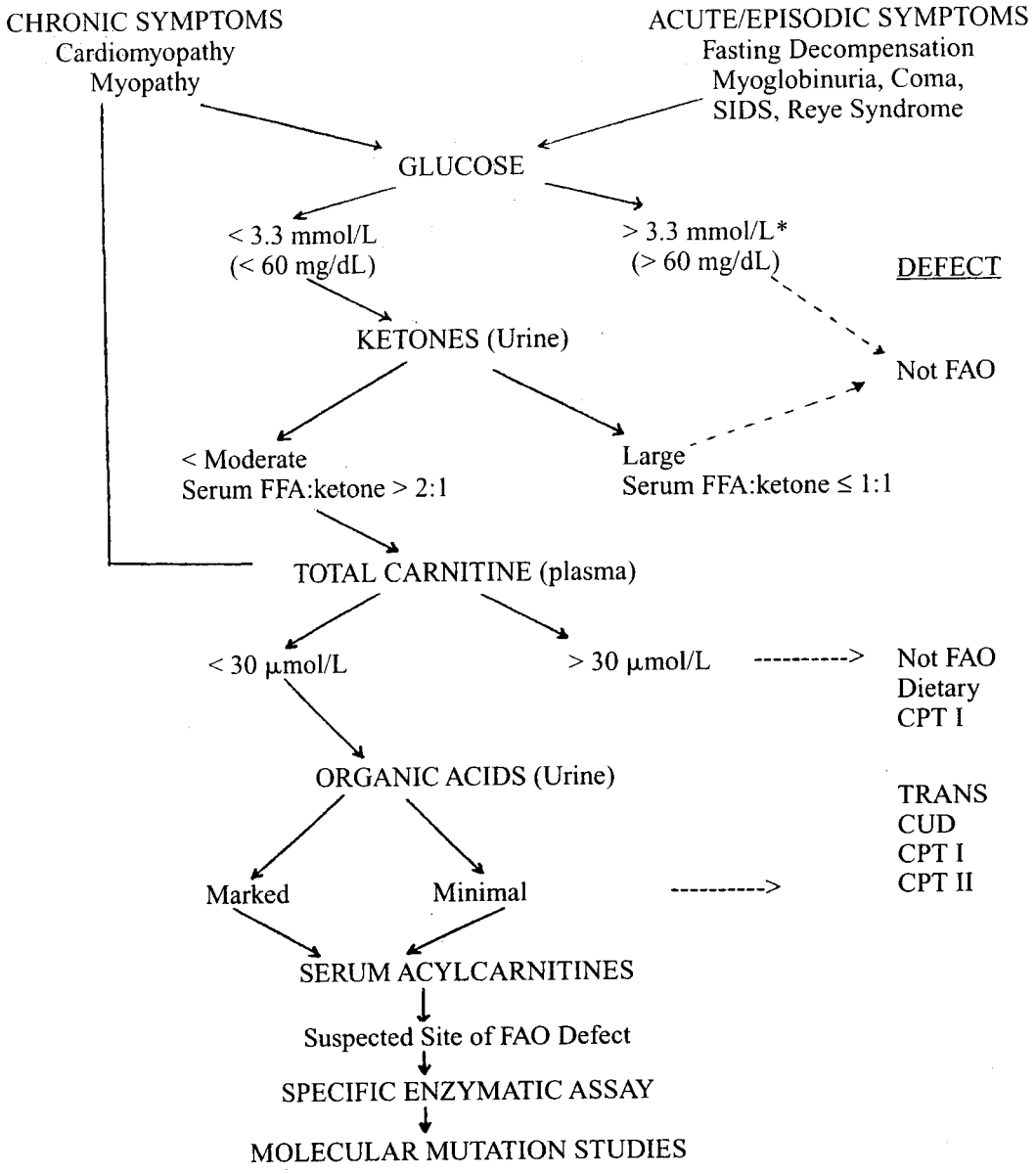
Advances in stable-isotope dilution mass spectrometry have improved the ability to detect metabolites in very small quantities in plasma or urine.[142] Acylglycines that are consistently excreted in small quantities in urine do not appear to have the same limitations as dicarboxylic acids. Useful glycine metabolites have been identified for several defects, including medium-chain acyl-CoA dehydrogenase, short-chain acyl-CoA dehydrogenase, electron-transferring flavoprotein, and electron-transferring flavoprotein CoQ oxidoreductase deficiencies. Increased concentrations of hexanoyl, suberyl-, and 3-phenylpropionylglycine are seen in medium-chain acyl-CoA dehydrogenase deficiency.[143]

Diagnostic Approaches and Screening Methods

1. *History and physical examination.* The key to investigation of these patients remains a careful history and clinical examination. The presentation may be acute and recurrent or more chronic and slowly progressive (Figure 4). The acute presentation is more typical, in which the child has a history of decreased oral intake during the preceding 24 to 36 hours followed by increasing lethargy and progressive obtundation or coma and seizures. A positive family history for sudden infant death syndrome, "near-miss" sudden infant death syndrome, or Reye's syndrome should increase the index of suspicion.

   Physical examination may reveal cardiomyopathy, myopathy, hypotonia, or hepatomegaly. Initial studies in a comatose child should include serum glucose and urine ketone measurements.[18] Determination of urine ketones may be complicated by dehydration, resulting in concentrated urine. If the blood glucose concentration is > 3.3 mmol/L (60 mg/dL) and it is accompanied by high concentrations of urinary ketones, this tends to rule out a disorder of fatty acid oxidation. However, if the blood glucose is < 3.3 mmol/L and the urine ketones are trace or small in amount, this would suggest the possibility of a fatty acid oxidation disorder and warrants further investigation. Most importantly, prior to the administration of intravenous glucose, samples from the acute episode should be obtained for evaluation of serum total and free carnitine, acylcarnitines, free fatty acids, and ketones and urine organic acids, acylglycines, and acylcarnitines. The serum free fatty acid to ketone ratio is a useful screen. The normal ratio is 1:1; if there is a block in fatty acid oxidation, this ratio increases to > 2:1. In addition, serum and urine samples obtained during the acute episode can be used to assess the integrity and hormonal regulation of biochemical pathways of glucose homeostasis.
2. *Total carnitine measurement.* A total plasma carnitine concentration of < 30 μmol/L suggests a fatty acid oxidation disorder but is not diagnostic. As previously discussed, there are a number of other genetic and acquired disorders that present with secondary carnitine deficiency (see Table 1). Total serum carnitine of 10 to 50% of normal, combined with an increase in the esterified fraction of carnitine (normal, 10 to 25% of total carnitine in fed state, 30 to 40% of total carnitine in fasted state), suggests an intramitochondrial β-oxidation defect with secondary carnitine deficiency. Total serum carnitine concentration of < 5% of normal with a normal esterified fraction, as well as evidence of progressive cardiomyopathy and myopathy, suggests the plasmalemmal carnitine transporter defect.[14,96] The exception is carnitine palmitoyltransferase I deficiency, which presents with recurrent episodes of hypoketotic, hypoglycemic encephalopathy, or Reye-like syndrome and an increased serum carnitine with a decreased esterified fraction owing to the block in palmitate esterification to carnitine
3. *Urinary organic acids.* As the production of fatty acid metabolites diminishes rapidly once normoglycemia is restored, it is critical to obtain urine specimens during the acute catabolic episode prior to intravenous glucose therapy. The chain length and species type of the organic acids may help to identify the specific site of fatty acid oxidation block.
4. *Fasting studies.* A fasting study may be considered for the purposes of distinguishing a defect in fatty acid oxidation from other causes of hypoglycemia if diagnostic samples have not been taken during an acute catabolic event. However, it must be strongly emphasized that if a fasting study is to be undertaken, it must be done under very carefully controlled hospital conditions with continuous monitoring and by physicians who are knowledgeable with respect to hypoglycemia, hypopituitarism, hyperinsulinism, and fatty acid oxidation disorders. Some authorities believe that fasting studies should not be performed in children with fatty acid oxidation disorders because diagnostic fasting may precipitate an acute metabolic crisis, leading to further morbidity or death. They suggest instead that loading tests with carnitine or phenlypropionate can be used to aid diagnosis.

   As suggested by Hale and Bennett,[18] there are pros and cons with each method. The advantages of a fasting study are (i) the duration of fasting tolerance can be determined under carefully controlled conditions, which may provide useful information regarding the long-term management of the affected patient and guidelines for prevention of acute catabolic episodes; (ii) the full spectrum of abnormal fasting adaptation can be studied through assessment of a number of laboratory parameters, includ-



EXAMPLES OF SPECIFIC STUDIES	RESULTS	DEFECT
Specific dicarboxylic acids	3-Hydroxy compounds	Trifunctional
Glycine conjugates	Phenylpropionylglycine	MCAD
Carnitine esters	Butyrylcarnitine	SCAD
Direct assay	Reduced SCHAD activity	SCHAD
Molecular probes	A985G mutation	MCAD

Figure 4. Approach to patients with potential fatty acid oxidation (FAO) disorder. Clinical signs and symptoms provide the initial suggestion of an inborn defect in fatty acid oxidation. Laboratory features include hypoglycemia with inappropriately low levels of ketones, decreased total plasma carnitine, and dicarboxylicaciduria. Further studies are guided by results of these general screening tools. FFA = free fatty acid; CPT = carnitine palmitoyltransferase; TRANS = carnitine acylcarnitine translocase; CUD = carnitine transporter defect; MCAD = medium-chain acyl-CoA dehydrogenase; SCAD = short-chain acyl-CoA dehydrogenase; SCHAD = short-chain L-3-hydroxyacyl-CoA dehydrogenase. *Does not absolutely exclude fatty acid oxidation defect. Adapted from Hale and Bennett.[18]

ing hormonal measurements; and (iii) the time to precipitation of acute clinical decompensation can be documented. As previously emphasized, the cardinal risk is the precipitation of an acute catabolic crisis leading to morbidity and death. It is preferable to gather as much information as possible through the collection of appro-

priate samples during an acute catabolic event. The purpose of the fasting study is to identify the defective metabolic pathway through analysis of temporal changes in substrates (glucose, free fatty acids, lactate, ketones), metabolites (carnitine, dicarboxylic acids), and relevant hormones (growth hormone, cortisol, insulin). The fast should be terminated when children demonstrate their first symptoms or have a blood glucose of < 3.3 mmol/L (60 mg/dL) or demonstrate moderate ketonuria. If at this point there is deficient ketogenesis, significant dicarboxylic aciduria, and a serum free fatty acid-to-ketone ratio > 2:1, there is presumptive evidence for a defect in fatty acid oxidation.

The primary advantage of loading tests and the measurement of specific metabolites is safety. However, these tests are useful only in certain fatty acid oxidation defects (eg, medium-chain acyl-CoA dehydrogenase deficiency); therefore, a negative test does not exclude all fatty acid oxidation defects. In addition, they do not evaluate the spectrum of fasting adaptation in the individual.

5. *Other studies.* Once there is presumptive evidence for a defect in fatty acid oxidation, the clinical picture, in combination with analysis of serum acylcarnitines and urinary organic acids and acylglycines, may suggest a specific site of defect as well as the chain-length specificity of the defect. Further investigations to identify the specific site of defect are as follows:
   i. *Fatty acid oxidation studies.* A further useful screening tool, provided that the defect is expressed in cultured skin fibroblasts, is the measurement of oxidation rates of [1-^{14}C]-labeled palmitate (C_{16}), octanoate (C_8), and butyrate (C_4) in fibroblasts to establish the chain-length specificity of the defect.[144] Incubation of fibroblasts with labeled palmitate and linoleate may yield diagnostically informative fibroblast acylcarnitine profiles.[145,146]
   ii. *Enzymatic assays.* Depending on the suspected site of defect, a direct enzyme assay may then be performed for the specific enzyme. These assays can be performed in cultured skin fibroblasts or in biopsied muscle specimens for carnitine palmitoyltransferase I and II, short-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, short-chain L-3-hydroxy-CoA dehydrogenase, and long-chain L-3-hydroxy-CoA dehydrogenase deficiencies. However, expression of certain of these defects may be tissue specific. For example, in muscle short-chain L-3-hydroxy-CoA dehydrogenase deficiency, the defect is primarily expressed in biopsied muscle but not in skin fibroblasts; therefore, it may be missed unless muscle is examined.[17] The carnitine-acylcarnitine translocase[147] and medium-chain acyl-CoA dehydrogenase deficiencies can be measured in cultured skin fibroblasts. Evidence for a defect in electron-transferring flavoprotein or electron-transferring flavoprotein CoQ relies on demonstration of a combined deficiency in the activities of short-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, and long-chain acyl-CoA dehydrogenase.
   iii. *Uptake studies.* For the carnitine transporter defect, diagnosis is confirmed by in vitro studies of carnitine uptake in cultured skin fibroblasts. These studies demonstrate negligible uptake of carnitine in affected patients throughout the physiologic range (0.1 to 50 μmol/L) of carnitine substrate concentrations (eg, 2% of normal control carnitine uptake at 5 μmol/L of carnitine), thereby precluding the calculation of the Michaelis binding constant and the maximal velocity in rate of uptake values.[14,148] This supports the concept that primary carnitine deficiency is attributable to a defect in the specific high-affinity, carrier-mediated carnitine transporter. In heterozygotes, carnitine uptake studies demonstrate normal Michaelis binding constant values but reduced maximal velocity in rate of uptake values of 13 to 44% of control,[14,96] suggesting a reduced number of normally functioning transporters. This is the most sensitive study for detection of the carrier state as serum carnitine concentrations in heterozygotes may be normal. Negligible carnitine uptake also has been demonstrated in the cultured lymphoblasts[149] and myoblasts[1] of affected children.
   iv. *Molecular studies.* Further molecular characterization of the specific defects include Western blotting to determine whether the defects are cross-reacting material positive, suggesting a kinetic deficiency, or cross-reacting material negative, suggesting decreased production of the affected enzyme. Western blotting also has been used in the determination of the amounts of α- and β-subunit of electron-transferring flavoprotein.[151] A number of enzymes have been cloned (eg, carnitine palmitoyltransferase I and carnitine palmitoyltransferase II, short-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, short-chain L-3-hydroxy-CoA dehydrogenase, and trifunctional protein), leading to the discovery of specific mutations that result in defective enzyme activity. Molecular probes can be used for the precise and rapid detection of specific defects in the population, as well as to determine the relative frequency of a mutation in a specific population. An example is the gene mutation found in more than 70% of children with medium-chain acyl-CoA dehydrogenase deficiency (A985G point mutation) in the UK population.[152] Mutations also have been demonstrated in the gene encoding the sodium ion-dependent, high-affinity, plasmalemmal carnitine transporter (carnitine/ organic cation transporter OCTN2).[153-161]

Treatment

Owing to the small numbers of patients with fatty acid oxidation disorders at any given institution, it is difficult to systematically evaluate in a prospective double-blind, controlled

clinical trial any single treatment regimen or to compare different regimens. However, the mainstay of therapy is the avoidance of precipitating factors, particularly prolonged fasting. The general treatment strategies are as follows:

1. *Avoidance of precipitating factors, such as prolonged fasting, prolonged aerobic exercise (> 30 minutes) and cold exposure leading to shivering thermogenesis.* The avoidance of prolonged fasting is key, specifically 6 to 10 hours for infants younger than 1 year of age or ≥ 12 hours for children between 1 and 4 years of age. In the event of progressive lethargy or obtundation or an inability to take oral feedings because of vomiting, the child should be taken immediately to the emergency room for intravenous glucose therapy. Intravenous glucose should be provided at rates sufficient to prevent fatty acid mobilization (8 to 10 mg/kg/min of glucose). In a young child weighing less than 15 kg, generally, 10% dextrose in a balanced electrolyte solution is administered at 1½ times the maintenance rates.[18] This should be adjusted in older individuals to achieve the same rate of glucose infusion. Intravenous glucose administration should be continued until the child is able to take oral feedings and the catabolic cascade has been reversed. It is prudent to institute intravenous glucose therapy as soon as there is concern regarding the status of the child as waiting until the occurrence of severe symptoms or a drop in blood glucose to < 3.3 mmol/L may result in serious morbidity or death. Similarly, prolonged exercise (> 30 minutes) should be avoided as this would be the time period in which there would be increased fat mobilization and increasing dependence on fatty acid oxidation for ATP production. A high-carbohydrate load prior to exercise and a rest period with a repeat carbohydrate load at 15 minutes are advisable. The entire period of exercise should not exceed 30 minutes at any given time. Because shivering thermogenesis is dependent upon fatty acid oxidation, it is also important to avoid cold exposure.
2. *High-carbohydrate, low-fat diet.* In general, it is advisable to institute a high-carbohydrate, low-fat diet with frequent feedings throughout the day, commensurate with the nutritional needs of the child given the age and development of the child. This is best implemented with the aid of a dietitian, aiming toward approximately 70 to 75% of calories from carbohydrate sources, 15% from protein, and approximately 10 to 15% from fat. It is very important to ensure that the child is receiving essential fatty acids; thus, supplementation may be required. In older children, an effective regimen consists of three regular meals per day with three equidistantly timed intermeal snacks, including a bedtime snack. Furthermore, in β-hydroxy-β-methylglutaryl-CoA lyase deficiency, a high-carbohydrate, low-fat, low-protein diet with leucine restriction should be implemented.[9]
3. *Uncooked cornstarch.* To delay the onset of overnight fasting, the institution of nightly uncooked cornstarch, in doses similar to those used in the treatment of glycogen storage disease (1 to 2 g/kg/day as a single nighttime dose), may be useful for prolonging the postabsorptive state.[114]

Specific measures for individual fatty acid oxidation disorders include the following:

1. *Carnitine.* The high-affinity plasmalemmal carnitine transporter defect is a clear indication for carnitine therapy. This disorder is characterized by carnitine-responsive cardiomyopathy, with or without weakness; hypoglycemic, hypoketotic encephalopathy; and failure to thrive, in which there are very low plasma and tissue concentrations of carnitine (generally < 5% of normal); lipid storage in muscle, heart, and liver; and severe renal leak of carnitine with absence of abnormal dicarboxylic aciduria.[14,96] In 22 patients with this condition treated with high-dose oral carnitine supplementation, dramatic improvement was observed in the cardiomyopathy and myopathy even within a few weeks and a reduction in heart size toward normal within a few months of initiating therapy. In addition, three children with significant failure to thrive prior to treatment demonstrated a marked improvement in growth after therapy.[14] The impairment in fasting ketogenesis was corrected with carnitine therapy in the one patient tested.[148] Eighteen of 19 patients treated with carnitine therapy for periods of 1 to 10 years continue to be healthy.[96] One of these 19 patients was moderately impaired with weakness and cardiomyopathy at age 20 years, which was thought to be owing to poor compliance with carnitine therapy. Thus, in the carnitine transporter defect, high-dose oral carnitine supplementation in a dosage of 100 mg/kg/day in four divided daily doses is critical and life saving, significantly reversing the pathology of this otherwise progressive and lethal disease.

   In the intramitochondrial β-oxidation defects with secondary carnitine deficiency, the results of carnitine therapy have been highly variable and insufficiently evaluated. The rationale for carnitine supplementation has been to limit the intracellular concentrations of potentially toxic acyl-CoA intermediates through transesterification and to thereby liberate critical intracellular free CoA.[18] However, there has been no prospective study to prove that carnitine administration has a beneficial effect. Stanley et al[16] studied one patient with medium-chain acyl-CoA dehydrogenase deficiency and demonstrated that the associated carnitine deficiency was not the cause of the defect in fatty acid oxidation, as evidenced by the lack of effect of carnitine replacement on fasting ketogenesis. After 3 months of oral carnitine therapy, this patient had no increase in plasma ketones and became hypoglycemic after 14 hours of fasting. Moreover, there is increasing evidence to suggest that in the long-chain fatty acid oxidation disorders, carnitine administration may have deleterious effects. In these disorders, there is an accumulation of long-chain acyl-CoAs proximal to the metabolic block, which, upon esterification, become long-chain acylcarnitines. The long-chain acylcarnitines

may have a detergent-like action on muscle membranes. Excessive amounts of palmitoyl-CoA and palmitoylcarnitine have been shown to have detergent properties on isolated canine myocytic sarcolemmal membranes and to potentiate free-radical–induced lipid membrane peroxidative injury in ischemia.[91] Long-chain acylcarnitines also have been shown to activate calcium channels in cardiac[92,93] and smooth muscle myocytes[93,94] and thus may potentiate the increase in cytosolic calcium associated with arrhythmogenesis observed in ischemic myocardium.[95] More recent evidence suggests, however, that there may be significant excretion of acylcarnitines in bile; two infants with long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency were shown to have marked biliary accumulation of C_{10} to C_{18} acylcarnitines in postmortem bile when analyzed by electrospray/tandem mass spectrometry.[162] This may serve as an important route of excretion for long-chain acylcarnitines. Further investigation of this issue is warranted.

2. *Medium-chain triglyceride oil.* Nutritional medium-chain triglyceride oil may be a useful treatment in long-chain fatty acid oxidation disorders as the medium-chain fatty acids would circumvent the block in long-chain fatty acid oxidation and facilitate ATP production from the remainder of the patent fatty acid oxidation pathway. Medium-chain triglyceride oil can be started at a dose of 0.5 g/kg/day divided in three daily doses, increasing to 1 to 1.5 g/kg/day as tolerated. The major side effect is diarrhea. The usefulness of this approach, however, may be limited by storage of excess medium-chain triglyceride as long-chain fats in adipocytes, which cannot be metabolized in long-chain fatty acid oxidation defects and would increase the long-chain fat load. The efficacy of this approach has been variable in trifunctional protein/long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency, ranging from highly beneficial to no significant response, and may depend on the individual case.[115] There have been several reports of successful treatment of the cardiomyopathy associated with very-long-chain acyl-CoA dehydrogenase deficiency with medium-chain triglyceride oil.[163,164]
3. *Riboflavin.* Certain cases of multiple acyl-CoA dehydrogenase deficiencies (eg, electron-transferring flavoprotein or electron-transferring flavoprotein CoQ–linked deficiencies) are responsive to riboflavin supplementation.[165] This may be attributable to a stabilizing effect of riboflavin on a mutant flavin-dependent enzyme. Riboflavin can be given in dosages of approximately 50 mg three times daily in infants and young children and 100 mg three times daily in older children.
4. *Specific therapies for long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency.* Several children with long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency and associated pigmentary retinopathy were shown to have a deficiency of the (n-3) polyunsaturated fatty acid docosahexaenoic acid. Subsequent supplementation with docosahexaenoic acid led to some improvement in visual function.[166] Furthermore, daily oral administration of cod liver oil extract containing high amounts of docosahexaenoic acid led to marked clinical and electrophysiologic recovery of the progressive peripheral sensorimotor axonopathy in one boy with the myoneuropathic form.[167,168] Oral prednisone has been shown to result in a dramatic reversal of the limb-girdle myopathy and marked reduction in episodic myoglobinuria in one boy with myoneuropathic long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency.[115]

Genetics and Presymptomatic Recognition

All known fatty acid oxidation disorders are inherited as autosomal recessive conditions. Therefore, both parents of children born with these disorders are obligate carriers of the mutant gene and have a 25% chance of bearing a normal child, 50% chance of bearing a carrier, and 25% chance of bearing an affected child. Identification of a disorder of fatty acid oxidation in a given family will have significant implications for siblings and their medical management. Screening for affected siblings is important as treatment with preventive measures is relatively simple and effective and may avoid significant morbidity and mortality.

One approach to screening is the evaluation of populations considered to be at risk for defects in fatty acid oxidation, such as families with a history of sudden infant death syndrome, near-miss sudden infant death syndrome, or Reye's syndrome. In a study of more than 100 confirmed cases of medium-chain acyl-CoA dehydrogenase deficiency, almost 20% were originally misdiagnosed as sudden infant death syndrome or near-miss sudden infant death syndrome.[18] Although screening of siblings following identification of an affected child benefits the family, this approach fails to identify individuals who do not belong to these high-risk groups.

Systematic screening of the general population has not been implemented, primarily owing to a lack of appropriate screening tools. Screening approaches considered to date include the analysis of urine or neonatal filter blood-spot cards for identification of specific fatty acid metabolites. Bennett et al[169] have shown that the incidence of dicarboxylic aciduria in an acutely ill pediatric population is approximately 1 in 12,000, and, in most cases, the pattern was consistent with a defect in fatty acid oxidation. Specific molecular probes have been used to screen for one of the more common defects, namely medium-chain acyl-CoA dehydrogenase deficiency. In a study using the specific molecular probe for the A985G mutation found in a majority of children with medium-chain acyl-CoA dehydrogenase deficiency in the United Kingdom, Blakemore et al[152] examined 410 consecutive neonatal blood-spot cards in a northern English population. Six individuals were found to be heterozygous, but none were homozygous for this particular mutation. These results suggested that the carrier frequency for this particular mutation is 1 in 68, corresponding to a frequency of approximately 1 in 18,500 births for the homozygous medium-chain acyl-CoA dehydrogenase-deficient state. In a more recent survey from the Pennsylvania newborn

screening program using tandem mass spectrometry and confirmed by molecular analysis for several mutations, the incidence of medium-chain acyl-CoA dehydrogenase deficiency was found to be higher at 1 in 8930 live births.[19]

HIGH-AFFINITY CARNITINE TRANSPORTER DEFECT

This is a potentially lethal autosomal recessive disease attributable to a genetic defect of the plasmalemmal high-affinity carnitine transporter. It is characterized by progressive, infantile-onset, hypertrophic and/or dilatative cardiomyopathy; recurrent hypoglycemic, hypoketotic encephalopathy; weakness; failure to thrive; and sudden infant death syndrome. Affected individuals have very low plasma and tissue carnitine concentrations (generally < 5% of normal); lipid storage in muscle, heart, and liver; and severe renal leak of carnitine.[14,96] Patients have markedly reduced carnitine uptake in cultured skin fibroblasts, lymphoblasts, myoblasts, and myotubes, as well as decreased renal reabsorption of carnitine.[14,96,148-150] This formerly lethal disorder is now highly treatable if diagnosed early and promptly treated with high-dose oral carnitine therapy, suggesting the existence of alternate cell membrane carnitine transporters. Therapy leads to rapid clinical improvement in cardiac function, strength, and growth and a cessation of episodic coma, provided that there is good lifelong compliance.[14,96] We have recently shown that early carnitine therapy may prevent the development of cardiomyopathy.

Carriers demonstrate intermediate rates of carnitine uptake and normal Michaelis binding constant values in cultured skin fibroblasts; this is the most sensitive test for carrier detection as serum carnitine concentrations may be low or normal in carriers.[14] The overall prevalence of heterozygotes has been estimated to be 1% in the Akita prefecture of Japan, providing an estimated incidence of high-affinity carnitine transporter deficiency of 1 in 40,000 births.[160] Among carriers older than 20 years of age, without other confounding diagnoses such as hypertension, approximately 33% were found to exceed the 95% upper limit on echocardiographic parameters. One carrier had clinically apparent cardiac hypertrophy, cardiomegaly, and supraventricular premature contraction. Multiple logistic analysis showed that the presence of mutations was a stronger independent risk factor than aging and hypertension for echocardiographic abnormalities. It was suggested that a low carnitine phenotype and aging and/or environmental factors may act synergistically to lower cardiac ATP production.

PHARMACOLOGIC AND TOXICOLOGIC RELEVANCE OF THE CARNITINE/ORGANIC CATION TRANSPORTER FAMILY

The carnitine/organic cation transporter OCTN2, the complementary DNA for the high-affinity carnitine transporter, has been cloned from a human placental trophoblast cell line.[170] OCTN2 belongs to the organic cation transporter family, which functions primarily in the elimination of cationic drugs and xenobiotics in kidney, intestine, liver, and placenta.[171-173] Tamai et al[174] identified OCTN2 as the physiologically important, high-affinity, sodium-dependent carnitine transporter in humans and found it to be strongly expressed in adult heart, kidney, skeletal muscle, and placenta. OCTN2 codes for a protein of 557 amino acids with 12 putative transmembrane domains. The human gene *OCTN2* has been sequenced as part of the Human Genome Project and maps to human chromosome 5q31.1.[175] In situ hybridization studies in the rat have shown that OCTN2 is expressed in kidney (proximal and distal tubules, glomeruli), heart (myocardium, valves, and arterioles), placenta (labyrinthine layer), and brain (cortex, hippocampus, and cerebellum).[176] Importantly, OCTN2 has been found to belong to the OCTN subfamily of carnitine/organic cation transporters, along with human OCTN1[177] and murine OCTN3,[178] which share high amino acid identity with hOCTN2 (> 75%), as well as the ability to transport carnitine with different affinities and other organic cations. Murine OCTN3 has a high affinity for carnitine,[178] whereas OCTN1 has a low affinity.[178,179]

In hOCTN2-transfected HEK293 cells, L-[^{3}H]-carnitine uptake has been shown to be significantly inhibited by a large number of xenobiotics in extensive clinical therapeutic use, including lipophilic organic cations (quinidine, verapamil, and emetine) and zwitterionic compounds such as β-lactam antibiotics (cephaloridine).[180,181] The anionic compounds valproic acid and probenecid were found to be moderate inhibitors. Certain compounds were shown to be directly transported by hOCTN2 in transfected cells (eg, tetraethylammonium, quinidine, verapamil, cephaloridine, and valproic acid). Given the role of hOCTN2 in the transport of acylcarnitines[96] and other cationic compounds, in combination with its inhibition by a variety of xenobiotics and its wide tissue distribution, hOCTN2 is of considerable pharmacologic and toxicologic importance. As certain drugs appear to compete with carnitine for the same substrate-binding site on OCTN2, it is likely that mutations causing high-affinity carnitine transporter deficiency are also associated with the loss of ability to transport these drugs, leading to increased renal clearance and hence decreased systemic half-life and therapeutic efficacy of these drugs. Currently, less is known regarding the pharmacologic characteristics of OCTN1[179] and OCTN3.[178] Given their wide tissue distribution and high amino acid identity with OCTN2, it will be important to fully characterize their pharmacologic profiles, which may also have specific therapeutic and toxicologic implications, particularly with respect to anticonvulsants such as valproic acid.

ROLE OF CARNITINE IN VALPROIC ACID-ASSOCIATED HEPATOTOXICITY

Carnitine deficiency in patients with epilepsy may result from multiple causes, including nutritional deficiency, an underlying inborn error of metabolism, and valproic acid therapy. Numerous studies have demonstrated a significant decrease

in total or free serum carnitine concentrations or both, in individuals taking multiple anticonvulsants including valproic acid[182–186] or valproic acid alone.[182] However, in one study of 21 children receiving valproic acid, mean serum carnitine concentration did not correlate with either the oral valproic acid dose or serum valproic acid concentration.[187] Importantly, serum concentration may not accurately reflect total tissue carnitine reserves, given that 90% of total body carnitine is stored in skeletal muscle.[15] This point was underscored in a study of three children receiving chronic valproic acid therapy for intractable seizures who were shown to have normal serum carnitine concentrations but clinically significant muscle carnitine deficiency.[188] Muscle carnitine concentrations provide a more accurate reflection of the true carnitine status of an individual. Renal loss of carnitine as valproylcarnitine esters has been examined by a number of investigators.[185,187,189] Several studies reported an increased ratio of acylcarnitine to total carnitine in the urine of patients receiving valproic acid, even though total urinary carnitine excretion was not increased.[185,187] Millington et al[189] documented decreased free carnitine and increased acylcarnitines in urine in two children on long-term valproic acid therapy and reported that valproylcarnitine constituted less than 10% of the total urinary acylcarnitine pool. Conversely, a marked increase in urinary carnitine excretion has been documented in a patient with valproic acid–associated hepatotoxicity.[190] It is unproven whether or not long-term valproic acid therapy, with continued urinary excretion of valproylcarnitine, depletes total body stores of carnitine, leading to a deficiency state.

We have previously demonstrated, in a cultured skin fibroblast model, that valproic acid induces serum and tissue carnitine depletion through inhibition of plasmalemmal carnitine uptake (including decreased renal reabsorption of free carnitine).[191] This effect is directly proportional to the duration and concentration of valproic acid exposure. The maximal inhibitory effect of valproic acid in cultured skin fibroblasts is completely reversible following greater than 5 days of valproic acid–free growth.[192] We have also shown that carriers of the high-affinity carnitine transporter defect who have intermediate rates of carnitine uptake in cultured skin fibroblasts are at increased risk for valproic acid–induced carnitine deficiency, placing them at heightened risk for life-threatening valproic acid hepatotoxicity.[193]

Four mechanisms by which valproic acid interferes with carnitine uptake have been proposed.[191] First, there may be direct inhibition of carnitine uptake into tissues and at the renal tubular reabsorptive site through the formation of valproylcarnitine esters, as valproic acid (2-propylpentanoic acid) is an 8-carbon fatty acid assumed to pass freely through the mitochondrial membrane in a carnitine-independent manner.[194] Stanley et al[96] found that the "muscle-kidney carnitine transport system" expressed in cultured skin fibroblasts was strongly inhibited by acylcarnitines (50% inhibition at 3.05 μmol/L L-carnitine, 4.6 μmol/L acetylcarnitine, 2.9 μmol/L octanoylcarnitine, and 0.37 μmol/L palmitoylcarnitine). Second, the formation of valproic acid metabolites (eg, 4-en valproate)[195] could lead to secondary inhibition of β-oxidation and accumulation of excessive acyl-CoA derivatives. Valproic acid and its metabolites were found to be potent inducers of microvesicular steatosis in rats,[195] and valproic acid has been shown to be toxic to rat liver mitochondria both in vitro[196] and in vivo.[197] The morphologic swelling of rat liver mitochondria could be prevented by the simultaneous administration of L-carnitine.[197] Valproic acid may inhibit β-oxidation by either sequestering CoA or by increasing the acyl-CoA-to-free CoA ratio.[195,198,199] Third, it has been suggested that the valproic acid metabolites, such as 3-keto-2-propylpentanoyl-CoA, are potent inhibitors of one or more intramitochondrial β-oxidation enzymes.[194,200] Fourth, a decrease in intracellular ATP owing to the inhibition of β-oxidation, pyruvate metabolism, and gluconeogenesis could decrease the efficiency of energy-dependent carnitine transport. Sequestration of free CoA through formation of valproyl-CoA[198,199] would decrease ATP production. Valproic acid also interferes with pyruvate uptake by rat brain mitochondria,[201] pyruvate oxidation,[199] oxidative phosphorylation,[196,202] and gluconeogenesis.[198,199,203]

Clinically, there has been great interest in the role of carnitine deficiency in predisposing a child on valproic acid therapy to the idiosyncratic, potentially life-threatening Reye-like syndrome,[204] which mimics that seen in inborn errors of fatty acid oxidation. Several patients with Reye-like syndrome triggered by valproic acid were shown to have carnitine deficiency,[183,190,205] whereas others did not.[182] Certain patients may have a preexisting carnitine deficiency owing to an inborn error of metabolism[206]; however, this has not been found in all.[182,183] To date, patients at greatest risk for valproic acid hepatotoxicity (1 in 500) are children under the age of 2 years with neurologic disabilities who are receiving multiple anticonvulsants.[207] The clinical scenario for fatal hepatotoxicity may include synergistic risk factors as follows. Seizures may be the presenting symptom of an inborn error of metabolism, such as a mitochondrial encephalomyopathy or a defect in fatty acid oxidation, which may also be associated with carnitine deficiency. Valproic acid may further exacerbate the underlying metabolic condition, for example, precipitate lactic acidosis in a mitochondrial disorder. Young children may be at higher risk for decreased tissue carnitine stores because of their decreased carnitine biosynthesis[5] and decreased intake. The addition of other anticonvulsants may act synergistically to increase potentially hepatotoxic valproic acid metabolites. Valproic acid–induced carnitine deficiency may interfere with long-chain fatty acid oxidation. Valproic acid also may reduce the available free CoA for β-oxidation, leading to the accumulation of potentially toxic short-chain fatty acids.[208] In every case, a thorough investigation should be done to determine whether the epileptic patient has an inborn error of metabolism predisposing him or her to this idiosyncratic complication.[15,209]

In the case of acute valproic acid–induced hepatotoxicity, Bohan et al[210] have demonstrated improved hepatic survival with L-carnitine therapy. In a study of 92 patients with

severe, symptomatic, valproic acid–induced hepatotoxicity," 48% of the 42 patients treated with L-carnitine survived compared with only 10% of the 50 patients treated solely with aggressive supportive care ($P < .001$). Furthermore, early intervention with intravenous rather than enteral L-carnitine was associated with the best hepatic survival outcome. Notably, most patients had features of chronic illness and most children appeared malnourished.

In summary, carnitine deficiency associated with valproic acid therapy may be multifactorial. One potential mechanism is the inhibition of plasmalemmal carnitine uptake into tissues, including decreased renal tubular reabsorption of free carnitine. The inhibition may be owing to increasing competition between free carnitine and acylcarnitines, including valproylcarnitine esters and short-chain acylcarnitines, at the plasmalemmal high-affinity carnitine transporter site. L-Carnitine supplementation may decrease the impairment of plasmalemmal free carnitine uptake into tissues through an increase in the free carnitine concentration at the transporter site. Increased free carnitine would also provide a greater buffering capacity for excessive potentially toxic acyl-CoAs, including valproyl-CoA, thereby decreasing secondary inhibition of fatty acid oxidation, pyruvate oxidation, and gluconeogenesis and increasing intramitochondrial free CoA.

As the potential toxicity of valproic acid may be attributable to multiple risk factors, each child should be assessed carefully prior to being considered for treatment with valproic acid therapy. Assessment includes a careful history and clinical examination, as well as serum carnitine (total and free) and lactate and urinary organic acids to screen for an underlying inborn error of metabolism, particularly a mitochondrial or fatty acid oxidation defect. These disorders may acutely decompensate with valproic acid therapy, thereby precluding its use. In the event of carnitine insufficiency owing to poor nutritional status or excessive renal loss (eg, cystinosis), restoration of carnitine stores through supplementation would appear to be indicated prior to the institution of valproic acid therapy to minimize risk factors for toxicity. As there is no evidence that L-carnitine administration adversely alters the anticonvulsant properties of valproic acid[211] or lowers the serum valproic acid concentration, it would seem prudent to give carnitine supplementation to children receiving valproic acid who have clinical or laboratory evidence of serum or tissue carnitine deficiency.[191]

CARNITINE AND THE KETOGENIC DIET

The ketogenic diet has been used in patients whose seizures are refractory to antiepilepsy drug therapy or who experience unacceptable side effects from antiepilepsy drugs.[212-214] The mechanism(s) by which the ketogenic diet exerts its anticonvulsant effect are not fully understood, although a number of theories have been suggested.[213,215-219] Ketones serve as the alternate source of fuel for the brain during times of hypoglycemia. Ketosis is associated with improved cerebral cellular energetics and elevated tissue ATP concentrations.[217] The brain derives most of its energy from the aerobic oxidation of glucose. However, under conditions of fasting stress or consumption of a high-fat diet, the brain will use ketones for energy. The ketogenic diet is high in fat and low in carbohydrates and protein (usually in a 3:1 or 4:1 ratio by weight), producing a ketosis that mimics the fasting state. This causes the brain to shift its main source of fuel from carbohydrates to fats. This metabolic shift appears to raise the seizure threshold, an effect that is rapidly reversed when the diet is discontinued.[214]

The ketogenic diet has been most effective in young children and also has been used in infants.[220,221] In both of these groups, the diet can be relatively easily monitored. The efficacy of the diet appears to vary with the patient's age and type of seizure.[222] Various studies have shown that approximately one third of patients have an excellent response (≥ 50% reduction in seizures with fewer antiepilepsy drugs and increased alertness). One third have a partial response (a decrease in seizures of < 50%), and the remaining third have no significant response.[212,216] More recent results suggest that the response may be even more favorable.[223]

Of importance, the ketogenic diet may serve to deplete carnitine stores by several mechanisms as follows. The diet may decrease carnitine intake owing to the moderately low protein content, increase the demand on carnitine use in the oxidation of fatty acids, and/or increase urinary acylcarnitine excretion.[224] Supplementation with L-carnitine should facilitate ketogenesis and thereby contribute to the anticonvulsant action of the diet. It would be medically prudent to supplement patients on the ketogenic diet with carnitine if (a) carnitine concentrations decrease as a result of the diet, (b) there is evidence of carnitine deficiency resulting in decreased systemic ketosis with exacerbation of symptomatic hypoglycemia, or (c) carnitine therapy replenishes carnitine stores and improves the clinical response.

Prior to starting the ketogenic diet, children should be screened for an underlying metabolic defect that would preclude use of the diet, particularly a defect in fatty acid oxidation,[225] as previously outlined. A child with a defect in fatty acid oxidation could be pushed into a catabolic crisis with the initial fast and suffer an episode of hypoketotic, hypoglycemic coma. Therefore, a careful history and clinical examination should be done prior to consideration of the child for the ketogenic diet. Determination of serum total and free carnitine and urinary organic acid screening should be performed, which may be particularly informative if samples are obtained during a carefully monitored, limited fast. However, it must be strongly emphasized that if a fasting study is to be undertaken, it must be done under very carefully controlled hospital conditions with continuous monitoring and by physicians who are knowledgeable with respect to hypoglycemia, hypopituitarism, hyperinsulinism, and fatty acid oxidation disorders. As described previously, the fast should be terminated when the child demonstrates his or her first symptoms, has a blood glucose < 3.3 mmol/L (60 mg/dL), or demonstrates moderate ketonuria. If, at this point, there is a deficient ketogenic response in the presence

of a significant dicarboxylic aciduria and a serum free fatty acid-to-ketone ratio of > 2:1, there is strong presumptive evidence for a defect in the fatty acid oxidation pathway. Serum carnitine and urinary organic acids are frequently normal during times of euglycemia. Serum acylcarnitine analysis tends to be more informative, even during euglycemia, and would be particularly helpful as it may suggest the specific site of block.

In addition, many children with chronic epilepsy exposed to multiple antiepilepsy drugs for several years may develop carnitine deficiency. This finding is supported by a recent study of children about to begin the ketogenic diet.[226] Of 12 children with chronic epilepsy in whom multiple standard antiepilepsy drugs had failed, total serum carnitine concentrations were deficient in 9 patients and acylcarnitine concentrations were increased in 6. Importantly, there was a strong correlation of symptomatic hypoglycemia on initiation of the ketogenic diet in 7 of the 9 patients with low total serum carnitine concentrations and in 5 of the 6 patients with increased serum acylcarnitine concentrations. Therefore, preexisting carnitine deficiency may be associated with adverse sequelae, such as symptomatic hypoglycemia, owing to insufficient carnitine-dependent ketogenesis during fasting, before initiation of the ketogenic diet. This underscores the need to evaluate the patient's carnitine status prior to initiation of the diet. In these cases, carnitine supplementation would appear to be indicated to replenish carnitine stores prior to beginning the diet.

An important concern must be raised against the concomitant use of valproic acid with the ketogenic diet. Because valproic acid is associated with potentially significant carnitine deficiency and inhibits fatty acid oxidation by several mechanisms, its administration to patients consuming the ketogenic diet creates the potential risk of mitochondrial dysfunction[222] and symptomatic hypoglycemia with insufficient ketogenesis. Both valproic acid and the ketogenic diet predispose to carnitine deficiency, and it is likely that this effect would be additive. As an inhibitor of fatty acid oxidation, valproic acid may attenuate the ketosis associated with the high-fat diet. Therefore, the concomitant use of valproic acid and the ketogenic diet is best avoided as it may place the individual in a metabolically compromised position and predispose to a catabolic crisis.

It would seem medically prudent to check the carnitine status of all children prior to initiation of the ketogenic diet and at regular intervals during the diet to ensure that clinically (eg, hypotonia, myopathy, lethargy, etc) or biochemically apparent carnitine deficiency is not present, which would require supplementation.

RECOMMENDATIONS FOR L-CARNITINE SUPPLEMENTATION IN CHILDHOOD EPILEPSY

Well-designed, randomized, double-blind, controlled prospective studies of the specific and general uses of L-carnitine supplementation in children with epilepsy are needed. In the absence of more specific information, clinical practice is based on a combination of observation, clinical experience, and theory. The following summarizes key points in the diagnosis and treatment of carnitine deficiency in childhood epilepsy.[222]

Carnitine deficiency is defined as a free serum carnitine concentration < 20 μmol/L; carnitine insufficiency is defined as an acylcarnitine-to-free carnitine ratio > 0.4 in the fed state. Muscle carnitine concentrations provide a more accurate estimate of total tissue carnitine reserves and may be depleted despite normal total serum carnitine concentrations. Clinical carnitine deficiency may present with evidence of hypotonia, weakness, lethargy, anorexia, nausea, vomiting, and constipation. A beneficial clinical response to L-carnitine supplementation supports the diagnosis of symptomatic carnitine deficiency.

Carnitine supplementation is clearly indicated for the high-affinity plasmalemmal carnitine transporter defect, valproic acid–associated hepatotoxicity, and valproic acid overdose. Conditions for which carnitine supplementation is strongly recommended include secondary carnitine deficiency syndromes, such as excessive renal loss (eg, renal tubular acidosis, cystinosis); patients undergoing hemodialysis with excessive clearance of carnitine; and premature infants who are unable to synthesize sufficient carnitine and who are receiving total parenteral nutrition without carnitine supplementation. In childhood epilepsy, carnitine supplementation would appear to be indicated in infants and young children receiving valproic acid with secondary carnitine deficiency, particularly in those younger than 2 years with a complex neurologic disorder who are receiving multiple anticonvulsants, patients with multiple risk factors for carnitine deficiency (eg, failure to thrive, poor nutritional intake, chronic illness, multiple anticonvulsants), and patients on the ketogenic diet with carnitine deficiency.

The major side effects of carnitine therapy are nausea, diarrhea, and fishy body odor at higher doses. The expense of the medication is also a consideration. In the treatment of the high-affinity plasmalemmal carnitine transporter defect, carnitine therapy is critical and life saving. However, basic experimental models suggest that carnitine administration may have deleterious effects in other long-chain fatty acid oxidation defects (eg, long-chain acyl-CoA dehydrogenase, very-long-chain acyl-CoA dehydrogenase, severe carnitine palmitoyltransferase II, long-chain L-3-hydroxyacyl-CoA dehydrogenase, and trifunctional protein deficiency) in terms of the membranotoxicity and arrhythmias associated with long-chain acylcarnitines.[92-94]

The clinical literature is limited regarding carnitine dosage recommendations. For the high-affinity plasmalemmal carnitine transporter defect, an oral L-carnitine dose of 100 mg/kg/day divided in four daily doses (maximum of 2 g/day) is generally recommended, although higher dosages have been used.[222] In secondary carnitine deficiency states, a starting dose of 25 to 50 mg/kg/day divided in four daily doses may be titrated depending on the restoration of carnitine stores. For valproic acid–associated hepatotoxicity or metabolic rescue of acutely catabolic patients,

intravenous administration of L-carnitine in higher doses (150 to 300 mg/kg/day) may be required.[222]

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Discussion

Dr De Vivo: Fatty acid oxidation defects usually spare the nervous system directly. Short-chain acyl-CoA dehydrogenase deficiency appears to be an exception to this rule. Neurologic problems seem to be overrepresented with impairments of cognition, behavior, and movement, and seizures are relatively frequent. Do you have any speculations on this clinical observation?

Dr Tein: In short-chain acyl-CoA dehydrogenase deficiency, there is a particularly high incidence of neurologic abnormalities including infantile-onset developmental delay, hyperactivity, central hypotonia or hypertonia, and seizures.[1,2] The mechanism for the neurotoxicity is particularly intriguing as there tends to be an absence of episodic hypoglycemic, hypoketotic encephalopathy because of the intact oxidation of medium- and long-chain fatty acids for sufficient ketogenesis. As the primary metabolites that accumulate in short-chain acyl-CoA dehydrogenase deficiency are short-chain fatty acid metabolites, this raises the question of whether the mechanism for the neurologic abnormalities may therefore be directly related to the accumulation of these short-chain fatty acid metabolites. [In short-chain acyl-CoA dehydrogenase deficiency, the carboxylation of butyryl-CoA to ethylmalonyl-CoA, by propionyl-CoA carboxylase,[3] becomes appreciable only when a large amount of butyryl-CoA accumulates in the matrix. Ethylmalonyl-CoA is then either hydrolyzed to free ethylmalonic acid or isomerized by methylmalonyl-CoA isomerase to methyl-succinyl-CoA. The latter pathway is probably less important because only smaller amounts of free methylsuccinic acid tend to be found.]

The neurotoxicity observed in short-chain acyl-CoA dehydrogenase deficiency may be attributable to several different mechanisms. First, a positive correlation has been demonstrated between elevated short-chain fatty acids and neurotoxicity in several clinical disorders as well as in experimental animal models. Consistent elevations in serum concentrations of the short-chain fatty acids propionate, butyrate and isobutyrate have been documented in a series of patients with Reye's syndrome early in the course of the disease.[4] The authors postulated that because the administration of short-chain fatty acids to experimental animals resulted in coma, seizures, and electroencephalographic (EEG) changes,[5,6] the elevations of short-chain fatty acid concentrations observed in their series of Reye's syndrome patients suggested that these fatty acids may play a role in the clinical manifestations of Reye's syndrome. Interestingly, clinical improvement appeared to correlate most closely with the clearance of short-chain fatty acids from the serum.[7] Furthermore, in a patient with Reye's syndrome studied throughout the course of the illness with continuous EEG monitoring, there was a high correlation between the degree of EEG abnormality, degree of encephalopathy, and elevations of the short-chain fatty acids (propionate, isobutyrate, butyrate, isovalerate, and valerate), whereas serum ammonia concentrations correlated poorly with the EEG and with the clinical state.[8] The EEG abnormalities consisted of slow background activity (1 to 2 Hz) with occasional multifocal spikes and polyspikes and frequent electrographic seizure acitivity that generalized from multiple spike foci.

In an animal model, Trauner and Huttenlocher demonstrated that the intravenous infusion of sodium octanoate into rabbits resulted in blood and brain levels of 200 to 700 μmol/L, during which time the rabbits exhibited marked hyperventilation, seizures, hypotonia, and decreased levels of consciousness and developed hyperammonemia and lactic acidemia.[9] EEG changes consisted primarily of slowing of background frequencies, and brief generalized tonic seizure activity was observed in approximately one third of the animals during the infusion. The high brain concentrations of octanoate measured in these animals indicated that

this compound readily crossed the blood-brain barrier and equilibrated with serum by 2 hours. The secondary hyperammonemia was mild, and the blood ammonia concentrations were lower than those required to produce cerebral symptoms in experimental animals[10]; therefore, the authors felt that the nervous system alterations may be a direct effect of the octanoate on brain metabolism rather than an indirect effect, such as that of hyperammonemia. The serum concentrations of octanoate were of the same order of magnitude as those seen in patients with Reye's syndrome.[11] The intravenous infusion of propionic, butyric, valeric, isovaleric, and octanoic acids were also shown to produce elevations in intracranial pressure in rabbits.[12]

In another Reye-like disorder, Jamaican vomiting sickness, ingestion of hypoglycin A, a plant toxin from unripe ackee fruit, produces prolonged vomiting, hypoglycemia, coma, and death.[13] When this substance was injected into rats, it caused markedly increased plasma concentrations of isovaleric acid and α-methylbutyric acid, branched pentanoic acids,[14] suggesting that these short-chain fatty acids play an etiologic role in the encephalopathy. Similarly, valproic acid (2-propylpentanoic acid) has been associated with a Reye-like syndrome in certain high-risk children likely owing to a multifactorial inhibition of mitochondrial β-oxidation, which has been fully discussed in the preceding section on valproic acid.[15–17]

Second, sodium butyrate in the 0.5- to 2-mmol/L dose range has been shown to induce aberrant tau phosphorylation and programmed cell death in human neuroblastoma cells.[18] Nuydens et al have demonstrated that this process is associated with increased morphologic differentiation.[18] Furthermore, the aberrant tau phosphorylation is followed by neurotoxicity. This neurotoxicity has been shown to have features of programmed cell death, such as fragmentation on a DNA agarose gel, fragmented nuclei, and chromatin condensation and inhibition by the protein synthesis inhibitor cycloheximide. The mechanisms by which sodium butyrate induces these modified tau proteins and neurotoxicity are largely unknown, but the data suggest an involvement of cytoskeletal proteins.

Third, animal models of short-chain acyl-CoA dehydrogenase deficiency have also been informative. In short-chain acyl-CoA dehydrogenase–deficient mice, Hinsdale et al found depressed messenger ribonucleic acid expression and enzyme activity for the urea cycle enzymes carbamyl phosphate synthetase I and argininosuccinate synthetase at 6 days of age.[19] This may predispose affected animals to hyperammonemia during times of acute catabolic crisis.

Finally, increased content of short- or medium-chain fatty acids and, in particular, their dicarboyxlic metabolites, from compensatory ω-oxidation, may cause secondary metabolic abnormalities, including an impairment of gluconeogenesis, β-oxidation, and the citric acid cycle,[20–22] leading to a further decrease in cellular adenosine triphosphate production. In summary, excessive short-chain fatty acid metabolites may therefore exert their toxic effects on the developing central nervous system through several different but synergistic mechanisms.

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