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Primary and Secondary Carnitine Deficiency Syndromes

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ABSTRACT

The objective of this article is to review primary and secondary causes of carnitine deficiency, emphasizing recent advances in our knowledge of fatty acid oxidation. It is now understood that the cellular metabolism of fatty acids requires the cytosolic carnitine cycle and the mitochondrial β-oxidation cycle. Carnitine is central to the translocation of the long chain acyl-CoAs across the inner mitochondrial membrane. The mitochondrial β-oxidation cycle is composed of a newly described membrane-bound system and the classic matrix compartment system. Very long chain acyl-CoA dehydrogenase and the trifunctional enzyme complex are embedded in the inner mitochondrial membrane, and metabolize the long chain acyl-CoAs. The chain shortened acyl-CoAs are further degraded by the well-known system in the mitochondrial matrix. Numerous metabolic errors have been described in the two cycles of fatty acid oxidation; all are transmitted as autosomal recessive traits. Primary or secondary carnitine deficiency is present in all these clinical conditions except carnitine palmitoyltransferase type I and the classic adult form of carnitine palmitoyltransferase type II deficiency. The sole example of primary carnitine deficiency is the genetic defect involving the active transport across the plasmalemmal membrane. This condition responds dramatically to oral carnitine therapy. The secondary carnitine deficiencies respond less obviously to carnitine replacement. These conditions are managed by high carbohydrate, low fat frequent feedings, and vitamin/cofactor supplementation (eg, carnitine, glycine, and riboflavin). Medium chain triglycerides may be useful in the dietary management of patients with inborn errors of the cytosolic carnitine cycle or the mitochondrial membrane-bound long chain specific β-oxidation system. (*J Child Neurol* 1995;10(Suppl):2S8–2S24).

Our understanding of disease states affecting carnitine metabolism has increased tremendously over the past 2 decades. The history of carnitine extends back to the beginning of the 20th century when it was first recognized as an important growth factor for the yellow meal worm, *Tenebrio molitor*. Its chemical structure was deduced in 1952,[1] and its role in human disease was recognized in 1973 when Engel and Angelini first described a young woman who had limb weakness and lipid storage myopathy.[2] The oxidation of long-chain fatty acids in vitro by muscle homogenates from this patient was stimulated by the addition of carnitine. The condition then described as muscle carnitine deficiency was established. In the same year, DiMauro and DiMauro described a patient with recurrent muscle complaints secondary to a deficiency of carnitine palmitoyltransferase type II.[3] In 1975, Karpati and associates described a young boy who had recurrent Reye syndrome–like episodes associated with marked decreases in the serum and tissue concentrations of carnitine.[4] These investigators termed this condition systemic carnitine deficiency.

Over the next 20 years, we have come to recognize a number of monoenzymopathies involving fatty acid oxidation, and some of the earlier cases required redefinition in light of newer observations. For example, some of the earlier patients with systemic carnitine deficiency have now been shown to have medium-chain acyl-coenzyme A (CoA) dehydrogenase deficiency.[5] Similarly, one or more of the patients with muscle carnitine deficiency appeared to have a tissue-specific defect of short-chain acyl-CoA dehydrogenase deficiency resulting in a lipid storage myopathy and limb weakness.[6]

The carnitine-responsive cardiomyopathy of childhood has emerged as the quintessential example of primary carnitine deficiency. Some of these cases were classified as examples of systemic carnitine deficiency in the past.[7] It is now clear that primary carnitine deficiency is a single example of a condition that is exquisitely sensitive to carnitine supplementation, and the molecular basis

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appears to involve the transporter system that actively transports carnitine across the plasma membrane. The correct classification of muscle carnitine deficiency remains unclear in most reported cases, with circumstantial evidence suggesting that several cases represent examples of tissue-specific monoenzymopathies with secondary carnitine deficiency of skeletal muscle.

The advances in our understanding of fatty acid oxidation have been extraordinary over this time interval. Several new enzyme defects have been described in this pathway, and recently we have been introduced to the membrane-bound pathway for the metabolism of very long chain fatty acids.[8,9] This pathway includes the very long chain acyl-CoA dehydrogenase[8] and the trifunctional enzyme complex that contains the catalytic activities for three enzymes.[9] A revised model for the metabolism of very long chain and long-chain fatty acids has been introduced as the result of these new observations.[10]

This report represents a review of primary and secondary deficiency syndromes, emphasizing some of the more recent advances that have occurred during the past 5 years.

CARNITINE FUNCTION AND METABOLISM

Carnitine (3-hydroxy-4-*N*-trimethylammonium butyrate) is a natural constituent of higher organisms, in particular, of cells of animal origin. It is a quaternary ammonium compound, water soluble, and only biologically active when in the L isoform.[11,12] Carnitine is able to form high-energy ester bonds with carboxylic acids at its β-hydroxyl position.[13]

Carnitine serves two major functions. The first is the transport of long-chain fatty acids into the mitochondrial matrix to undergo β-oxidation and generate energy, mainly in liver, heart, and skeletal muscle. This carnitine-mediated transport is carried out by the action of carnitine palmitoyltransferase I and II and the specific acylcarnitine translocase. The second major function of carnitine is to modulate the intracellular CoA homeostasis. Acyl-CoA esters arising from β-oxidation and other mitochondrial processes are transesterified by carnitine through the action of carnitine-acyltransferases.[11,12]

$$\text{acyl-CoA} + \text{carnitine} \underset{\text{carnitine-acyltransferases}}{\leftrightarrows} \text{acylcarnitine} + \text{CoA}$$

Acylcarnitines can cross the mitochondrial membrane in exchange for free carnitine via the translocase. This pathway permits the regeneration of intramitochondrial free CoA, especially under conditions where acyl-CoA esters are produced at a rate faster than they can be used.[12]

Carnitine in humans is derived from dietary intake and endogenous synthesis. Major dietary sources are red meat, poultry, fish, and dairy products.[14] Variable amounts of carnitine are absorbed (54% to 87%) from the small intestine to the systemic circulation.[15,16] The amount of absorbed carnitine may modify the extent of synthesis of carnitine.[16] In humans, carnitine is synthesized in liver and kidney from protein-bound lysine and methionine. Skeletal and heart muscle cannot synthesize carnitine. Therefore, these tissues are entirely dependent on carnitine uptake from the blood. The transport of carnitine into tissues is against a concentration gradient, permitting tissue carnitine concentrations to be 20- to 50-fold higher than plasma levels.[11] This active carnitine uptake into cells is performed by a specific high-affinity transporter that is sodium dependent and functions from low (K_m, 0.5 to 10 μmol/L) to intermediate concentrations (10 to 200 μmol/L).[7,17–19] The membrane protein active in the transport has not been isolated or characterized. Two functional systems for carnitine uptake have been described in human cultured myoblasts and fibroblasts, one of high affinity and one of intermediate affinity.[17,19] The transporter has been shown to be of the high-affinity type in human cultured heart cells[20] and of the intermediate-affinity type in renal tubular and epithelial intestinal cells.[21,22] Human liver cells and brain use a low-affinity carnitine transporter with K_m of 500 μmol/L and 1000 μmol/L, respectively.[23] In humans, 98% of total body carnitine is in skeletal muscle, 0.6% in extracellular fluid, and only 1.6% in liver and kidney.[24] In tissues and physiologic fluids, carnitine is present in a free and an esterified form. The proportion of esterified carnitine may vary considerably with nutritional conditions, exercise, and disease states. The great majority of carnitine esters are represented by acetylcarnitine. Under conditions of undisturbed intermediary metabolism, acylcarnitine esters account for 22% of total carnitine in serum, 13% in muscle and liver, and as much as 50% to 60% of total carnitine in urine.[24] Due to the reversible transesterification of the acyl-CoAs with carnitine and the fact that acylcarnitine can cross the mitochondrial membrane, the intramitochondrial relationship between acyl-CoA and free CoA is reflected in the extramitochondrial acylcarnitine to free carnitine ratio. This acylcarnitine to free carnitine ratio is very sensitive to changes in mitochondrial metabolism. It is considered normal when it is 0.25 and abnormal when it is greater than 0.4. The equilibrium between acyl-CoA and acylcarnitine is rapid and useful in lowering acyl-CoA levels in the presence of an adequate carnitine supply.[13]

Plasma carnitine concentrations are mainly regulated by the kinetics of carnitine reabsorption by the kidney. The proximal renal tubule reabsorbs more than 90% of filtered carnitine at normal physiologic concentrations, and the apparent renal plasma excretory threshold for free carnitine is 40 μmol/L, which is close to the normal plasma carnitine concentration (about 50 μmol/L).[12]

CARNITINE DEFICIENCY

Carnitine deficiency can be defined as a state of carnitine concentration in plasma or tissues that is below the requirement for the normal function of the organism. In clinical practice, plasma levels are commonly used to diagnose carnitine deficiency; however, these values do not always reflect the tissue carnitine concentrations.

Carnitine requirements depend on many factors, such as age, diet, tissue dependence on β-oxidation, and

metabolic conditions (stress, fed versus fasting, and rest versus exercise).[14] The balance between functional carnitine requirements and carnitine levels determines whether carnitine deficiency is clinically significant.[5,14] Clinical and biochemical data suggest that tissue carnitine levels may have to fall to less than 10% to 20% of normal before the biologic effects can be clinically significant.[5] Carnitine deficiency can be *primary* or *secondary*.

PRIMARY CARNITINE DEFICIENCY

Primary carnitine deficiency is defined as a decrease of intracellular carnitine content that impairs fatty acid oxidation and that is not associated with another identifiable systemic illness that might deplete tissue carnitine stores.[25] The criteria for this condition are: (1) severe reduction of plasma or tissue carnitine levels, (2) evidence that the low carnitine levels impair fatty acid oxidation, (3) correction of the disorder when carnitine levels are restored, and (4) absence of other primary defects in fatty acid oxidation.[26]

Depending on the tissue distribution of the low carnitine content, primary carnitine deficiency can be divided into systemic or muscular carnitine deficiency. In the *systemic* form, there is a profound reduction of carnitine in plasma and also in the affected tissues, whereas in the *muscular* form the low content is restricted to muscle.

Systemic Carnitine Deficiency

Pathogenesis

Possible causes of systemic carnitine deficiency include defective biosynthesis, increased degradation, and defective transport affecting uptake or release of carnitine from tissues. No evidence of defective biosynthesis or excessive degradation has been found in patients with systemic carnitine deficiency.[27] At present, there is evidence that the defect in this disorder involves the transport of carnitine from serum to cell in affected tissues. It has been conclusively demonstrated that carnitine transport is abnormal in the high-affinity carnitine uptake system in fibroblasts.[7,18,26,28] Despite the fact that abnormal transport has not been proven in other tissues in systemic carnitine deficiency, clinical and biochemical data suggest that the transport system may be affected in these tissues as well: The excessive urinary excretion of carnitine points to a renal transport defect. The very small increase in carnitine content in muscle when plasma carnitine levels are raised with oral treatment suggests that the transport defect exists also in muscle. A low and delayed plasma response to orally administered L-carnitine in one patient points to an intestinal transport defect.[29] Uptake in liver seems not to be affected due to its different kinetic properties and the great increase in carnitine content with carnitine replacement.[7,18,26,29]

Pathophysiology

Intracellular carnitine deficiency hinders the entry of long-chain fatty acids into the mitochondrial matrix; thus, no long-chain substrates are available for β-oxidation and energy production.[5] The modulation of the intramitochondrial free CoA is also affected, causing increased acyl-CoA esters in the mitochondria, affecting pathways of intermediary metabolism requiring CoA (Krebs cycle, pyruvate oxidation, amino acid oxidation, and mitochondrial and peroxisomal β-oxidation).[30]

In 1988, Koizumi et al discovered a strain of mice affected with microvesicular steatosis, hypoglycemia, hyperammonemia, cardiac hypertrophy, and growth retardation that showed a good response to carnitine treatment.[31] The carnitine concentration in blood, liver, and skeletal muscle of these animals is low. Its sodium-dependent transport of carnitine in kidney is 20% of normal. This strain of mice seems to be a useful animal model for clarifying the molecular mechanisms of the renal reabsorption of carnitine and for understanding the pathophysiology of patients with systemic carnitine deficiency.[32]

Clinical Manifestations

About 30 patients with systemic carnitine deficiency have been described in the literature. Although the biochemical studies are not complete in some of them,[33,34] all these cases fulfill the diagnostic criteria of primary carnitine deficiency mentioned above.[7,18,26,28,29,33–35] In almost half of the patients, there is the antecedent of a deceased sibling due to a cardiac disease or a sudden death.[7,18,35] When tissues of these siblings are studied, the most frequent findings are fatty infiltration in liver and heart muscle,[18,28,29,35] and when carnitine is measured in these tissues, it is very reduced.[28,33] Consanguinity is present in some families, and ethnic origins are varied.[7,18,35]

Patients are normal at birth and may appear healthy for several years before they develop signs of the disease. However, some patients can have earlier clinical problems such as failure to thrive, recurrent respiratory infections, or recurrent attacks resembling hypoglycemia.[7,18,35]

There is no sex predominance. The mean age at onset is 2 years, with onset ranging from 1 month to 7 years of age. Three different types of presentation have been described: progressive cardiomyopathy, hypoketotic hypoglycemic encephalopathy, and myopathy. All forms of presentation may coexist in the same family.[7,18,35] Progressive cardiomyopathy is the most common form of presentation and usually manifests at an older age. Generally, echocardiograms and electrocardiograms show dilated cardiomyopathy, peaked T waves, and signs of ventricular hypertrophy. In a few patients, heart carnitine concentration has been measured, showing levels below 5% of normal.[28,34,35] Cardiac function responds poorly to general treatment with digoxin and diuretics. If no carnitine replacement is administered, progressive congestive heart failure leads to death.[7,18,35]

Acute encephalopathy associated with hypoketotic hypoglycemia is more commonly seen in younger infants. Usually, these acute episodes are triggered by viral illness associated with vomiting or reduced oral intake. Change to a diet poor in carnitine content has also been described

as a contributory factor.[26] Patients present variable degrees of decreased consciousness, generally associated with hepatomegaly. When liver biopsy is done, steatosis and low carnitine content (less than 6% of normal) are demonstrated. Glucose and ketone bodies are inappropriately low, transaminases and ammonia can be moderately elevated, and other laboratory abnormalities can be present, such as metabolic acidosis, prolonged prothrombin time, or elevated creatine kinase. Unlike intramitochondrial fatty acid disorders, no abnormal organic acids are found in urine. Although the clinical picture is dominated by the encephalopathy, most of these patients also present signs of cardiac involvement. If no carnitine replacement is given, the patients suffer recurrent episodes of encephalopathy.[7,18,35]

Myopathy as an isolated form of presentation is rare. However, it is more common when associated with cardiomyopathy or encephalopathy. Usually it manifests with mild motor delay, hypotonia, or slowly progressive proximal weakness. Serum creatine kinase level can be normal or slightly elevated. Electromyography and nerve conduction studies have not been informative. Muscle biopsy shows very low carnitine concentrations (less than 6% of normal) and fatty infiltration.[7,18,35] In some cases, very low carnitine concentrations and similar morphologic abnormalities can be found in muscle in the absence of clinical signs of muscle involvement.[26,34]

Cognitive delay[18,35] and central nervous system dysfunction, such as pyramidal signs[26,33] and minimal athetoid movements,[34] have been described in some patients secondary to severe hypoglycemic encephalopathy and cardiac or respiratory arrest.[26,33,34] In some cases, there are no clear reasons for the central nervous system dysfunction.[18,35]

Carnitine deficiency has been found to be a cause of gastrointestinal dysmotility.[36] This could explain why some patients with systemic carnitine deficiency have gastrointestinal manifestations: Recurrent episodes of abdominal pain and diarrhea that resolved with carnitine treatment were described in one patient,[18] and recurrent vomiting was described in a symptomatic heterozygote.[35] Moreover, pyloric stenosis and gastroesophageal reflux described in two patients of the same family, suggested involvement of smooth muscle.[34]

Anemia has been found in one quarter of the patients. Red blood cell features are variable, but frequently there is a mild to moderate hypochromic anemia.[7,18,35] One patient had a severe hypochromic anemia with low iron levels that required blood transfusions.[18]

Diagnosis

The diagnosis of systemic carnitine deficiency is made when a compatible clinical picture and laboratory evidence of carnitine deficiency exist. The carnitine levels in plasma and tissues are usually below 10% of normal, and the acylcarnitines are proportionately reduced. Therefore, the acylcarnitine to free carnitine ratio is normal. Renal fractional excretion of free carnitine exceeds 100% of the filtered load.[26] The diagnosis is definitively made when carnitine uptake in fibroblasts shows negligible transport.[7,18,35]

Treatment

The mainstay of treatment is oral carnitine at daily doses of 100 to 200 mg/kg. At this dose, carnitine is able to reach the systemic circulation by passive diffusion through the intestine. With this treatment, patients achieve variable plasma levels. Carnitine concentrations increase slightly in skeletal muscle and reach nearly normal levels in liver. Fasting ketogenesis is recovered, and there is a significant improvement in cardiac function, strength, and growth.[7,18,35] Beneficial changes of personality[7] and improvement of cognitive performance[18] also have been described. Intermittent diarrhea and fishy body odor have been described in some patients as side effects of carnitine replacement.[29]

The Heterozygote State

The parents of these patients have moderately low or normal carnitine values in plasma, and when carnitine transport is studied in fibroblasts, they show intermediate values of uptake.[7,18,35] These data suggest an autosomal recessive pattern of inheritance and indicate that the carnitine uptake study in fibroblasts cultured from heterozygotes is a sensitive test to diagnose this state. A symptomatic heterozygote with cardiac and muscle involvement has been described.[35] This finding suggests that carnitine replacement should be considered in these cases even without clinical manifestations of carnitine deficiency, especially in stress situations like fasting, vomiting, and intercurrent viral illness.

Muscle Carnitine Deficiency

Severe reduction in muscle carnitine levels and normal serum carnitine concentrations characterizes muscle carnitine deficiency.[37–39] This disorder is restricted to muscle, with no renal leak of carnitine or signs of liver involvement. This type of disorder is considered primary. Therefore, affected patients should fulfill the diagnostic criteria mentioned above.

Pathogenesis

At present, no definitive biochemical defect has been discovered in muscular carnitine deficiency. Some evidence suggests that the muscle carnitine transporter is affected: The first patient presented deficient oxidation of long-chain fatty acids in muscle homogenates that was corrected by the addition of carnitine.[2] However, treatment with carnitine generally does not replenish muscle stores.[40]

Studies in cultured muscle cells at different stages of differentiation have shown changes in the kinetic properties of the low-affinity transport system, suggesting the existence of a muscle-specific carnitine transporter that gradually develops during myogenesis and is eventually fully expressed in the adult tissue.[19] The investigators postulate that a defect in this developmentally regulated carrier may be the cause of human muscle carnitine defi-

ciency. Mesmer and Lo did not find a deficit of carnitine uptake in myoblasts cultured from a patient affected with muscle carnitine deficiency. However, a faster rate of carnitine efflux was found in these myoblasts, resulting in reduced intracellular carnitine levels.[41]

Short-chain acyl-CoA dehydrogenase deficiency has been documented in cultured fibroblasts of a patient with the myopathic form of carnitine deficiency.[42] Other fatty acid oxidation defects, either generalized or tissue specific, could also be responsible for this entity. Several factors are compatible with this speculation: (1) Fatty acid oxidation defects were not excluded in most of the cases.[39] (2) Carnitine concentrations in other tissues such as liver or heart were not available in most of the patients.[39] (3) There was little or no clinical response to carnitine therapy in some patients.[43–45] (4) In many patients, elevated esterified carnitine levels, suggesting secondary carnitine deficiency, were found in either plasma[44,46–48] or muscle.[44,45,47–49] (5) One patient had an increase in a urinary dicarboxylic acid (adipic acid), which can be present in a number of fatty acid oxidation defects.[44] Urinary organic acids were not reported in most of the cases.[2,43,45,46,49–56]

The description of a symptomatic heterozygote for systemic carnitine deficiency who had low muscle carnitine concentration[35] raises the possibility that some of the patients with "muscle carnitine deficiency" may in fact be heterozygotes for the systemic form.

Clinical Manifestations, Diagnosis, and Treatment

More than 20 patients have been described.[37–40] Symptoms of muscle carnitine deficiency can appear in the first years of life[40] but usually occur later, during the 2nd or 3rd decade.[38] Patients have progressive proximal muscular weakness of variable degree. Some of them can present with exercise intolerance, myalgias, or myoglobinuria. Cardiomyopathy has also been described in some of these patients.[38,40]

Muscle biopsy shows lipid storage myopathy, and peripheral nerve involvement has been described in some.[38] In infancy, muscle fat infiltration is rare.[40] Muscle carnitine levels are about 20% of normal or less. Plasma carnitine levels are normal or slightly reduced.[38,40] Intermediate levels of carnitine in skeletal muscle of some parents suggests autosomal recessive inheritance.[38]

Some of the patients benefit from carnitine treatment. The response is variable, ranging from moderate improvement to normalization of muscle strength. The increases in muscle carnitine levels are variable, but in general, carnitine stores are not completely replenished.[38,40]

SECONDARY CARNITINE DEFICIENCY

Secondary carnitine deficiency, which manifests with a decrease in the levels of carnitine in plasma or tissues, may be associated with genetically determined metabolic errors, acquired medical conditions, or iatrogenic states.[27]

Genetically Determined Metabolic Errors

Carnitine deficiency is an associated phenomenon of a large number of metabolic disorders (Table 1). The most

Table 1. Carnitine Deficiency: Etiology

- Primary carnitine deficiency*
  - Systemic carnitine deficiency
  - Muscle carnitine deficiency
- Secondary carnitine deficiency
  - Genetically determined metabolic errors
    - Fatty acid oxidation disorders
      - Carnitine cycle
        - Carnitine palmitoyltransferase I†
        - Translocase
        - Carnitine palmitoyltransferase II: infantile and adult
      - β-Oxidation cycle
        - Acyl-CoA dehydrogenases
          - Short-chain acyl-CoA dehydrogenase
          - Medium-chain acyl-CoA dehydrogenase
          - Long-chain acyl-CoA dehydrogenase
          - Very long chain acyl-CoA dehydrogenase
          - Multiple acyl-CoA dehydrogenases: severe, mild, and riboflavin responsive
        - Short-chain 3-hydroxyacyl-CoA dehydrogenase
        - Trifunctional protein
        - 2,4-dienoyl-CoA reductase
    - Branched-chain amino acid disorders
      - Isovaleric acidemia
      - Propionic acidemia
      - Methylmalonic acidemia
      - 3-Methylcrotonyl-CoA carboxylase deficiency
      - 3-Methyl-glutaconic aciduria
      - 3-Hydroxymethylglutaryl-CoA lyase deficiency
      - 2-Methylacetoacetyl-CoA thiolase deficiency
    - Glutaric aciduria I
    - Mitochondrial disorders
      - Multiple and isolated respiratory chain deficiencies
    - Other genetic defects
      - 5-Methylene tetrahydrofolate reductase deficiency
      - Adenosine deaminase deficiency
      - Ornithine transcarbamylase deficiency
      - Carbamoylphosphate synthase I deficiency[57]
    - Dysgenetic syndromes
      - Williams-Beuren syndrome[58]
      - Ruvalcaba-Myhre-Smith syndrome[59,60]
  - Acquired medical conditions‡
    - Decreased biosynthesis
      - Cirrhosis
      - Chronic renal disease
      - Extreme prematurity
    - Decreased intake
      - Chronic total parenteral nutrition
      - Malnutrition
      - Lacto-ovovegetarians and strict vegetarians
      - Soy protein infant formula without added L-carnitine
      - Malabsorption (cystic fibrosis, short-gut syndrome, celiac disease)
    - Decreased body stores/increased requirements
      - Pregnant and lactating women[61]
      - Extreme prematurity
      - Intrauterine growth retardation
      - Infant of carnitine-deficient mother
      - Critically ill patients (increased catabolism)[61]
      - Acquired immune deficiency syndrome[62]
    - Increased loss
      - Fanconi syndrome
      - Renal tubular acidosis
  - Iatrogenic factors‡
    - Hemodialysis
    - Valproate
    - Pivampicillin[63]
    - Emetine[64]
    - Zidovudine[65]

*This category includes systemic and myopathic carnitine deficiency to the extent that they have been studied. However, some of these cases are now attributed to general or tissue specific enzyme defects in fatty acid oxidation.
† See text.
‡ Often multifactorial.
CoA = coenzyme A.

characteristic, representative causes of secondary carnitine deficiency are metabolic disorders associated with impaired oxidation of acyl-CoA intermediates in the mitochondria. These include fatty acid oxidation disorders and amino acid oxidation defects. In these disorders, plasma and tissue carnitine levels are in the range of 25% to 50% of normal.[6] The acylcarnitine to free carnitine ratio is increased due to an absolute or relative elevation of the acylcarnitine esters.

Pathogenesis of Secondary Carnitine Deficiency

Intramitochondrial block in fatty acid or amino acid oxidation contributes to the accumulation of the acyl-CoA

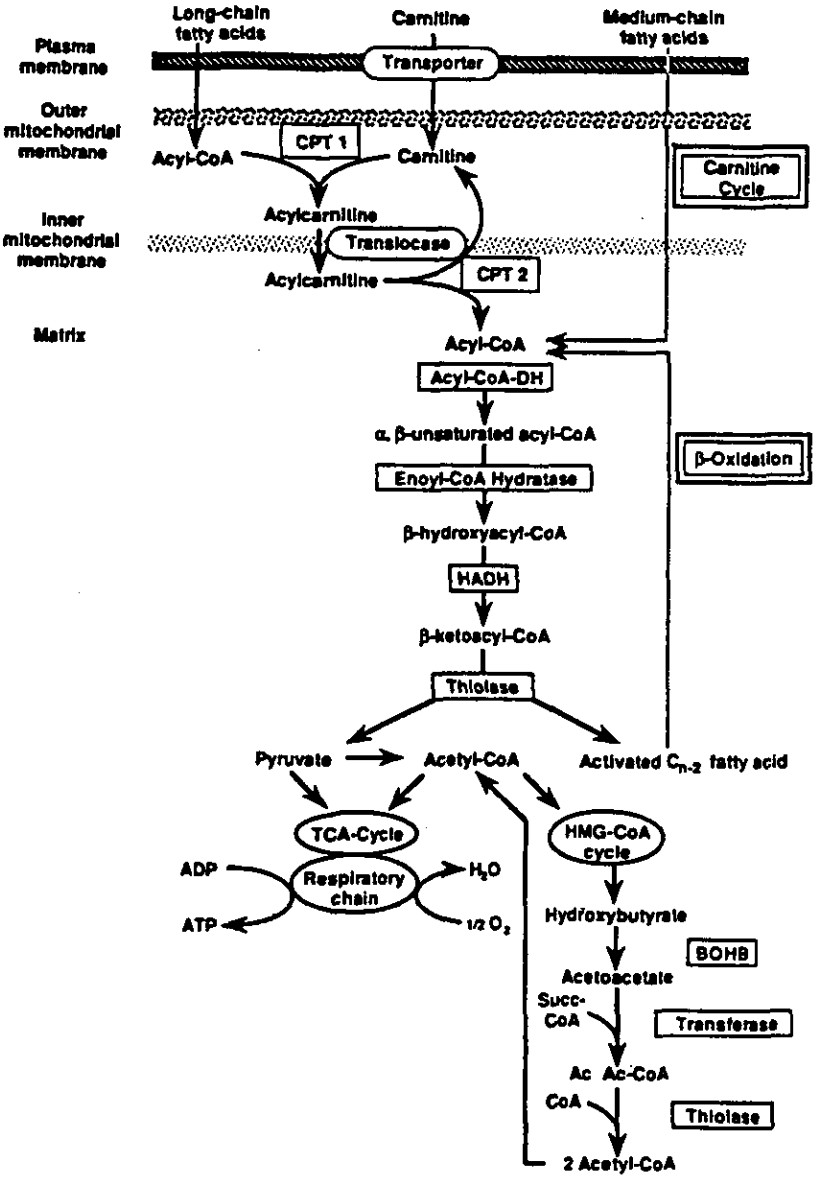

Figure 1. Schematic representation of fatty acid oxidation and ketone body synthesis. Fatty acid oxidation is subdivided into the carnitine cycle and the β-oxidation spiral. The carnitine cycle includes the plasma membrane transporter, carnitine palmitoyltransferase I, carnitine-acylcarnitine translocase system and carnitine palmitoyltransferase II. β-Oxidation involves four sequential enzymatic steps. Very long and long-chain fatty acids are metabolized initially by the membrane bound system as shown in Figure 2. Subsequent oxidation of shortened-chain fatty acids is accomplished by the matrix system. The end-product of fatty acid oxidation is the formation of acetyl-CoA and activated C_{n-2} fatty acid. Acetyl-CoA may enter the Krebs cycle or the β-hydroxy-β-methylglutaryl-CoA cycle to form ketone bodies in the liver. CoA = coenzyme A; CPT = carnitine palmitoyltransferase; DH = dehydrogenase; HADH = 3-hydroxyacyl-CoA dehydrogenase; TCA = tricarboxylic acid; HMG-CoA = hydroxymethyl-glutaryl-CoA; ADP = adenosine diphosphate; ATP = adenosine triphosphate; BOHB = β-hydroxybutyric acid; Succ-CoA = succinyl-CoA; Ac Ac-CoA = acetoacetyl-CoA. From Siegel GS, Agranoff BW, Albers WR, Molinoff PB (eds): *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, 5th ed. New York, Raven Press, 1994, by permission.

intermediates at or near the site of the metabolic block. As was mentioned earlier, the transesterification of these acyl-CoAs with carnitine leads to the formation of acylcarnitine and to the release of free CoA. Acylcarnitines are transported out of the mitochondria and out of the cell and finally excreted in the urine.[12,13] The plasma acylcarnitine profile depends on the accumulated acyl-CoAs, the alternative metabolic pathways they may undergo, and the substrate specificity of the carnitine-acyltransferases.[13] The acylcarnitine profile in urine depends also on the flux of carnitine[66] and the type of acylcarnitine, ie, long-chain acylcarnitines are rarely detected in urine.[5,66]

The postulated mechanism of carnitine deficiency in these disorders has been an imbalance between the urinary excretion of the accumulated acylcarnitines and the sum of dietary intake and synthesis.[12,67] A recent study of the evolution of carnitine deficiency in patients with fatty acid oxidation defects and organic acidurias after a period of carnitine repletion demonstrated a low renal threshold of carnitine excretion as a contributory mechanism, probably due to the inhibition of carnitine transport in renal cells by the acylcarnitines.[68]

Fatty acid oxidation defects are the most frequent cause of carnitine deficiency among the genetically determined metabolic errors that cause secondary carnitine deficiency. Moreover, in the last 5 years, several new enzyme deficiencies of this metabolic pathway have been described, increasing the list of causes of carnitine deficiency. For these reasons, we will emphasize the description of these disorders.

Fatty Acid Oxidation Defects

The fatty acid oxidation defects can be subdivided into *defects of the carnitine cycle* for the transport of the long-chain fatty acids into the mitochondria and *defects of the β-oxidation spiral*, that occurs within the mitochondria (Figures 1 and 2).

The following enzymes are involved in the carnitine cycle: carnitine transporter, carnitine palmitoyltransferase I, carnitine palmitoyltransferase II, and carnitine-acylcarnitine translocase. The spiral of β-oxidation includes the four enzymatic steps that shorten progressively saturated fatty acids by two carbon fragments: acyl-CoA dehydrogenation, 2-enoyl-CoA hydration, 3-hydroxyacyl-CoA dehydrogenation, and 3-ketoacyl-CoA thiolytic cleavage[10] (Figures 1 and 2).

Degradation of unsaturated or polyunsaturated fatty acids by β-oxidation leads to the formation of intermediates that are dependent on other enzyme reactions.[10] Disorders in this pathway have also been described.[69]

Pathophysiology of Fatty Acid Oxidation Defects

Defects of fatty acid oxidation impair energy production in the cardiac muscle and in the aerobically exercising skeletal muscle. Under conditions of fasting or stress, the breakdown of energy production is more generalized.[5] The defect results in the underproduction of acetyl-CoA and, subsequently, a dysfunction of the Krebs cycle and of hepatic ketogenesis. Fatty acids that cannot be oxidized accumulate in the liver and are shunted into alternative pathways, resulting in the production of characteristic organic acids. As with acylcarnitine, acylglycine esters are formed and accumulate, offsetting the sequestration of CoA by the accumulation of acyl-CoA intermediaries. The plasma and urinary profiles of the accumulated organic acids, acylcarnitines, and acylglycines are suggestive and sometimes specific for the enzyme defect.[70]

Clinical Manifestations of Fatty Acid Oxidation Defects

Defects of fatty acid oxidation have specific clinical and metabolic signatures.[71-74] These disorders appear to be inherited in an autosomal recessive fashion. Consanguinity and the antecedent of a dead sibling with lipid accumulation in tissues or sudden death is a feature in some families. The age of onset is variable. Acute metabolic decompensation usually occurs in infancy, whereas cardiac and skeletal muscle disease manifest later. The most typical presentation is recurrent episodes of metabolic decompensation triggered by fasting or common viral illness. The episodes consist of altered consciousness that sometimes are com-

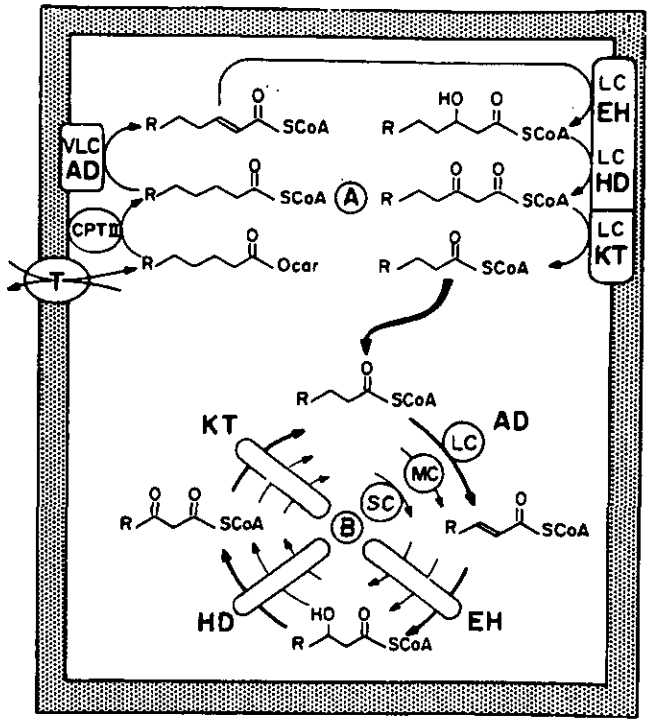

Figure 2. Model of the functional and physical organization of β-oxidation enzymes in mitochondria.[10] Long-chain acylcarnitines enter the mitochondrial matrix by the action of the carnitine palmitoyltransferase II at the inner mitochondrial membrane to yield long-chain acyl-CoAs, which undergo one or more cycles of chain shortening catalyzed by the membrane-bound long-chain specific β-oxidation system. Chain-shortened acyl-CoAs are further degraded by the well-known β-oxidation system present in the mitochondrial matrix. *A*, β-oxidation system active with long-chain acyl-CoAs. *B*, β-oxidation system active with long-chain, medium-chain, and short-chain acyl-CoAs. LC = long chain; EH = 2-enoyl-CoA hydratase; CoA = coenzyme A; VLC = very long chain; AD = acyl-CoA dehydrogenase; HD = 3-hydroxyacyl-CoA dehydrogenase; CPT = carnitine palmitoyltransferase; KT = 3-ketoacyl-CoA thiolase; T = acylcarnitine translocase; MC = medium chain; SC = short chain. Kindly provided by Dr H. Schulz.

plicated by seizures, apnea, or cardiorespiratory arrest. The acute encephalopathy can be accompanied by liver involvement, hypotonia, or cardiac dysfunction. Hypoketotic hypoglycemia is characteristic, often with moderate increases of serum transaminases and ammonia. Metabolic acidosis, elevated serum creatine kinase levels, hyperuricemia, or altered coagulation may also be present. Liver biopsy during the crisis shows microvesicular and macrovesicular steatosis. Abnormal organic acids are found in the urine if the defect is in the β-oxidation spiral, whereas these metabolites are absent in the carnitine cycle defects.[71–74]

Patients may have a history of failure to thrive, developmental delay, or nonspecific abdominal problems before the onset of the acute encephalopathy. Cardiomyopathy and lipid storage myopathy are also characteristic features. Less frequent clinical manifestations are cardiac arrhythmias, neuropathy, recurrent myoglobinuria, pigmentary retinopathy, and renal abnormalities[71–74] (Table 2).

Treatment of Fatty Acid Oxidation Defects

The general dietary management in patients with fatty acid oxidation disorders consists of avoidance of fasting; the intake of high-carbohydrate, low-fat meals at frequent intervals; and supplements of carnitine, riboflavin, or glycine.

Defects of Carnitine Cycle

Carnitine Transporter

Defective transport of carnitine was discussed earlier as the quintessential example of primary carnitine deficiency.

Carnitine Palmitoyltransferase I

Although we are including carnitine palmitoyltransferase I deficiency in this section, this is the only enzyme defect of fatty acid oxidation not associated with secondary carnitine deficiency.[75] Because we are also giving an overview of the fatty acid oxidation defects, we consider it of interest to discuss this disorder as well.

Carnitine palmitoyltransferase I is located in the inner face of the outer mitochondrial membrane and catalyzes the conversion of long-chain acyl-CoA to long-chain acylcarnitine. Carnitine palmitoyltransferase I deficiency has been described in about 10 cases.[75–79] Patients present in infancy with acute metabolic encephalopathy. No cardiac abnormalities have been described, except for mild cardiomegaly in one patient.[77] No clinical skeletal muscle involvement has been described, although one case had muscle lipid accumulation.[78] Renal tubular acidosis has been noted in one patient.[78] Unlike other fatty acid oxidation disorders, plasma carnitine levels are elevated[75,79] or normal.[77,78] Liver and muscle carnitine concentrations were normal when measured.[76,78,79] The acylcarnitine profile is normal.[66] Carnitine palmitoyltransferase I activity is deficient in liver and cultured fibroblasts but not in skeletal muscle.

Carnitine-Acylcarnitine Translocase

This enzyme catalyses the transmembrane transfer of acylcarnitines in exchange for carnitine (Figures 1 and 2). Its deficiency has been described in two patients.[80,81] Both presented in the newborn period with severe metabolic encephalopathy and arrhythmia. One patient died at 8 days of life with pulmonary hemorrhage after repeated episodes of hypoketotic hypoglycemia and massive macrovesicular steatosis.[81] The other patient manifested recurrent episodes of hypoketotic hypoglycemic enceph-

Table 2. Main Clinical and Biochemical Characteristics of Fatty Acid Oxidation Disorders

Disorder	*AME*	*Cardiac Signs*	*Myopathy*	*Myoglobinuria*	*Others*	*Hypoketotic Hypoglycemia*	*Carnitine Deficiency*	*Abnormal Organic Acids*
Carnitine cycle								
Transporter	+	+++	+	–	Gastrointestinal dysfunction, anemia	+	+++	–
CPT I	+++	–	–	–	—	+++	–*	–
Translocase	+++	+++	++	–	—	+++	+++	–
CPT II Infantile	++	++	±	–	Renal dysplasia	++	+++	–
CPT II Adult	–	–	±	+++	—	–	±	–
β-Oxidation cycle								
Short-chain AD	+	–	+	–	—	±	++	+++
Medium-chain AD	+++	–	±	–	—	+++	+++	+++
Long-chain AD	+++	++	+	±	—	+++	+++	+++
Very long chain AD	++	++	+	+	—	++	–†	+++
ETF severe	+++	–	–	–	Renal dysplasia, congenital anomalies	+++	NR	+++
ETF mild	+++	±	±	–	—	+++	+++	+++
Riboflavin responsive	+++	±	±	–	—	+++	+++	+++
Short-chain HD	+++	++	++	++	—	+++	+++	+++
Long-chain HD/ trifunctional protein	+++	++	++	±	Neuropathy, retinopathy	+++	+++	+++
2,4-Dienoyl CoA reductase	–	–	–	–	Microcephaly, dysmorphism, failure to thrive, emesis	–	+++	–

*Elevated carnitine levels.

† Elevated acylcarnitine levels in one patient and normal carnitine levels in another.

AME = acute metabolic encephalopathy with or without liver involvement; + = found in 25% to 50% of cases; +++ = found in more than 75% of cases; – = absent; CPT = carnitine palmitoyltransferase; ++ = found in 50% to 75% of cases; ± = found in less than 25% of cases; AD = acyl-coenzyme A dehydrogenase; ETF = electron transfer flavoprotein; NR = not reported; HD = 3-hydroxyacyl-coenzyme A dehydrogenase.

alopathy, persistent hyperammonemia, generalized weakness, liver involvement, and signs of cardiac hypertrophy. He died at 37 months of age of aspiration pneumonia.[80] Plasma free carnitine was reduced, and long-chain acylcarnitines were elevated. The enzyme deficiency was demonstrated in cultured fibroblasts.

Carnitine Palmitoyltransferase II

Carnitine palmitoyltransferase II is located in the inner face of the inner mitochondrial membrane and catalyzes the conversion of long-chain acylcarnitine to long-chain acyl-CoA. More than 50 patients with this deficiency have been described. Classically, carnitine palmitoyltransferase II deficient patients present in late childhood or early adulthood with recurrent episodes of muscle cramping or myoglobinuria provoked by fasting, exercise, or stress.[3] The ketone response to fasting can be delayed. Carnitine levels in plasma and tissues are in general normal.[82] A missense mutation has been identified in one patient.[83]

A severe infantile form has also been described in seven patients.[84–89] Most of them present in the neonatal period, and the evolution is fatal.[85–87,89] Acute metabolic encephalopathy,[84,87,88] cardiomyopathy, arrhythmias,[84,86–88] and renal dysplasia[85,86] are the most frequent features. One patient with dysmorphic features, severe weakness, areflexia, and hypotonia in the neonatal period has also been described.[89] Low plasma and tissue carnitine levels are accompanied by elevated long-chain acylcarnitines. Carnitine palmitoyltransferase II activity is deficient in fibroblasts, liver, and muscle. A more severe degree of enzyme deficiency was found in patients with the infantile form (less than 10%), compared with the adult form (more than 25%).[90] Different molecular defects have been found in patients with the infantile presentation.[88,91,92]

Defects of the β-Oxidation Spiral

Acyl-CoA Dehydrogenases

The acyl-CoA dehydrogenases catalyze the first step of β-oxidation[10] (Figures 1 and 2). There are four different types of enzymes, depending on the length of the substrate chain: short-chain acyl-CoA dehydrogenases act on fatty acyl-CoA substrates of four to six carbon atoms, medium-chain act on substrates of four to 14 carbons, long-chain act on substrates of 10 to 18 carbons, and very long chain act on substrates of 14 to 24 carbon atoms.

Short-Chain Acyl-CoA Dehydrogenase

Seven patients affected with short-chain acyl-CoA dehydrogenase deficiency have been described.[6,42,93–96] Three patients presented in the neonatal period with altered consciousness, hypertonicity, and metabolic acidosis.[94,96] The others presented with myopathy at different ages, in infancy,[42,95] childhood,[93] and adulthood.[6] Muscle biopsy showed lipid storage and carnitine deficiency.[6,42,93] Failure to thrive, developmental delay, frequent emesis, poor feeding,[42] and recurrent respiratory infections[95] are also features of this deficiency. No cardiac abnormalities have been described. Plasma carnitine is normal or low, and short-chain acylcarnitine levels are elevated.[6,42,95,96] Characteristic abnormal urinary organic acids in acute[94,96] and chronic states[6,95,96] are ethylmalonic acid, methylsuccinic acid, and also butyrylglycine and butyrylcarnitine. The enzyme deficiency has been demonstrated in fibroblasts[42,94–96] and skeletal muscle.[6,93] Two distinct mutations have been found in one patient.[97]

Medium-Chain Acyl-CoA Dehydrogenase

This is the most frequent enzyme deficiency of fatty acid oxidation, with more than 100 cases described. These patients are distinguished by recurrent episodes of hypoglycemic hypoketotic encephalopathy without muscle or cardiac involvement.[25] A recent follow-up study showed that 37% of patients have developmental or behavioral problems, 17% have proximal muscle weakness, 17% have seizures, 13% have failure to thrive, and 10% have cerebral palsy.[98] The characteristic organic acids during the acute episodes are dicarboxylic acids of medium chain length. The presence of the glycine conjugates (hexanoylglycine, suberylglycine, and phenylpropionylglycine) in urine, even when patients are asymptomatic, are specific markers of the deficiency.[99] Plasma carnitine deficiency is found in 96% of cases in the fed state.[100] The presence of plasma and urine six- to 10-carbon saturated and unsaturated acylcarnitines, mainly octanoylcarnitine, is specific for medium-chain acyl-CoA dehydrogenase deficiency.[66] The enzyme deficiency is demonstrated in cultured fibroblasts, leukocytes, and other tissues (liver, skeletal muscle, and heart). A point mutation at codon 985 that causes a substitution of a lysine for a glutamate is found in most of the patients.[101]

Long-Chain Acyl-CoA Dehydrogenase

About 20 cases with long-chain acyl-CoA dehydrogenase deficiency have been described.[71] The clinical picture is reminiscent of medium-chain acyl-CoA dehydrogenase deficiency, but it tends to be more severe, with earlier presentation and more frequent recurrent attacks.[102] Cardiomyopathy and skeletal muscle involvement are prominent features. Recurrent episodes of myoglobinuria have been described in patients after puberty. In urine, acetylcarnitine but no acylglycines are found, and medium- to long-chain dicarboxylic acids are characteristic.[102] Elevated long-chain acylcarnitines, mainly C14:1 acylcarnitine, are present in plasma.[66,102] The tissue carnitine deficiency in these patients tends to be more severe (10%).[5] The enzyme deficiency is demonstrated in fibroblasts. At present, no molecular defect underlying long-chain acyl-CoA dehydrogenase deficiency has been reported. Some of these patients may represent very long chain acyl-CoA dehydrogenase deficiency.

Very Long Chain Acyl-CoA Dehydrogenase

Since the purification of this enzyme in 1992,[8] four cases of very long chain acyl-CoA dehydrogenase deficiency have been described.[103–105] The clinical phenotype is heterogeneous. Two patients presented in early infancy with fatal hypoketotic hypoglycemic encephalopathy, liver

involvement, and cardiomyopathy.[103] One patient presented at 36 hours of life with ventricular fibrillation and respiratory arrest after one night of fasting.[104] Another patient developed recurrent episodes of myoglobinuria at the age of 16 years.[105] Variable profiles of organic acids have been found in these patients: Medium-chain dicarboxylic aciduria was found in two patients,[103,104] medium-chain hydroxydicarboxylic aciduria was discovered in another patient,[103] and no abnormal organic acids were detected in another.[105] Plasma carnitine was normal in one patient,[104] whereas elevated long-chain acylcarnitines were found in another.[105] The enzyme deficiency has been found in fibroblasts,[103–105] platelets, and skeletal muscle.[105]

Some patients who were originally diagnosed as having long-chain acyl-CoA dehydrogenase deficiency have subsequently been proven to have deficiency of very long chain acyl-CoA dehydrogenase.[106] Restudying these patients will permit a better understanding of the clinical phenotype of both long-chain and very long chain acyl-CoA dehydrogenase deficiencies.

Multiple Acyl-CoA Dehydrogenase Deficiency

Electron-transfer flavoprotein and electron-transfer flavoprotein coenzyme Q oxidoreductase carry electrons to the respiratory chain from the flavin-dependent acyl-CoA dehydrogenases. These enzymes catalyze the dehydrogenation of several metabolic pathways: fatty acid oxidation, branched-chain amino acid oxidation, and lysine oxidation. For this reason, these enzyme deficiencies are also called multiple acyl-CoA dehydrogenase deficiency.

There are three distinct clinical presentations: (1) a severe neonatal form with congenital abnormalities, (2) a severe neonatal form without congenital abnormalities, and (3) a mild, later-onset form.[107] Congenital abnormalities include facial dysmorphism with low-set ears, hypertelorism, high forehead, hypoplastic midface, rocker-bottom feet, muscular defects of the anterior abdominal wall, anomalies in the external genitalia, and enlarged kidney with cystic dysplasia. Patients with the neonatal form with or without congenital abnormalities present in the first 24 to 48 hours of life with lethargy, hypotonia, hepatomegaly, hypoglycemia, and metabolic acidosis, and often with an unusual odor. Usually, patients with congenital abnormalities die in the 1st week of life. Patients without congenital anomalies survive longer, and they may develop a fatal cardiomyopathy in a few months, or they may manifest recurrent metabolic decompensations. The mild, later-onset form is extremely variable. Later infancy, childhood, and adulthood onset are possible. Clinically, they can have recurrent metabolic decompensations or progressive lipid storage myopathy. An extrapyramidal movement disorder with dystonic features has been described in one patient.[108] Plasma and urine organic acids in the severe forms are characterized by six- to 12-carbon dicarboxylic, ethylmalonic, glutaric, isovaleric, isobutyric, and methylbutyric acids and related acylglycines and acylcarnitines. The mild form is characteristic for the adipic and ethylmalonic acids. Plasma and tissue carnitine deficiency with elevated esterified fraction is associated with the mild form.[39,109] Plasma C4 to C18:1 acylcarnitines have been found in asymptomatic patients.[66] Electron-transfer flavoprotein and electron-transfer flavoprotein coenzyme Q oxidoreductase deficiencies are diagnosed in fibroblasts. The three types of presentation can be due to either enzyme deficiency. However the severe form with congenital anomalies is usually due to electron-transfer flavoprotein coenzyme Q oxidoreductase deficiency. Several mutations have been described.[110,111]

Some patients with the mild form of multiple acyl-CoA dehydrogenase deficiency have responded dramatically to riboflavin treatment, implying that there is, in these cases, a defect in flavin metabolism, but no primary biochemical defect has been demonstrated.[39] If treatment is suspended, symptoms reappear.[39]

2-Enoyl-CoA Hydratases

The 2-enoyl-CoA hydratases catalyze the second step of β-oxidation[10] (Figures 1 and 2). Two types have been described: one that acts with short-chain substrates (crotonase), and another that acts with longer substrates. The latter belongs to a trifunctional protein that harbors the enzymes for the second, third, and fourth steps of β-oxidation for the long-chain substrates[9,10] (Figure 2).

At present, no deficiencies of short-chain 2-enoyl-CoA hydratase have been described, whereas deficiency of the long-chain 2-enoyl-CoA hydratase has been found associated with other defective enzyme activities (see below).

3-Hydroxyacyl-CoA Dehydrogenases

The 3-hydroxyacyl-CoA dehydrogenases catalyze the third step of β-oxidation[10] (Figures 1 and 2). Two types have been described: one that acts with short-chain substrates, and another that acts with longer substrates and belongs to the trifunctional protein.[9,10]

Short-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency

Two patients have been described.[112,113] One patient presented in infancy with recurrent hypoglycemic encephalopathy and liver involvement, with death occurring at age 11 months.[112] The other patient manifested recurrent myoglobinuria, hypoketotic hypoglycemic encephalopathy, and cardiomyopathy at age 16 years, with eventual fatal outcome.[113] Analysis of urine organic acids showed dicarboxylic and 3-hydroxydicarboxylic aciduria.[112,113] Plasma carnitine was deficient, with an elevated esterified fraction,[112,113] predominantly short-chain acylcarnitines.[112] The enzyme deficiency was demonstrated in fibroblasts in one patient[112] and in skeletal muscle but not in fibroblasts in the other.[113]

Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency/Trifunctional Protein Deficiency

More than 20 patients have been diagnosed as having long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency.[114–128A] The mean age of onset is 12 months. The presentation in the majority of cases is an acute hypoketotic hypoglycemic

encephalopathy with severe hepatic involvement. Some patients present with failure to thrive, developmental delay, and nonspecific gastrointestinal problems. As with long-chain acyl-CoA dehydrogenase deficiency, recurrent metabolic crises, cardiomyopathy, and skeletal muscle involvement are the most prominent features. Recurrent myoglobinuria has also been described in two patients.[121,127] Moreover, long-chain 3-hydroxyacyl-CoA dehydrogenase–deficient patients have distinctive features: pigmentary retinopathy[37,114,115,122-124] and peripheral neuropathy.[115,124,127] Urinary organic acids are characteristic for the medium- to long-chain 3-hydroxydicarboxylic acids. Plasma carnitine levels are low, and long-chain acylcarnitine levels are increased. In liver and skeletal muscle, the carnitine profile is similar.

The deficient activity of long-chain 3-hydroxyacyl-CoA dehydrogenase was diagnosed in fibroblasts. Activity of other enzymes of long-chain fatty acid oxidation was assessed in eight cases.[118,120,126,128,128A] In two patients, hydration was in the low range of normal and thiolytic cleavage was partially deficient, implying that a defect of the trifunctional protein might be present.[129] In two patients, the activities of hydratase and thiolase for long-chain substrates were in the lower range of normal.[118,128,128A] In the other four patients, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency was a singular abnormality.[118,126,128, 128A]

Since the description of the trifunctional protein harboring the activities of the last three steps of the β-oxidation for long-chain substrates, three patients have been diagnosed with a deficiency of this multifunctional protein.[129-131] One patient presented in infancy with recurrent episodes of muscle weakness, hypotonia, and anorexia, triggered by minor infections. At 4½ years of age, the patient died during a severe attack accompanied by elevated serum levels of creatine kinase, ammonia, and lactic and pyruvic acids. Necrosis with minimal lipid storage was found in skeletal muscle, whereas steatosis and some degree of fibrosis were found in liver.[129] The other two patients had early hypoketotic hypoglycemic encephalopathy,[130,131] one of them with fatal cardiomyopathy.[130] In two cases, the urinary organic acids were also remarkable for 3-hydroxydicarboxylic aciduria.[130,131] The carnitine profile in these patients has not been reported. Analysis of the three different enzyme activities of the trifunctional protein has been performed in fibroblasts,[129-131] muscle, heart, and liver.[129] The activities of the three enzymes were diminished to different degrees.

In a recent study,[132] long-chain 3-hydroxyacyl-CoA dehydrogenase–deficient patients were divided into two groups: group 1 consisted of only one patient with deficient activities in the three components of the multifunctional protein, and group 2 comprised 26 patients with deficient long-chain 3-hydroxyacyl-CoA dehydrogenase and partial long-chain 3-ketothiolase activities.[132] Twenty-four of the group 2 patients were homozygotes for a mutation involving the α-subunit of the mitochondrial trifunctional protein in the long-chain 3-hydroxyacyl-CoA dehydrogenase encoding region. The other two cases were heterozygotes for this mutation.[132]

3-Ketoacyl-CoA Thiolase

The 3-ketoacyl-CoA thiolase catalyzes the fourth step of β-oxidation[10] (Figures 1 and 2). Two types of 3-ketoacyl-CoA thiolase have been demonstrated in the mitochondrial matrix: one functions in ketone body and isoleucine metabolism and the other is required for β-oxidation.[10] A long-chain 3-ketoacyl-CoA thiolase, which is a component of the trifunctional protein, also has been identified. It is bound to the mitochondrial membrane.[9]

At present, no deficiency of the matrix 3-ketoacyl-CoA thiolase involved in β-oxidation has been proven. Deficiencies of the membrane-bound long-chain 3-ketoacyl-CoA thiolase have been found associated with other defective enzyme activities (see above).

2,4-Dienoyl-CoA Reductase

2,4-Dienoyl-CoA reductase is an enzyme necessary for the β-oxidation of unsaturated fatty acids. One patient has been described with this enzyme deficiency.[69] The patient presented with dysmorphic features, microcephaly, hypotonia, failure to thrive, feeding problems, and vomiting. He died at 4 months of age with respiratory acidosis. Hyperlysinemia was found in plasma. Carnitine in plasma was deficient, with an elevated esterified fraction due to accumulation of 2-*trans*,4-*cis*-decadienoylcarnitine. Urinary organic acids were normal except for the presence of the unusual carnitine ester decadienoylcarnitine. Deficient enzyme activity was demonstrated in liver and skeletal muscle.

Other Genetically Determined Metabolic Errors Associated With Secondary Carnitine Deficiency

Patients with branched-chain amino acid disorders share some metabolic abnormalities with fatty acid oxidation defects because there is also a block of acyl-CoA oxidation. Low plasma and tissue total carnitine levels, increased acylcarnitine to free carnitine ratios, and excretion of disease-specific acylcarnitines reflect the abnormal acyl-CoA species accumulating at or near the site of the metabolic block.[5,13] Major diagnostic urinary acylcarnitine profiles are isovalerylcarnitine in isovaleric acidemia, glutarylcarnitine in glutaric acidemia type I and 3-methylglutarylcarnitine in 3-hydroxy-3-methylglutaryl-CoA lyase deficiency.[13] The urinary pattern of propionylcarnitine and acetylcarnitine is typical of both propionic and methylmalonic acidemias, whereas excretion of tiglylcarnitine is suggestive of 2-methylacetoacetyl-CoA thiolase deficiency.[13] Administration of an oral carnitine load to increase the acylcarnitine excretion can be used as a noninvasive test when insufficient carnitine availability in these patients causes low excretion of acylcarnitines.[70]

Secondary carnitine deficiency is also associated with other genetically determined metabolic errors (Table 1). Multiple and varied factors contribute to the deficiency in these disorders, such as impairment of carnitine biosynthesis or increased urinary loss of carnitine.[5,27,133] In patients

with impairment of the mitochondrial respiratory chain, such as cytochrome *c* oxidase (COX) deficiency, there is decreased energy metabolism that has been demonstrated to compromise the energy-dependent carnitine uptake in vitro.[134] This effect would interfere with the carnitine transport in tissues, including renal reabsorption,[134] thus explaining the low plasma and tissue levels in these patients.[135]

In some dysgenetic syndromes (Table 1) muscle lipid storage associated with low muscle carnitine levels has been reported.[58,59] No definitive biochemical defects have been detected, except for one patient with Bannayan-Riley-Ruvalcaba syndrome who had deficient short-chain 3-hydroxyacyl-CoA dehydrogenase and long-chain 3-hydroxyacyl-CoA dehydrogenase activities in cultured skin fibroblasts.[60] More patients with this dysgenetic syndrome should be studied to further characterize the role of this finding.

Carnitine Therapy in Fatty Acid Oxidation Defects and Organic Acidurias

The role of carnitine supplementation in fatty acid oxidation disorders and other organic acidurias has not been assessed systematically. Carnitine supplement is given to correct the existing carnitine deficiency and to allow removal of toxic intermediates from tissues while restoring CoA levels.[25] In some patients, carnitine therapy improved the general clinical condition and decreased the frequency of the metabolic attacks, but in other patients, replacement therapy was ineffective.[39,71,100]

A number of studies of renal carnitine excretion during carnitine replacement in patients with carnitine deficiency due to fatty acid oxidation defects or other organic acidurias have appeared in the last few years.[68,136–138] Enhanced excretion of relevant carnitine esters was documented in all of them. Whether this increased excretion reflects increased production through the enhancement of acyl-CoA oxidation or increased elimination of the toxic acyl-CoA intermediates is still a matter of debate.

Carnitine therapy in long-chain fatty acid oxidation defects has been questioned because it promotes long-chain acylcarnitine formation, and these esters may contribute to ventricular arrhythmogenesis and membrane dysfunction.[139]

Acquired Medical Conditions

Acquired metabolic conditions, particularly those affecting the liver and kidney, may secondarily affect carnitine homeostasis (Table 1). Multiple mechanisms may play a role in secondary carnitine deficiency[133]: Diminished carnitine biosynthesis may be associated with extreme prematurity, cirrhosis, and chronic renal disease. Decreased intake due to diets with low carnitine content or decreased reabsorption in malabsorption syndromes may also cause carnitine deficiency. Reduced body stores and increased requirements for carnitine may accompany diverse clinical conditions such as pregnancy, prematurity, or increased catabolism in critically ill patients. Excessive renal losses have been associated with Fanconi syndrome and renal tubular acidosis.[27,61,133]

Iatrogenic Factors

Patients with chronic kidney failure undergoing hemodialysis develop carnitine deficiency due to a dramatic loss of carnitine into the dialysate fluid.[27] Several drugs have been associated with carnitine deficiency (Table 1). Considerable attention has been focused on valproic acid. Valproic acid is a branched-chain fatty acid used in the treatment of epilepsy. Like natural fatty acids, valproic acid forms CoA thioesters and carnitine esters (valproylcarnitine).[140] Chronic therapy is associated with decreased serum carnitine levels.[141] Muscle carnitine deficiency can exist in the presence of normal serum levels.[142] The prevalence of carnitine deficiency in valproic acid–treated patients (4% to 76%) varies with different studies, probably reflecting differences in the nature of the population studied.[141] Serious and less common side effects of valproic acid treatment are potentially life-threatening hepatotoxicity, Reye-like syndrome, and pancreatitis.[27,140] Their relation with carnitine homeostasis remains unclear.[141] Valproic acid–induced toxicity is considered to be secondary to mitochondrial dysfunction. A number of mechanisms have been postulated, including sequestration of CoA by valproic acid and its metabolites (4-ene-valproic acid, 2,4-diene-valproic acid), causing a secondary disturbance of intermediary metabolism, and direct inhibition of fatty acid oxidation enzymes by valproic acid metabolites.[140]

Multiple mechanisms of valproic acid–associated carnitine deficiency have been considered: First, a number of studies have reported an increased acylcarnitine to total carnitine ratio in the urine, although total carnitine in urine was not increased.[141] In valproic acid–treated patients, excretion of valproylcarnitine constitutes less than 10% of the total urinary acylcarnitine pool,[143] whereas medium-chain acylcarnitines have been found to be excreted at the same level as in medium-chain acyl-CoA dehydrogenase–deficient patients.[144] Decreased renal tubular reabsorption of free carnitine has also been reported.[145] During long-term valproic acid therapy, continued urinary excretion of acylcarnitines might gradually deplete total body stores of carnitine, resulting in a deficient state.[141]

Second, it has been demonstrated that valproic acid impairs the plasma membrane carnitine uptake in vitro in cultured fibroblasts and that this effect is directly proportional to the duration of exposure and concentration of valproic acid.[146] This carnitine transport impairment may explain serum depletion caused by decreased renal tubular reabsorption of carnitine and muscle depletion caused by decreased muscle uptake.[146] The authors proposed that this inhibition may be due to increasing competition between free carnitine and acylcarnitines, including valproylcarnitines and short-chain acylcarnitines at the plasmalemmal transporter site.[146]

Finally, carnitine deficiency in valproic acid–treated patients may result also from preexisting metabolic disorders causing secondary carnitine deficiency, such as organic acidurias, urea cycle defects, or mitochondrial respiratory disorders, nutritional carnitine deficiency,

treatment with other antiepileptic drugs, or a combination of these factors.[141]

The valproic acid effect on the carnitine uptake together with the existence of an underlying inborn error involving energy metabolism may precipitate fatal complications. A patient with cytochrome *c* oxidase deficiency with fatal hepatic failure apparently triggered by valproic acid administration has been reported.[147] We believe that, in addition, heterozygosity for primary carnitine deficiency or other fatty acid oxidation disorders may predispose to serious complications after valproic acid treatment. Therefore, every recognized case should be studied in exhaustive detail to determine whether the epileptic patient has an associated inborn error of metabolism.[27]

Oral carnitine supplement normalizes plasma carnitine concentrations in patients treated with valproic acid.[148] Most patients who have valproic acid–associated carnitine deficiency manifest no symptoms of disease, making it very difficult to evaluate the beneficial effect of carnitine supplementation. Carnitine treatment has been helpful in a few patients with muscle weakness and failure to thrive,[149] whereas no substantial benefit has been detected in others.[148] From the biochemical point of view, carnitine supplementation in valproic acid–treated patients permits a decrease in the plasmalemmal carnitine transport inhibition by increasing the free carnitine concentration at the transporter site, provides a greater buffering capacity for the excessive potentially toxic acyl-CoA, and increases the intramitochondrial free CoA, thereby decreasing mitochondrial dysfunction.[146]

Although the role of carnitine is debatable in this situation, clinical wisdom suggests that carnitine should be administered prophylactically to all children under 2 years of age treated with valproic acid and selectively when there is laboratory or clinical evidence of carnitine deficiency. There is no evidence that carnitine administration adversely alters the anticonvulsant properties of valproic acid or lowers the valproic acid concentration.[27,145]

SUMMARY

Tremendous advances have been made in our understanding of carnitine metabolism, and a reorganization of our thinking, including the proper usage of terminology, has been necessary. Primary carnitine deficiency now is clearly defined. The only clear example of this condition is the carnitine-responsive cardiomyopathy of childhood that is exquisitely sensitive to carnitine supplementation and is due to a genetic defect of the carnitine transport system located in the plasma membrane. The definitions of systemic carnitine deficiency and muscle carnitine deficiency are less clear, and these patients need to be reclassified in light of recent advances. Some of these patients have a primary genetic defect involving the plasma membrane transporter, whereas other patients have generalized or tissue-specific monoenzymopathies such as medium-chain acyl-CoA dehydrogenase deficiency or short-chain acyl-CoA dehydrogenase deficiency. Most of the monoenzymopathies involving fatty acid oxidation are associated with secondary carnitine deficiency. Exceptions include carnitine palmitoyltransferase I deficiency and the adult form of carnitine palmitoyltransferase II deficiency. The serum and tissue carnitine concentrations in these two conditions tend to be high or normal in most cases. In contrast, the infantile presentation of carnitine palmitoyltransferase II, is associated with decreased carnitine concentrations. Dicarboxylic aciduria tends to distinguish defects involving the carnitine cycle from defects involving the β-oxidation spiral. The free and bound carnitine fractions are decreased proportionately in primary carnitine deficiency. There is a disproportionately high bound carnitine fraction in the secondary carnitine deficiency syndromes associated with defects of acyl-CoA oxidation. Secondary carnitine deficiency syndromes also can result from acquired medical conditions and from iatrogenic factors. Valproate, a commonly used anticonvulsant medication, produces secondary carnitine deficiency and interferes directly with the active transport of carnitine across the plasma membrane. As a result, patients given valproate may develop tissue carnitine deficiency in the presence of relatively normal serum carnitine concentrations. It remains unclear whether carnitine supplementation should be initiated in all cases of secondary carnitine insufficiency syndromes associated with inborn metabolic errors, acquired medical conditions, and iatrogenic states. However, the insidious nature of the symptoms associated with carnitine insufficiency suggest that replacement therapy should be considered when low serum or tissue carnitine concentrations are documented.

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References

1. Carter HE, Bhattacharyya PK, Werdman KR, Fraenkel G: Chemical studies on vitamin B_t isolation and characterization as carnitine. *Arch Biochem Biophys* 1952;38:405–416.
2. Engel AG, Angelini C: Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: A new syndrome. *Science* 1973;179:899–902.
3. DiMauro S, DiMauro PMM: Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science* 1973;182:929–930.
4. Karpati G, Carpenter S, Engel AG: The syndrome of systemic carnitine deficiency. *Neurology* 1975;25:16–24.
5. Stanley CA: New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. *Adv Pediatr* 1987;34:59–88.
6. Turnbull DM, Barlett K, Stevens DL, et al: Short chain acyl-CoA dehydrogenase deficiency associated with a lipid-storage myopathy and secondary carnitine deficiency. *N Engl J Med* 1984;311:1232.
7. Stanley CA, De Leeuw S, Coates PM, et al: Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake. *Ann Neurol* 1991;30:709–716.

8. Izai K, Uchida Y, Orii T, et al: Novel fatty acid beta-oxidation enzymes in rat liver mitochondria. I: Purification and properties of very-long-chain acyl-coenzyme A dehydrogenase. *J Biol Chem* 1992;267:1027–1033.
9. Uchida Y, Izai K, Orii T, Hashimoto T: Novel fatty acid beta-oxidation enzymes in rat liver mitochondria. II: Purification and properties of enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J Biol Chem* 1992;267:1034–1041.
10. Kunau WH, Dommes V, Schulz H: Beta oxidation of fatty acids in mitochondria, peroxisomes and bacteria. A century of continued progress. *Prog Lipid Res*, in press.
11. Bremer J: Carnitine. Metabolism and functions. *Physiol Rev* 1983;63:1420–1480.
12. Rebouche CJ: Carnitine metabolism and function in humans. *Ann Rev Nutr* 1986;6:41–66.
13. Bohles H, Evangeliou A, Bervoets K, et al: Carnitine esters in metabolic disease. *Eur J Pediatr* 1994;153(Suppl 1):57–61.
14. Rebouche CJ: Carnitine function and requirements during life cycle. *FASEB J* 1992;6:3379–3386.
15. Hallgrimur G, Li BUK, Shug AL, Olse WA: Studies of carnitine metabolism in relation to intestinal absorption. *Am J Physiol* 1985;248:G313–G319.
16. Rebouche CJ, Chenard CA: Metabolic fate of dietary carnitine in human adults: Identification and quantification of urinary and fecal metabolites. *J Nutr* 1991;121:539–546.
17. Rebouche CJ, Engel AG: Carnitine transport in cultured muscle cells and skin fibroblasts from patients with primary systemic carnitine deficiency. *In Vitro* 1982;18:495–500.
18. Tein I, DeVivo DC, Bierman F, et al: Impaired skin fibroblasts carnitine uptake in primary systemic deficiency manifested by childhood carnitine-responsive cardiomyopathy. *Pediatr Res* 1990;28:247–255.
19. Martinuzzi A, Vergani MR, Angelini C: L-Carnitine uptake in differentiating human cultured muscle. *Biochim Biophys Acta* 1991;1095:217–222.
20. Bahl JJ, Bressler: The pharmacology of carnitine. *Ann Rev Pharmacol Toxicol* 1987;27:257–277.
21. Shaw RD, Li BUK, Hamilton JW, et al: Carnitine transport in rat small intestine. *Am J Physiol* 1983;245:G376–G381.
22. Rebouche CJ, Engel AG: Kinetic compartmental analysis of carnitine in human carnitine deficiency syndromes. Evidence of alterations in tissues carnitine transport. *J Clin Invest* 1984; 73:857–867.
23. Bieber LL: Carnitine. *Ann Rev Biochem* 1988;57:261–283.
24. Engel AG, Rebouche CJ: Carnitine metabolism and inborn errors. *J Inherit Metab Dis* 1984;7(Suppl 1):38–43.
25. Roe CR, Coates PM: Acyl CoA dehydrogenase deficiencies, in Scriver CR, Beaudet AL, Sly WS, Valle D (eds): *The Metabolic Basis of Inherited Diseases*. New York, McGraw-Hill, 1989, pp 889–914.
26. Treem WR, Stanley CA, Finegold DN, et al: Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle and fibroblasts. *N Engl J Med* 1988;319:1331–1336.
27. DeVivo DC, Tein I: Primary and secondary disorders of carnitine metabolism. *Int Pediatr* 1990;5:134–141.
28. Erikson BO, Lindstedt S, Nordin I: Hereditary defect in carnitine membrane transport is expressed in skin fibroblasts. *Eur J Pediatr* 1988;147:662–668.
29. Waber LJ, Valle D, Neill C, et al: Carnitine deficiency presenting as familial cardiomyopathy: A treatable defect in carnitine transport. *J Pediatr* 1982;101:700–705.
30. Brass EP: Overview of coenzyme A metabolism and its role in cellular toxicity. *Chem Biol Int* 1994;90:203–214.
31. Koizumi T, Nikaido H, Hayakama J, et al: Infantile disease with microvesicular fatty infiltration of viscera spontaneously occurring in the C3H-H-2° strain of mouse with similarities to Reye's syndrome. *Lab Anim* 1988;22:83–87.
32. Horiuchi M, Kobayashi K, Yamaguchi S, et al: Primary defect of juvenile visceral steatosis (jvc) mouse with systemic carnitine deficiency is probably in renal carnitine transport system. *Biochim Biophys Acta* 1993;1226:25–30.
33. Chapoy PR, Angelini C, Brown WJ, et al: Systemic carnitine deficiency. A treatable inherited lipid storage disease presenting as Reye's syndrome. *N Engl J Med* 1980;303:1389–1394.
34. Tripp ME, Katcher ML, Peters HA, et al: Systemic carnitine deficiency presenting as familial endocardial fibroelastosis. *N Engl J Med* 1981;305:385–390.
35. Garavaglia B, Uziel G, Dworzak F, et al: Primary carnitine deficiency: Heterozygote and intrafamilial variation. *Neurology* 1991;41:1691–1693.
36. Weaver LT, Rosenthal SR, Gladstone W, Winter HS: Carnitine deficiency: A possible cause of gastrointestinal dysmotility. *Acta Paediatr* 1992;81:79–81.
37. Engel AG: Carnitine deficiency syndromes and lipid storage myopathy, in Engel AG, Banker BQ (eds): *Myology: Basic and Clinical*. New York, McGraw-Hill, 1986, pp 1663–1696.
38. Angelini C, Trevisan G, Isaya G, et al: Clinical varieties of carnitine and carnitine palmitoyltransferase deficiency. *Clin Biochem* 1987;20:1–7.
39. DiDonato S: Disorders of lipid metabolism affecting skeletal muscle: Carnitine deficiency syndromes, defects in the catabolic pathway and Chanarin disease, in Engel AG, Franzini-Amstrong CL (eds): *Myology: Basic and Clinical*, 2nd ed. New York, McGraw-Hill, 1994, pp 1587–1609.
40. Shapira Y, Glick B, Harel S, et al: Infantile idiopathic myopathic carnitine deficiency: Treatment with L-carnitine. *Pediatr Neurol* 1993;9:35–38.
41. Mesmer OT, Lo TCY: Hexose transport properties of myoblasts isolated from a patient with suspected muscle carnitine deficiency. *Biochem Cell Biol* 1990;68:1372–1379.
42. Coates PM, Hale DE, Finochiaro G, et al: Genetic deficiency of short-chain acyl-CoA dehydrogenase in cultured fibroblasts from a patient with muscle carnitine deficiency and severe skeletal muscle weakness. *J Clin Invest* 1988;81:171.
43. Hart ZH, Chang C, DiMauro S, et al: Muscle carnitine deficiency and fatal cardiomyopathy. *Neurology* 1978;28:147–151.
44. Carroll JE, Brooke MH, DeVivo DC, et al: Carnitine "deficiency": Lack of response to carnitine therapy. *Neurology* 1980;30:618–626.
45. Trevisan CO, Reichman H, DeVivo DC, DiMauro S: Beta-oxidation enzymes in normal human muscle and in muscle from a patient with an unusual form of myopathic carnitine deficiency. *Muscle Nerve* 1985;8:672–675.
46. Angelini C, Govoni E, Bragaglia MM, et al: Carnitine deficiency: Acute postpartum crisis. *Ann Neurol* 1978;4:558–561.
47. DiDonato S, Cornelio F, Storchi G, Rimoldi M: Hepatic ketogenesis and muscle carnitine deficiency. *Neurology* 1979;29:780–785.
48. Buscaino GA, Cocchiararo M, Marolda M, et al: "Lipid storage myopathy" with muscle carnitine deficiency only. *Acta Neurol (Napoli)* 1982;1:1–13.
49. Prockop LD, Engel WK, Shug AL: Nearly fatal muscle carnitine deficiency with full recovery after replacement therapy. *Neurology* 1983;33:1629–1631.
50. Markesbery WR, McQuillen MP, Procopis PG, et al: Muscle carnitine deficiency. *Arch Neurol* 1974;31:320–324.
51. Vandyke DH, Griggs RC, Markesbery W, DiMauro S: Hereditary carnitine deficiency of muscle. *Neurology* 1975;25:154–159.

52. Isaac H, Hefrron JJA, Badenhorst M, Pickerin A: Weakness associated with the pathological presence of lipid in skeletal muscle: A detailed study of a patient with carnitine deficiency. *J Neurol* 1976;39:1114–1123.
53. Angelini C, Lucke S, Cantarutti F: Carnitine deficiency of skeletal muscle: Report of a treated case. *Neurology* 1976;26:633–637.
54. Scarlato G, Albizzati MG, Cerri C, Frattola L: A case of lipid storage myopathy with carnitine deficiency: Biochemical and electromyographic correlations. *Eur Neurol* 1977;16:222–229.
55. Avigan J, Askanas V, Engel K: Muscle carnitine deficiency: Fatty acid metabolism in cultured fibroblasts and muscle cells. *Neurology* 1983;33:1021–1026.
56. Colin AA, Jaffe M, Shapira Y, et al: Muscle carnitine deficiency presenting as familial fatal cardiomyopathy. *Arch Dis Child* 1987;62:1170–1172.
57. Mori T, Tsuchiyama A, Nagai K, et al: A case of carbamoylphosphate synthetase-I deficiency associated with secondary carnitine deficiency—L-carnitine treatment of CPS-I deficiency. *Eur J Pediatr* 1990;149:272–274.
58. Voit T, Kramer H, Thomas C, et al: Myopathy in Williams-Beuren syndrome. *Eur J Pediatr* 1991;150:521–526.
59. Berkley R, Budden SS, Buist NRM: Dominantly inherited megalencephaly, muscle weakness, and myoliposis: A carnitine-deficient myopathy within the spectrum of the Ruvalcaba-Myhre-Smith syndrome. *J Pediatr* 1993;123:70–75.
60. Fryburg JS, Pelegano JP, Bennet MJ, Bebin M: Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (L-CHAD) deficiency in a patient with the Bannayan-Riley-Ruvalcaba syndrome. *Am J Med Genet* 1994;52:97–102.
61. Tanphaichitr V, Leelahagul P: Carnitine metabolism and human carnitine deficiency. *Nutrition* 1993;9:246–254.
62. Simone CD, Tzantzoglou S, Jrillo E, et al: L-Carnitine deficiency in AIDS patients. *AIDS* 1992;6:203–205.
63. Holme E, Greter J, Jacobson CE, et al: Carnitine deficiency induced by pivampicilline and pivmecillinam therapy. *Lancet* 1989;2:469–473.
64. Kuntzer T, Reichman H, Bogousslavsky J, Regli F: Emetine-induced myopathy and carnitine deficiency. *J Neurol* 1990; 237:495–496.
65. Dalakas MC, Leon-Monzon ME, Bernardini I, et al: Zidovudine-induced mitochondrial myopathy is associated with muscle carnitine deficiency and lipid storage. *Ann Neurol* 1994;35: 482–487.
66. Millington DS, Terada N, Chace DH, et al: The role of tandem mass spectrometry in the diagnosis of fatty acid oxidation disorders, in Coates PM, Tanaka K (eds): *International Symposium on Clinical, Biochemical, and Molecular Aspects of Fatty Acid Oxidation. New Developments in Fatty Acid Oxidation.* New York, Wiley-Liss, 1992, pp 339–354.
67. Chalmers RA, Roe CR, Stacey E, Hoppel CL: Urinary excretion of L-carnitine and acyl-carnitines by patients with disorders of organic acid metabolism: Evidence for secondary insufficiency of L-carnitine. *Pediatr Res* 1984;18:1325–1328.
68. Stanley CA, Berry GT, Bennet MJ, et al: Renal handling of carnitine in secondary carnitine deficiency disorders. *Pediatr Res* 1993;34:89–97.
69. Roe CR, Millington DS, Norwood DL, et al: 2,4-Dienoyl-coenzyme A reductase deficiency: A possible new disorder of fatty acid oxidation. *J Clin Invest* 1990;85:1703–1707.
70. Roe CR, Millington DS, Maltby DA: Diagnostic and therapeutic implications of acylcarnitine profiling in organic acidurias associated with carnitine insufficiency, in Borum PR (ed): *Clinical Aspects of Human Carnitine Deficiency.* New York, Pergamon Press, 1986, pp 97–107.
71. Hale DE, Benett MJ: Fatty acid oxidation disorders: A new class of metabolic diseases. *J Pediatr* 1992;121:1–11.
72. Saudubray JM, Mitchell G, Bonnefont P, et al: Approach to the patient with a fatty acid oxidation disorder, in Coates PM, Tanaka K (eds): *International Symposium on Clinical, Biochemical, and Molecular Aspects of Fatty Acid Oxidation. New Developments in Fatty Acid Oxidation.* New York, Wiley-Liss, 1992, pp 271–288.
73. Stanley CA: Disorders of fatty acid oxidation, in Fernandes J, Saudubray JM, Tada K (eds): *Inborn Metabolic Diseases.* Berlin, Springer-Verlag, 1991, pp 395–410.
74. Rhead WJ: Inborn errors of fatty acid oxidation in man. *Clin Biochem* 1991;24:319–329.
75. Stanley CA, Sumarzo F, Hale DE, et al: Elevated plasma carnitine in the hepatic form of CPT I deficiency. *J Inherit Metab Dis* 1992;15:985–989.
76. Bougneres PF, Saudubray JM, Marsac C, et al: Fasting hypoglycemia resulting from hepatic carnitine palmitoyl-transferase deficiency. *J Pediatr* 1981;98:742–746.
77. Demaugre F, Bonnefont JP, Mitchell G, et al: Hepatic and muscular presentations of CPT deficiency: Two distinct entities. *Pediatr Res* 1988;24:308–311.
78. Bonnefont JP, Haas R, Wolf J, et al: Deficiency of carnitine palmitoyltransferase I. *J Child Neurol* 1989;4:197–202.
79. Vianey-Saban C, Mousson B, Bertrand C, et al: Carnitine palmitoyl transferase I deficiency presenting as a Reye-like syndrome without hypoglycemia. *Eur J Pediatr* 1993;152:334–338.
80. Stanley CA, Hale DE, Berry GT, et al: Brief report: A deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N Engl J Med* 1992;327:19–23.
81. Pande SV, Brivet M, Slama A, et al: Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculoventricular block. *J Clin Invest* 1993;91:1247–1252.
82. Zierz S: Carnitine palmitoyltransferase deficiency, in Engel AG, Franzini-Amstrong CL (eds): *Myology: Basic and Clinical,* 2nd ed. New York, McGraw-Hill, 1994, pp 1577–1586.
83. Taroni F, Verderio E, Dworzak F, et al: Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients. *Nat Genet* 1993;4: 314–320.
84. Demaugre F, Bonnefont JP, Colonna M, et al: Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death. *J Clin Invest* 1991;87:859–864.
85. Witt DR, Theobald M, Santa Maria M, et al: Carnitine palmitoyltransferase type II deficiency: Two new cases and successful prenatal diagnosis. *Am J Hum Genet* 1991;49:109, Abstract 535.
86. Zinn AB, Hoppel C: An unusual form of carnitine palmitoyltransferase B (CPT B) deficiency associated with neonatal cardiomyopathy and renal dysorganogenesis. *Am J Hum Genet* 1991;49:109, Abstract 536.
87. Hug G, Bove K, Senking S: Lethal neonatal multiorgan deficiency of CPT II. *N Engl J Med* 1991;325:1862–1864.
88. Taroni F, Verderio E, Fiorucci S, et al: Molecular characterization of inherited carnitine palmitoyltransferase II deficiency. *Proc Natl Acad Sci U S A* 1992;89:8429–8433.
89. Land JM, Mistry S, Squier W, Hope O: Neonatal carnitine palmitoyltransferase deficiency: A case with muscular presentation, in Coates PM, Tanaka K (eds): *International Symposium on Clinical, Biochemical, and Molecular Aspects of Fatty Acid Oxidation. New Developments in Fatty Acid Oxidation.* New York, Wiley-Liss, 1992, pp 309–315.
90. Demaugre F, Bonnefont JP, Brivet M, et al: Hepatic and muscular approach to carnitine palmitoyltransferase II deficiencies, in Coates PM, Tanaka K (eds): *International Symposium on*

Clinical, Biochemical, and Molecular Aspects of Fatty Acid Oxidation. New Developments in Fatty Acid Oxidation. New York, Wiley-Liss, 1992, pp 301–308.

91. Bonnefont JP, Cepanec C, Munnich A, et al: Infantile form of CPT II deficiency: Identification of a missense mutation in the CPT II gene. *Am J Hum Genet* 1992;51:165, Abstract 647.
92. Gellera C, Witt DR, Verderio E, et al: Molecular study of lethal neonatal carnitine palmitoyltransferase II (CPT II) deficiency. *Am J Hum Genet* 1992;51:168, Abstract 660.
93. DiDonato S, Cornelio F, Gellara C, et al: Short-chain acyl-CoA dehydrogenase deficiency. *Muscle Nerve* 1986;9:178, Abstract 33.6.
94. Amendt BA, Greene C, Sweetman L, et al: Short-chain acyl-CoA dehydrogenase deficiency: Clinical and biochemical studies in two patients. *J Clin Invest* 1987;79:1303.
95. Sewell AC, Herwig J, Bohles H, et al: A new case of short-chain acyl-CoA dehydrogenase deficiency with isolated ethylmalonic aciduria. *Eur J Pediatr* 1993;152:922–924.
96. Dawson DB, Waber L, Hale DE, Bennet MJ: Transient organic aciduria and persistent lactacidemia in a patient with short-chain acyl-CoA dehydrogenase deficiency. *J Pediatr* 1995;126: 69–71.
97. Naito E, Indo Y, Tanaka K: Identification of two variant short chain acyl-coenzyme A dehydrogenase alleles, each containing a different point mutation in a patient with short chain acyl-coenzyme A dehydrogenase deficiency. *J Clin Invest* 1989;85: 1575–1582.
98. Iafolla AK, Thompson RJ, Coates RR: Medium-chain acyl-CoA dehydrogenase deficiency: Clinical course in 120 affected children. *J Pediatr* 1994;124:409–415.
99. Rinaldo P, O'Shea JJ, Coates PM, et al: MCAD deficiency. Diagnosis by stable isotope dilution measurement of urinary *n*-hexanoylglycine and 3-phenylpropionylglycine. *N Engl J Med* 1988;319:1308–1313.
100. Touma EH, Charpentier C: Medium chain acyl-CoA dehydrogenase deficiency. *Arch Dis Child* 1992;67:142–145.
101. Matsubara Y, Narisawa K, Miyabayashi S, et al: Identification of a common mutation in patients with medium-chain acyl-CoA dehydrogenase deficiency. *Biochem Biophys Res Commun* 1990;171:498–505.
102. Treem WR, Stanley CA, Hale DE, et al: Hypoglycemia, hypotonia and cardiomyopathy: The evolving clinical picture of long-chain acyl-CoA dehydrogenase deficiency. *Pediatrics* 1991;87: 328–333.
103. Aoyama T, Uchida Y, Kelley RI, et al: A novel disease with deficiency of mitochondrial very-long-chain acyl-CoA dehydrogenase. *Biochem Biophys Res Commun* 1993;191:1369–1372.
104. Bertrand C, Largilliere C, Zabot MT, et al: Very long chain acyl-CoA dehydrogenase deficiency: Identification of a new inborn error of mitochondrial fatty acid oxidation in fibroblasts. *Biochim Biophys Acta* 1993;1180:327–329.
105. Ogilvie I, Ourfarzan M, Jackson S, et al: Very long chain acyl-CoA dehydrogenase deficiency presenting with exercise-induced myoglobinuria. *Neurology* 1991;44:467–473.
106. Yamaguchi S, Indo Y, Coates PM, et al: Identification of very-long-chain acyl-CoA dehydrogenase deficiency in three patients previously diagnosed with long-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 1993;34:111–113.
107. Frerman FE, Goodman SI: Glutaric acidemia type II and defects of the mitochondrial respiratory chain, in Scriver CR, Beaudet AL, Sly WS, Valle D (eds): *The Metabolic Basis of Inherited Diseases.* New York, McGraw-Hill, 1989, pp 915–932.
108. Loehr JP, Goodman SI, Frerman FE: Glutaric acidemia type II: Heterogeneity of clinical and biochemical phenotypes. *Pediatr Res* 1990;27:311–315.
109. Bell RB, Bronell AKW, Roe CR, et al: Electron transfer flavoprotein ubiquinone oxidoreductase (EFT:QO) deficiency in an adult. *Neurology* 1990;40:1779–1782.
110. Freneaux E, Sheffield VC, Molin L, et al: Glutaric acidemia type II. Heterogeneity in beta-oxidation flux, polypeptide synthesis, and complementary DNA mutations in the alpha subunit of electron transfer flavoprotein in eight patients. *J Clin Invest* 1992;90:1679–1686.
111. Colombo I, Finocchiaro G, Garavaglia B, et al: Mutations and polymorphisms of the gene encoding the beta-subunit of the electron transfer flavoprotein in three patients with glutaric acidemia type II. *Hum Mol Genet* 1994;3:429–435.
112. Hale DE, Thorpe C: Short-chain 3-OH-acyl-CoA dehydrogenase deficiency. *Pediatr Res* 1989;25:199A, Abstract 1776.
113. Tein I, De Vivo DC, Hale DE, et al: Short-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency in muscle: A new cause for recurrent myoglobinuria and encephalopathy. *Ann Neurol* 1991;30:415–419.
114. Glasgow AM, Engel AG, Bier DM, et al: Hypoglycemia, hepatic dysfunction, muscle weakness, cardiomyopathy, free carnitine deficiency and long-chain acylcarnitine excess responsive to medium-chain triglyceride diet. *Pediatr Res* 1983;17:319–326.
115. Poll-The BT, Bonnefont JP, Ogier H, et al: Familial hypoketotic hypoglycemia associated with peripheral neuropathy, pigmentary retinopathy and C6-C14 hydroxydicarboxylic aciduria. A new defect in fatty acid oxidation. *J Inherit Metab Dis* 1986; 11(Suppl 2):183–185.
116. Hale DE, Thorpe C, Braat K, et al: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Res* 1989;25:199A, Abstract 1176.
117. Duran M, Wanders RJA, deJager JP, et al: 3-Hydroxydicarboxylic aciduria due to long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with sudden infant death. *Eur J Pediatr* 1991;150:190–195.
118. Hangenfeldt L, von Doblen U, Holme E, et al: 3-Hydroxydicarboxylic aciduria—a fatty acid oxidation defect with severe prognosis. *J Pediatr* 1990;116:387–392.
119. Rochiccioli F, Wanders RJA, Aubourg P, et al: Deficiency of long-chain acyl-CoA dehydrogenase: A cause of lethal myopathy and cardiomyopathy in early childhood. *Pediatr Res* 1990;28: 657–662.
120. Jackson S, Barlett K, Land J, et al: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Res* 1991;29:406–411.
121. Dionisi Vici C, Burlina AB, Bertini E, et al: Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Pediatr* 1991; 118:744–746.
122. Pryzrembel H, Jakobs C, Ijlst L, et al: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1991;14: 674–680.
123. Ribes A, Riudor E, Navarro C, et al: Fatal outcome in a patient with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1992;15:278–279.
124. Bertini E, Dionisi Vici C, Garavaglia B, et al: Peripheral sensory-motor polyneuropathy, pigmentary retinopathy and fatal cardiomyopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Eur J Pediatr* 1992;151:121–126.
125. Moore R, Glasgow JFT, Bingham MA, et al: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency diagnosis, plasma carnitine fractions and management in a further patient. *Eur J Pediatr* 1993;152:433–436.
126. Sewell AC, Bender SW, Wirth S, et al: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: A severe fatty acid oxidation disorder. *Eur J Pediatr* 1994;153:745–750.

127. Tein I, Donner EJ, Hale DE, Murphy EG: Clinical and neurophysiologic response of myopathy and neuropathy in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency to oral prednisone. *Pediatr Neurol* 1995;12:68–76.

128. Venizelos N, Ijlst L, Wanders RJA, Hangelfeldt L: Beta oxidation enzymes in fibroblasts from patients with 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Res* 1994;36:111–114.

128A. Treem W, Rinaldo P, Hale DE, et al: Acute fatty liver of pregnancy and long-chain 3-hydroxyacyl-Coenzyme A dehydrogenase deficiency. *Hepatology* 1994;19:339–345.

129. Jackson S, Kler RS, Barlett K, et al: Combined enzyme defect of mitochondrial fatty acid oxidation. *J Clin Invest* 1993;90: 1219–1225.

130. Wanders RJA, Ijlst L, Poggi F, et al: Human trifunctional protein deficiency: A new disorder of mitochondrial fatty acid beta-oxidation. *Biochem Biophys Res Commun* 1992;188:1139–1145.

131. Kamijo T, Wanders RJA, Saudubray J, et al: Mitochondrial trifunctional protein deficiency. *J Clin Invest* 1994;93:1740–1747.

132. Ijlst L, Wanders RJA, Ushikubo S, et al: Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Identification of the major disease-causing mutation in the alfa subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta* 1994;1215:347–350.

133. Winter SC, Szabo-Aczel S, Curry CJR, et al: Plasma carnitine deficiency. *Am J Dis Child* 1987;141:660–665.

134. Tein I, DeVivo DC, Ranucci D, DiMauro S: Skin fibroblast carnitine uptake in secondary carnitine deficiency disorders. *J Inherit Metab Dis* 1993;16:135–146.

135. Campos Y, Huertas R, Bautista J, et al: Muscle carnitine deficiency and lipid storage myopathy in patients with mitochondrial myopathy. *Muscle Nerve* 1993;16:778–781.

136. Rinaldo P, Welch RD, Previs SF, et al: Ethylmalonic/adipic aciduria: Effects of oral medium-chain triglycerides, carnitine and glycine on urinary excretion of organic acids, acylcarnitine and acylglycine. *Pediatr Res* 1991;30:216–221.

137. Rinaldo P, Schmidt-Sommerfeld E, Posca AP, et al: Effects of treatment with glycine and L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1993;122: 580–584.

138. Van Hove JLK, Kahler SG, Millington DS, et al: Intravenous L-carnitine and acetyl-L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency and isovaleric acidemia. *Pediatr Res* 1994;35:96–101.

139. Corr PB, Gross RW, Sabel BE: Amphipathic metabolites and membrane dysfunction in ischemic myocardium. *Circ Res* 1988; 55:135–154.

140. Fromenty B, Pessayre D: Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol Ther*, in press.

141. Coulter DL: Carnitine, valproate and toxicity. *J Child Neurol* 1990;6:7–14.

142. Shapira Y, Gutman A: Muscle carnitine deficiency in patients using valproic acid. *J Pediatr* 1991;118:646–649.

143. Millington DS, Bohan TP, Roe CR, et al: Valproyl-carnitine: A novel drug metabolite identified by fast atom bombardment and thermospray liquid chromatography-mass spectrometry. *Clin Chim Acta* 1985;145:69–76.

144. Schmidt-Sommerfeld E, Penn D, Rinaldo P, et al: Urinary medium-chain acyl-carnitines in medium-chain acyl-CoA dehydrogenase deficiency, medium-chain triglyceride feeding and valproic acid therapy: Sensitivity and specificity of the radioisotopic exchange/high performance liquid chromatography method. *Pediatr Res* 1992;31:545–551.

145. Matsuda I, Ohtani Y, Ninomiya N: Renal handling of carnitine in children with hyperammonemia associated with valproate therapy. *J Pediatr* 1986;109:131–134.

146. Tein I, DiMauro S, Xie Z-W, DeVivo DC: Valproic acid impairs carnitine uptake in cultured human skin fibroblasts. An in vitro model for the pathogenesis of valproic acid associated carnitine deficiency. *Pediatr Res* 1993;34:281–287.

147. Chabrol B, Mancini J, Chretien D, et al: Valproate-induced hepatic failure in a case of cytochrome c oxidase deficiency. *Eur J Pediatr* 1994;153:133–135.

148. Melegh B, Pap M, Bock I, Rebouche CJ: Relation of carnitine and carnitine precursors lysine, E-*N*-trimethyllysine, and G-butyrobetaine in drug-induced carnitine depletion. *Pediatr Res* 1993;34:460–464.

149. Bratton SL, Garden AL, Bohan TP, et al: A child with valproic acid associated carnitine deficiency and carnitine responsive cardiac dysfunction. *J Child Neurol* 1992;7:413–416.