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L-Carnitine

GUEST EDITOR
David L. Coulter

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Editorial

Introduction to the Special Issue on Carnitine

David L. Coulter, MD

Carnitine is a relatively simple, ubiquitous molecule that can be considered essential for life because of its critical role in energy metabolism. The essential role of carnitine in metabolism is also reflected in the mechanisms that have evolved to maintain stable carnitine levels. Thus, carnitine is absorbed from the diet, synthesized from dietary precursors, and reabsorbed from the kidney, and compensatory mechanisms exist to adjust the relative contributions of each of these processes to maintain carnitine homeostasis.

The role of carnitine in health and disease has become increasingly apparent, and an extensive biochemical and clinical literature has developed. These studies suggest that an understanding of carnitine is relevant for many areas of clinical medicine, including nutrition, gastroenterology, endocrinology, cardiology, and nephrology. The purpose of this special issue of the *Journal of Child Neurology* is to highlight the relevance of carnitine for child neurology. The peer-reviewed articles included in this special issue discuss aspects of carnitine metabolism and deficiency that child neurologists may encounter in their clinical practice.

Carter, Abney, and Lapp provide the biochemical background necessary to understand the role of carnitine in clinical neurology. They point out that the pathways for carnitine biosynthesis are incompletely developed at birth, so premature and newborn infants are more dependent on dietary sources of carnitine. Borum develops this theme further in her article and notes that neonates on parenteral nutrition are particularly stressed because they cannot make carnitine and are receiving no carnitine in the diet. Carnitine supplementation in these infants seems to promote metabolism and improve growth. Coulter's review of carnitine deficiency in epilepsy also notes that carnitine deficiency is common in preterm infants with seizures, but it is not yet clear that carnitine supplementation will prevent seizures.

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Pons and DeVivo discuss the clinical disorders in which child neurologists may encounter carnitine deficiency. Although many of these are rare, prompt recognition is essential because carnitine treatment can be very effective. They include acquired medical disorders and iatrogenic factors that may induce carnitine deficiency. Perhaps the most common clinical situation in which child neurologists may encounter carnitine deficiency is the treatment of epilepsy. Coulter's article discusses this situation in detail, developing the theme that sufficient data exist to identify risk factors that predict which patients with epilepsy are most likely to have carnitine deficiency. The status of carnitine treatment in epilepsy is also reviewed. Astute readers may note some discrepancy in treatment recommendations between these articles, which reflects alternative clinical responses to incomplete data regarding the effectiveness of carnitine treatment. Clearly, more data are needed to resolve these issues, but these articles provide the currently available data from which readers may draw their own conclusions.

Several other clinical situations arise in which child neurologists may see patients with carnitine deficiency. Child neurologists are increasingly involved in the care of patients with human immunodeficiency virus infection and acquired immune deficiency syndrome (AIDS). Mintz reviews the evidence for carnitine deficiency in these patients. Studies of carnitine treatment in these patients are difficult to do because of the complicated nature of the illness, but evidence is accumulating to support the possible effectiveness of carnitine in AIDS. Winter's review of carnitine in pediatric cardiomyopathy is included in this issue because child neurologists may be asked to evaluate these patients in consultation, and pediatricians' awareness of the role of carnitine in these patients is limited.

Finally, child neurologists should consider several other situations in which carnitine deficiency may occur but for which there are currently few or no data. For example, the data in Coulter's article suggest risk factors to identify carnitine deficiency in patients receiving valproic acid. Because valproic acid is increasingly used in psychiatry to treat children with a variety of disorders including aggression and disorders of mood and affect, the effect of valproic acid on carnitine metabolism may also become apparent in these patients. Valproic acid is

also being used to prevent migraine and could have effects on carnitine metabolism in these patients as well.

The articles included in this special issue should provide the information needed to assist child neurologists in their evaluation and management of these and other patients. The authors hope that these articles will stimulate further research to clarify the role of carnitine deficiency and treatment in pediatrics and neurology.

Note from the Editor-in-Chief

The policy of the *Journal of Child Neurology* is that all editorial materials (including invited articles for supplemental issues) are peer reviewed. For this special supplementary issue on carnitine, the following individuals (in addition to members of the Editorial Board) have graciously assisted in peer review of articles: A. Chadwick Cox, Jeanie B. McMillin, Van S. Miller, and Hugo W. Moser. I also acknowledge the sponsorship of this special issue by Sigma-Tau Pharmaceuticals Inc.

Original Article

Biosynthesis and Metabolism of Carnitine

A. Lee Carter, PhD; Tom O. Abney, PhD; David F. Lapp, PhD

ABSTRACT

This review article presents the biosynthesis, metabolism, sources, levels, and general functions of carnitine. Emphasis is placed on the expression of carnitine deficiency and insufficiency as well as the causes of these conditions. The various functions of carnitine are discussed as they may relate to disease treatment. (*J Child Neurol* 1995;10(Suppl):2S3–2S7).

Carnitine (β-hydroxy-γ-trimethylammonium butyrate) (Figure 1) is found throughout nature including most human tissues.[1] In the meal worm, *Tenebrio molitor*, carnitine is essential for life, hence it has the designation of vitamin B_T.[2] In higher animals, the major sources of carnitine are de novo synthesis and the diet. Normal levels of carnitine in human plasma have been determined for all ages.[3] Plasma carnitine increases during the 1st month of life and remains at a steady-state level for the rest of life.[3] Abnormal levels of tissue and plasma carnitine have been associated with a number of pathologic conditions. The first recognized physiologic function of carnitine was the transport of fatty acyl-coenzyme A (CoA) across the inner mitochondrial membrane for β-oxidation.[4] Recent reports have suggested that carnitine has other functions. These involve two areas: (1) carnitine may act as an acyl sink in order to maintain adequate cellular levels of free CoA,[5,6] and (2) carnitine may interact with membranes to change their physiochemical properties.[7,8]

Dietary carnitine is believed to be actively transported across the intestine in a sodium-dependent manner.[9,10] It is excreted intact by the kidney either as free carnitine or as acylcarnitine.[11] Carnitine is not degraded in humans except by some types of intestinal bacteria.[11]

CARNITINE BIOSYNTHESIS

Carnitine is synthesized from the essential amino acids lysine[12] and methionine,[13] which have been incorporated into a protein. In human tissue proteins, lysine residues are trimethylated by protein-dependent methyltransferases that use *S*-adenosyl methionine as the methyl group donor.[14] Free lysine is not methylated. When the proteins are degraded, the trimethyllysine released cannot be used for the synthesis of new proteins due to absence of a transfer RNA for trimethyllysine. Its levels are therefore sufficient for carnitine biosynthesis.

The biosynthetic pathway (Figure 2) of carnitine from ε-*N*-trimethyllysine involves several enzymes and cofactors. The first enzyme is ε-*N*-trimethyllysine hydroxylase, which hydroxylates ε-*N*-trimethyllysine at the three position.[15] This is the only mitochondrial enzyme in the pathway. It has an activity that is similar in function to proline hydroxylase and requires α-ketoglutarate, ascorbate, and Fe^{++}. The enzyme has proven difficult to isolate and has not been studied in detail.

The second enzyme, β-hydroxy-ε-*N*-trimethyllysine aldolase, catalyzes the cleavage of glycine from β-hydroxy-ε-*N*-trimethyllysine, leaving γ-trimethylaminobutyraldehyde.[16] This enzyme is reported to be similar to serine hydroxymethyltransferase.[15] This enzyme requires pyridoxal phosphate as a cofactor. Although the K_m for trimethyllysine is much higher than that for other substrates, eg, serine and threonine, no other enzyme has been implicated in this reaction.[16]

The next enzyme, γ-trimethylaminobutyraldehyde dehydrogenase, is a cytosolic enzyme that catalyzes the production of γ-butyrobetaine from γ-trimethylaminobutyraldehyde[17] with the transfer of the hydrogen ions to oxidized nicotinamide adenine dinucleotide. The synthesis of butyrobetaine can occur in most cells.[18] Trimethyllysine and butyrobetaine are found in blood and urine.[19]

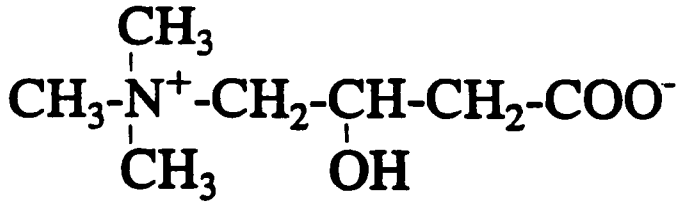

Figure 1. The structural formula of carnitine.

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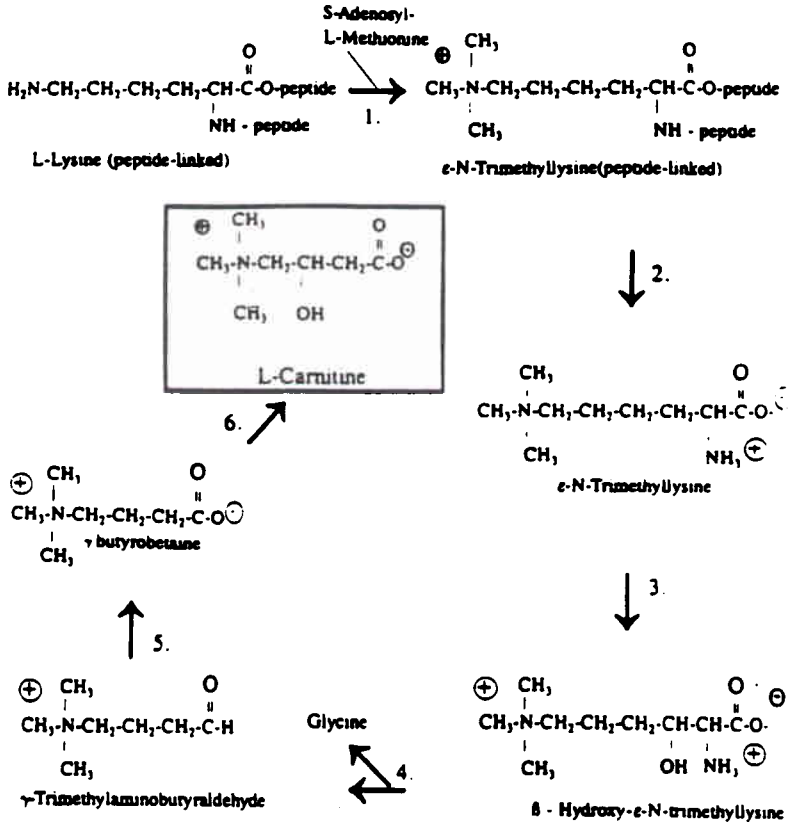

Figure 2. The biosynthesis of carnitine in mammals. Each number refers to an enzymatic activity. 1 = *S*-adenosylmethionine: L-lysine methyltransferase; 2 = protein hydrolysis; 3 = ε-*N*-trimethyllysine hydroxylase; 4 = β-hydroxy-ε-*N*-trimethyllysine aldolase; 5 = γ-trimethylaminobutyraldehyde dehydrogenase; 6 = γ-butyrobetaine hydroxylase.

The last enzyme in the carnitine pathway, γ-butyrobetaine hydroxylase, is similar to ε-*N*-trimethyllysine hydroxylase in that it requires α-ketoglutarate, ascorbate, and Fe^{++}.[20] It catalyzes the conversion of γ-butyrobetaine to carnitine. It is a cytosolic enzyme that is found in only a few tissues. In humans, this enzyme is found in the kidney, liver, perhaps the testis, and possibly the brain. The highest specific activity is found in the kidney.[21] This enzyme is missing in the rat kidney, and so the liver becomes the main site of synthesis. In the rat, it has been shown to be induced by thyroxine.[22] γ-Butyrobetaine hydroxylase is difficult to isolate, due in part to its instability in dilute solutions.

The carnitine biosynthetic pathway also requires ferrous ions and a number of vitamins: ascorbate, niacin, and pyridoxine. The net effect of this pathway is the removal of the amino acid glycine from trimethyllysine for reutilization and the production of one molecule of reduced nicotinamide adenine dinucleotide. The regulation of carnitine biosynthesis is currently not well defined. Therefore, it is essential that additional research be conducted to gain a better understanding of the treatment of patients with carnitine.

DEVELOPMENT OF THE CARNITINE BIOSYNTHETIC PATHWAY

The activity of γ-butyrobetaine hydroxylase in the 1st week of life is about 12% of that found in normal adults and increases linearly to about 30% of the normal adult level during the first 30 months of life.[23] This reduced amount of enzymatic activity is still more than adequate to produce carnitine in an efficient manner in the neonatal system.[23] Under normal conditions, trimethyllysine and butyrobetaine are quickly converted to carnitine, and only small amounts of the carnitine precursors are found in urine. Because newborn infants and premature infants are generally in an anabolic state and not degrading large amounts of protein, the levels of carnitine precursors might be limiting. For these reasons, newborn infants of all lengths of gestation may require an exogenous source of carnitine.[24] Carnitine is found in breast milk, and many soy-based commercial formulas are supplemented with carnitine.[25,26] The requirement for carnitine of premature infants is considered in another article in this supplement.

DIETARY SOURCES OF CARNITINE

Carnitine is found in high concentrations in meat and milk products, the largest amount being in red meat. Carnitine is absent or in low amounts in plants and plant products. Most soy-based commercial infant formulas now have carnitine added.[26] Approximately 15% of the carnitine ingested is absorbed in the intestine.[9] If excessive amounts of carnitine are ingested, diarrhea may result, which can be resolved by discontinuing carnitine therapy.[27]

Table 1. Carnitine Concentrations of Selected Human Tissues

Tissue	*Carnitine Level, nmol/g Wet Weight*	*Source*
Skeletal muscle	1140–3940	Angelini et al[29]
Heart	610–1300	Angelini et al[29]
Kidney	330–600	Angelini et al[29]
Liver	500–1000	Angelini et al[29]
Brain	500–1000	Angelini et al[29]
Plasma	41.4–66.6	Harper et al[30]

CARNITINE LEVELS IN NORMAL INDIVIDUALS

Normative values for total carnitine in plasma have been established for all age groups: approximately 25 μmol/L during infancy and 54 μmol/L in old age.[3] Reported urine values are highly variable.[28] This variability may be due in part to the circadian nature of excretion. The distribution of carnitine in major tissues is shown in Table 1. Muscle carnitine concentrations are greater than those in the heart or liver. This indicates that the muscle may be a site of carnitine storage.

The amount of carnitine in tissues is affected by factors other than dietary availability and synthesis. Free choline taken orally causes an increase in carnitine uptake and a decrease in carnitine excretion.[31] There are differences between the sexes in that females have lower circulating levels of carnitine than males.[3] Juvenile diabetic subjects under good control tend to have an elevated acylcarnitine to free carnitine ratio (carnitine insufficiency) (A.L. Carter and H. Wohltman, personal communication, 1991). Animal studies have indicated that both the sex hormones[32] and the glucagon to insulin ratio[33] have an effect on carnitine levels. Total plasma carnitine levels of less than 20 μmol/L in all age groups are usually considered deficient.

Carnitine in tissues and fluids is present either as free carnitine or as carnitine esters. In plasma, carnitine is present mainly in the form of free carnitine, with small amounts of acylcarnitine (approximately 10% to 15%).[34] Most plasma acylcarnitine is present as acetylcarnitine.[34] In urine, free carnitine generally accounts for 75% or less of the total carnitine. Acylcarnitines are represented by a relatively large amount of acetylcarnitine and small amounts of other acylcarnitines.[34] The relative amounts of acylcarnitine are often expressed as a ratio of acylcarnitine to free carnitine. An acylcarnitine to free carnitine ratio greater than 0.4 is considered abnormal. This state is referred to as carnitine insufficiency,[20] indicating that more carnitine is needed to handle any increased need for the production of acylcarnitines.

CARNITINE AND FATTY ACID OXIDATION

The first role ascribed to carnitine is the ability to shuttle activated long-chain fatty acids into the mitochondria for β-oxidation.[35] This process is now recognized to be under the control of at least three different proteins: carnitine palmitoyltransferase I, acylcarnitine translocase, and carnitine palmitoyltransferase II. Carnitine palmitoyltransferase I catalyzes the transfer of the fatty acid moiety from long-chain fatty acyl-CoA to carnitine.[36] This enzymatic activity is inhibited by malonyl-CoA,[37] the first unique metabolite of cytosolic fatty acid biosynthesis. Malonyl-CoA can be found in tissues that cannot synthesize fatty acids but have the capacity to oxidize fatty acids,[38] such as cardiac muscle. The carnitine palmitoyltransferase I step is the rate-limiting step in the β-oxidation of fatty acids.

The second step in this process is the transfer of the long-chain acylcarnitine from the outside to the inside of the mitochondrial membrane. This transfer is catalyzed by a mitochondrial translocase.[39] This enzyme catalyzes the transfer of one long-chain acylcarnitine molecule into the mitochondria and the export of one molecule of free carnitine or acylcarnitine out of the mitochondria.

The final step is the conversion of long-chain acylcarnitine to long-chain acyl-CoA in the mitochondrial matrix, a reaction catalyzed by carnitine palmitoyltransferase II.[40] The enzyme is located on the matrix side of the inner mitochondrial membrane. Until recently, there has been some disagreement as to whether the polypeptide chains containing the catalytic activity of the two carnitine palmitoyltransferases are the same or different.[41] The controversy centered around whether the malonyl-CoA binding site of carnitine palmitoyltransferase I was located on the same polypeptide chain as the catalytic subunit. Brown and coworkers[42] expressed a complementary DNA for rat liver carnitine palmitoyltransferase I in yeast and established that the catalytic activity and malonyl-CoA sensitivity resides in a single polypeptide. There is also little agreement as to whether the carnitine palmitoyltransferases in various organs are identical or represent different isoforms.[41]

Since the discovery that long-chain fatty acids must be transported into the mitochondria as the carnitine derivative, one question often arises: Is the amount of free carnitine available in the cell a rate-limiting factor for β-oxidation of long-chain fatty acids? For example, the amount of carnitine required for maximum activity of the carnitine palmitoyltransferase is equal to less than 2% of the total amount of free carnitine present in most tissues. Therefore, with a substantial decrease in the total amount of free carnitine in tissues, fatty acid oxidation can still proceed at normal rates.

OTHER FUNCTIONS OF CARNITINE

Besides the carnitine palmitoyltransferases discussed above, there are a number of other acyltransferases that catalyze the transfer of acyl groups of varying lengths; however, they exhibit preferences for specific chain lengths. Table 2 contains a list of acylcarnitine transferases and the K_m for each substrate. Although they all function near equilibrium in vitro, the K_ms suggest that under physiologic conditions, some of these enzymes may catalyze the reaction in only one direction.

Peroxisomes contain a fatty oxidation system that is capable of oxidizing long-chain fatty acids to a length of

Table 2. K_ms of Carnitine Acyl-CoA Transferases

Enzyme	Substrates*				Source
	Carnitine	Acyl-CoA	CoA	Acylcarnitine	
CAT	120	37	37	350	Bremer[43]
COT (peroxisomes, liver)	155	15	780	100	Bremer[43]
CPT II	250–450	10–20	5	40–140	Bremer[43]
CPT I	35	25	—	—	McGarry et al[38]

CoA = coenzyme A; CAT = carnitine acetyltransferase; COT = carnitine octanoyltransferase; CPT = carnitine palmitoyltransferase.
*μmol/L.

approximately six to eight carbons.[44] The peroxisome contains a carnitine acetyltransferase and a carnitine octanoyltransferase[5] that use long-chain fatty acids. These two transferases seem to be involved in the oxidation of fatty acids by this organelle. There is a long-chain carnitine transferase located in the endoplasmic reticulum whose function is still unknown.[45]

Carnitine transferases are thought to function in the regulation of the free CoA/acyl-CoA ratio in tissues and organelles. It has been suggested that in sperm[46] and macrophages[47] acetylcarnitine may be a storage form of energy for the cell. Carnitine has been postulated to function in the removal of poorly metabolized acyl-CoAs to prevent CoA sequestration. It is becoming increasingly clear that a main function of carnitine is the regulation of free CoA in cells and perhaps in different cellular organelles.

Some observations concerning the physiologic effects of carnitine cannot be explained at the present time by the acylation of fatty acyl-CoA derivatives. Examples include a role in limiting the effects of doxorubicin cardiotoxicity,[48] the stabilization of red blood cell membranes,[9] and the enhancement of Ca^{++} transport.[49] These effects seem to occur to the same extent whether D- or L-carnitine is used. This indicates that these effects are caused by the interaction of carnitine with membranes in a physical reaction. The most likely site for these reactions to occur is in association with cardiolipin.[50]

PHARMACEUTICAL USE OF CARNITINE

The most frequently encountered clinical manifestation of abnormal carnitine metabolism involves a total carnitine level below normal. In evaluating the causes of low carnitine levels as discussed in the subsequent articles of this supplement, several different conditions should be considered: (1) Is there an effect of exogenous carnitines on carnitine biosynthesis or does the patient have an adequate capacity to synthesize carnitine? (2) Is there adequate carnitine in the diet and is there sufficient absorption? (3) Is the excretion of carnitine normal? All of these questions should be addressed to determine whether there is a primary defect in the handling of carnitine.

A second type of aberration in carnitine metabolism occurs when there is an abnormally high amount of acylcarnitine relative to free carnitine. This condition, called carnitine insufficiency, can occur in any tissue and may be independent of the plasma carnitine levels. Treatment of individuals with carnitine insufficiency may require administering carnitine irrespective of the total amount of carnitine present to correct the acylcarnitine to free carnitine ratio. This is discussed in detail elsewhere in this supplement.

Finally, a third type of question often asked is: Are there any underlying conditions manifested in the patient that require treatment with carnitine even though carnitine levels in plasma are normal? For example, patients with cardiomyopathy caused by a transport defect in the heart usually present with normal to high carnitine levels.[51] In treating a patient with carnitine, it is important to focus on the clinical state of the patient. Improvement in the expected characteristic of the patient is generally more important than simply achieving a certain level of carnitine or a specific ratio of acylcarnitine to free carnitine in the plasma or tissues. If symptoms improve, carnitine therapy is probably needed as long as the underlying problem that led to the symptoms is still present.

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Original Article

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.[57]

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.[5] 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

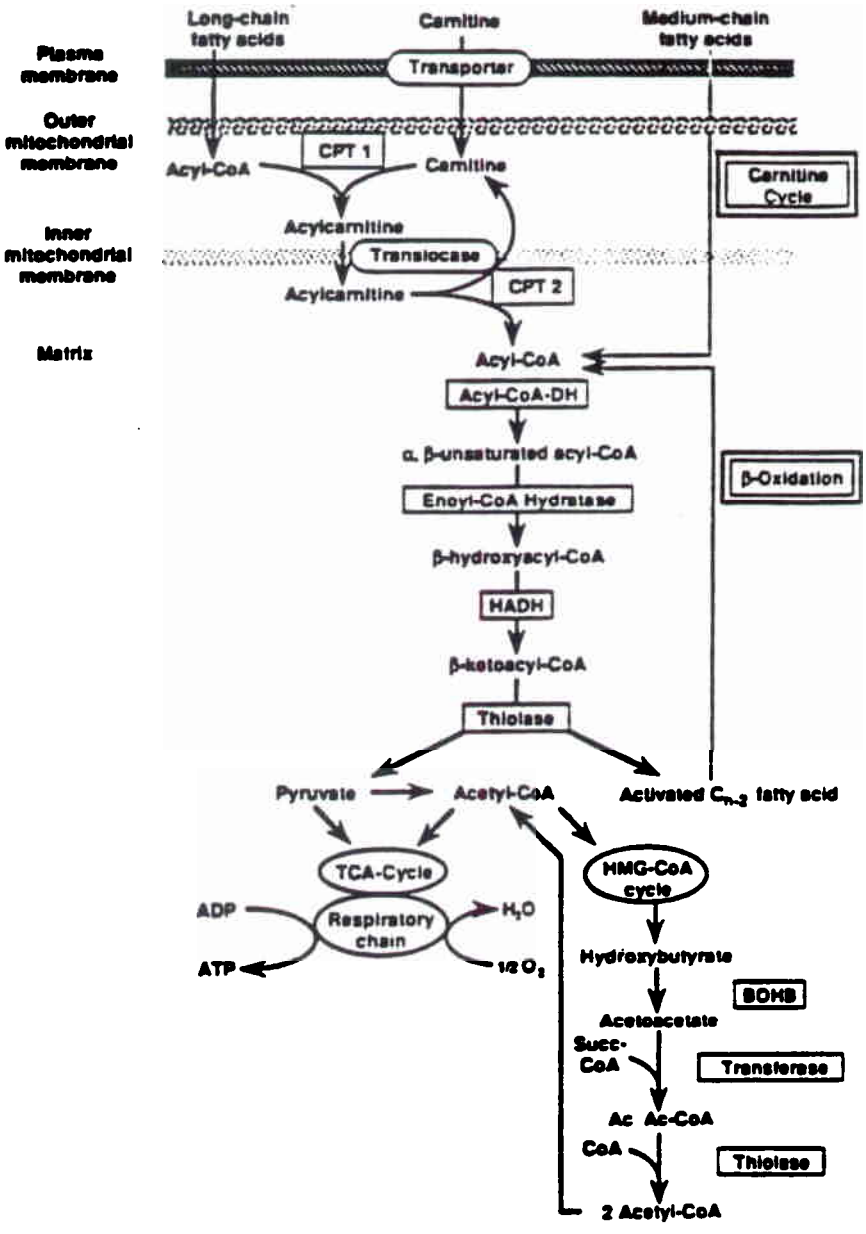

Figure 1. Schematic representation of fatty acid oxidation and ketone body synthesis. Fatty acid oxidation is subdivided into the carnitine cycle and the β-oxidation spiral. The carnitine cycle includes the plasma membrane transporter, carnitine palmitoyltransferase I, carnitine-acylcarnitine translocase 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-

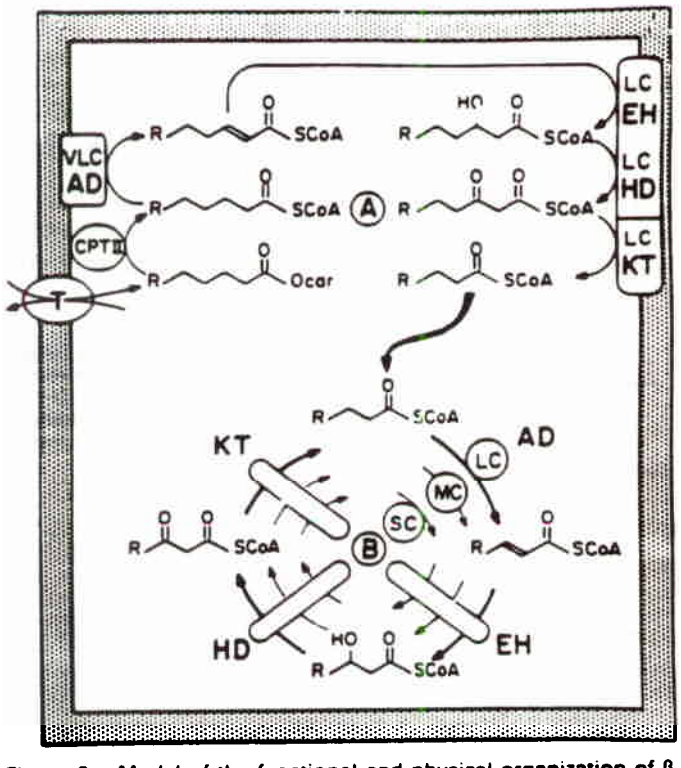

Figure 2. Model of the functional and physical organization of β-oxidation enzymes in mitochondria.[10] Long-chain acylcarnitines enter the mitochondrial matrix by the action of the carnitine palmitoyltransferase II at the inner mitochondrial membrane to yield long-chain acyl-CoAs, which undergo one 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, 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.[46] 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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Original Article

Carnitine in Neonatal Nutrition

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ABSTRACT

Experimental evidence from several investigators suggests that carnitine is a conditionally essential nutrient for neonates. If carnitine is a conditionally essential nutrient for the neonate, most neonates on total parenteral nutrition in the United States are not receiving adequate nutritional support. The metabolic functions of carnitine are varied and important in several aspects of neonatal physiology. All neonates receiving breast milk receive dietary carnitine and most neonates receiving enteral infant formulas receive dietary carnitine at a level similar to that of the breast-fed neonate. However, most neonates on total parenteral nutrition receive no dietary carnitine. Investigators have been testing the working hypothesis that carnitine is a conditionally essential nutrient for the neonate for many years. This review discusses (1) data supporting the hypothesis, (2) reasons why it has not been either proved or disproved by now, and (3) the author's view of a prudent approach to dietary carnitine supplementation of neonates. (*J Child Neurol* 1995;10(Suppl):2S25–2S31).

If carnitine is a conditionally essential nutrient for the neonate, most neonates on total parenteral nutrition in the United States are not receiving adequate nutritional support. Essential nutrients are those nutrients required in the diet of healthy adults because metabolic requirements are greater than the individual's biosynthetic capability. Conditionally essential nutrients are those nutrients that are required in the diet of only certain individuals. A special physiologic condition causes those individuals to have metabolic requirements that are greater than their biosynthetic capability. Immaturity is one of the physiologic conditions frequently associated with conditionally essential nutrients.

For almost 20 years, several investigators have been testing the working hypothesis that dietary carnitine is essential for the neonate.[1] More than 10 years ago, available data supporting the carnitine conditional essentiality hypothesis for neonates resulted in carnitine being added to most enteral infant formulas not containing endogenous carnitine. Today in the United States, all neonates receiving breast milk receive dietary carnitine, and most neonates receiving enteral infant formulas receive dietary carnitine at a level similar to that of the breast-fed neonate. However, most neonates on total parenteral nutrition receive no dietary carnitine. This review addresses the questions: What are the data supporting the carnitine conditional essentiality hypothesis for neonates? Why has the carnitine conditional essentiality hypothesis for neonates not been either proved or disproved by now? What is the prudent approach to dietary carnitine supplementation of neonates?

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METABOLIC FUNCTIONS OF CARNITINE IN THE NEONATE

Carnitine is typical of many metabolites in that the first well-described function of the compound is often assumed to be the only function, and investigators turn their attention to other issues. As a result, additional metabolic roles of the compound may go unrecognized for many years. This scenario describes the last 35 years of research focusing on carnitine.

One extremely important function of carnitine is the transport across membranes of carboxylic acids that have been activated to the coenzyme A (CoA) level (Figure 1). Because all membranes are impermeable to CoA compounds, once a carboxylic acid is activated to CoA, it is trapped in its subcellular location. Conversion of CoA compounds to carnitine compounds makes the carboxylic acid transportable while maintaining the high energy state of the molecule. Thus, the ability of carnitine to confer "transportability" to a high-energy carboxylic acid means that it can facilitate the delivery of a needed substrate, the elimination of a toxin, and the transport of high energy from one subcellular or cellular location to another. The critical role of carnitine in delivering long-chain fatty acid CoA compounds to the mitochondrial matrix and therefore in facilitating β-oxidation of long-chain fatty acids is still often referred to as the function of carnitine. How-

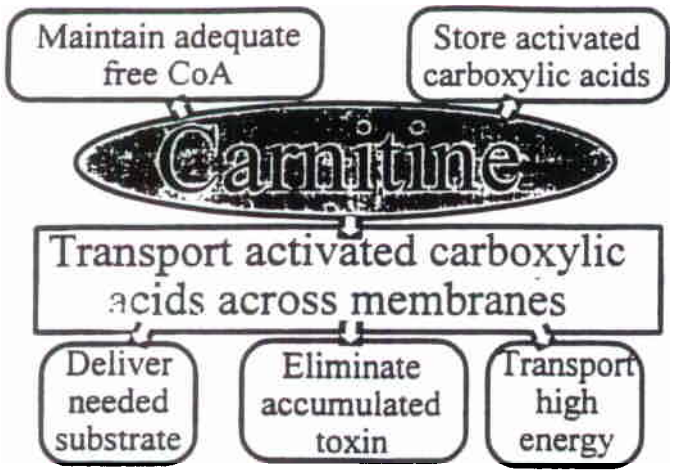

Figure 1. Diagram of five categories of metabolic functions performed by carnitine. Two of the functions (maintaining adequate free CoA and storing activated carboxylic acid) require acylcarnitine transferase activity; transport of the carnitine esters across membranes is not an absolute requirement. The other three categories of metabolic function (delivering needed substrates, eliminating accumulated toxin, and transporting high energy) require transport of the carnitine ester across membranes.

ever, there is a large body of knowledge that identifies additional metabolic functions for carnitine that may have more clinical importance for neonates than the first function described. Because medium-chain fatty acids are activated to the CoA level within the mitochondrial matrix in liver and outside the mitochondrial matrix in other tissues, carnitine is not needed for the oxidation of medium-chain fatty acids in liver but is needed for oxidation of medium-chain fatty acids in skeletal muscle and cardiac muscle. Although it is customary to think of fatty acids as the needed substrates being delivered to the site of further metabolism, several investigators have shown that we are experiencing tunnel vision. The needed substrates may include other energy substrates such as glucose metabolites, acetoacetate, and metabolites of amino acids (especially branched-chain amino acids).[2]

As diagrammed in Figure 1, the "transportability" of carboxylic acids is also important in the removal of toxins from a particular subcellular or cellular location. The toxins may be either compounds that are not normally found in metabolism or normal metabolites that have accumulated to abnormally high and toxic concentrations. The fact that the carnitine ester maintains the high energy of the CoA compound results in the transport of metabolic energy from one subcellular or cellular location to another.[3]

CoA compounds are critical metabolites in a wide variety of pathways, but the total CoA concentration in the cell is low. Because the carnitine concentration of a cell is much higher than the CoA concentration, the conversion of a carboxylic acid at the CoA level to the carnitine ester can replenish a dwindling free CoA pool and permit the continuation of metabolism that is dependent on such a pool. This function of carnitine impacts many different metabolic pathways.

Because the carnitine ester is a high-energy compound, the acylcarnitine pool also functions to store activated carboxylic acids that may have many functions that are only now being recognized, such as facilitating the remodeling of membranes.[4,5] There are many reviews of carnitine metabolism and function,[6–9] including those in this supplement.

CARNITINE STATUS OF PRETERM NEONATES VERSUS TERM NEONATES

Assessment of carnitine status is complicated by the fact that blood and urine are not necessarily indicative of the metabolic pools of carnitine in tissues. Plasma, red blood cells, liver, and skeletal muscle appear to be from different metabolic pools of carnitine in adult humans.[10] In addition, the accretion of carnitine in these compartments appears to differ during gestation. Work with experimental animals[11,12] and autopsy tissues of neonates of varying gestational ages receiving no carnitine and dying within 24 hours of birth[13] have shown that there is a significant accretion of carnitine by muscle tissue during the last trimester of gestation. Postnatally, the skeletal muscle and liver carnitine concentrations continue to increase.[14]

The fetal rat is a major contributor to its own tissue carnitine.[15] In the rat, the rapid accretion of tissue carnitine is so great that at weaning most of the tissue carnitine has been acquired since birth. Approximately 50% of this acquired carnitine comes from milk and approximately 50% from endogenous synthesis in the infant rat.[15] Dietary carnitine also appears to be a major factor in the accretion of carnitine in humans postnatally. Plasma and red blood cell carnitine concentrations of full-term neonates receiving either breast milk or formula containing carnitine increase approximately 1.5- to 2.0-fold during the first 2 weeks of life. They continue to increase until at 3 months of age they are 2.5- to 3.0-fold higher than those in cord blood.[16] In Figure 2, the total plasma carnitine concentration data from cord blood of full-term healthy neonates are set to 1.0 and used to normalize data from all other samples. Preterm neonates have higher plasma and red blood cell carnitine concentrations at birth than do full-term neonates. In contrast to the increase in plasma carnitine seen in full-term neonates postnatally, preterm neonates receiving carnitine-free total parenteral nutrition have plasma and red blood cell carnitine concentrations at 3 weeks of age that are only one third the concentration found in cord blood of full-term neonates. Carnitine supplementation of the total parenteral nutrition at a dose of approximately 50 μmol/kg daily for 1 week and then at 100 μmol/kg daily increased the plasma concentrations at 3 weeks of age to levels approximately 30% higher than the breast-fed full-term neonates.

In Figure 3, the total red blood cell carnitine concentration data from cord blood of full-term healthy neonates are set to 1.0 and used to normalize data from all other samples. The data in Figure 3 show trends similar to those in Figure 2 with one major exception. Whereas carnitine

supplementation of total parenteral nutrition increases the plasma carnitine concentrations of the preterm neonates, the red blood cell carnitine concentrations remain low.

Oral supplementation of infants requiring long-term total parenteral nutrition who were able to tolerate small enteral feedings increased the plasma carnitine, acetoacetate, and β-hydroxybutyrate concentrations compared to the placebo-treated infants.[17]

DIETARY SOURCES OF CARNITINE FOR THE NEONATE

Carnitine biosynthesis requires the essential nutrients lysine, methionine, vitamin B_6, vitamin C, niacin, and iron. A diet deficient in any of these will adversely affect the neonate's ability to make carnitine.

Human milk contains approximately 60 to 70 nmol/mL of carnitine[18,19] and is a very bioavailable source of carnitine. The dietary carnitine intake for the breast-fed neonate is approximately 2 to 5 mg/kg daily. Milk of all species measured contains carnitine. Cow milk contains approximately twice the concentration of human milk, and when it is used to prepare formula, the carnitine concentration of the formula is approximately the same as the concentration of human milk. Infant formulas based on soy protein have no endogenous carnitine.[20] Several investigators have shown that the infants receiving carnitine-free formula have altered carnitine status.[21,22] Therefore, most commercial infant formulas based on soy protein are now supplemented with carnitine at levels similar to that of human milk.

In contrast to enteral nutrition formulas for neonates that contain carnitine, none of the parenteral nutrition solutions contain carnitine. Thus, the most metabolically stressed neonates are the ones who are routinely receiving no endogenous carnitine.

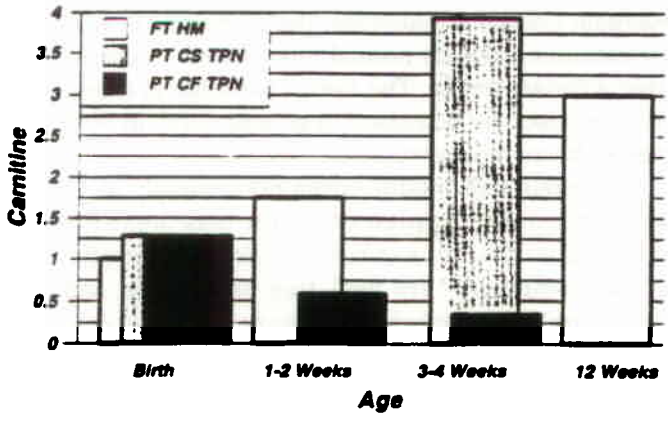

Figure 2. Plasma carnitine concentration data of infants (at different ages and receiving different dietary carnitine intake) normalized to the plasma carnitine data of cord blood from full-term neonates. The units for the original carnitine concentration data are nmol/mL. The full-term neonates received human milk (FT HM), and the preterm neonates received either total parenteral nutrition containing no carnitine (PT CF TPN) or total parenteral nutrition containing carnitine at 50 μmol/mL and then 100 μmol/mL (PT CS TPN). Figure is adapted from data reported by Borum et al.[16]

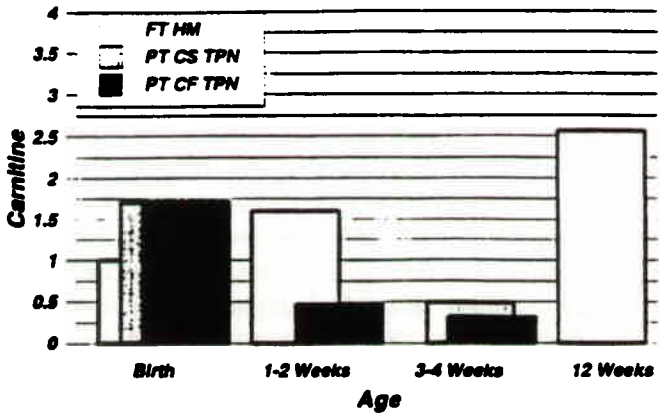

Figure 3. Red blood cell carnitine concentration data of infants (at different ages and receiving different dietary carnitine intake) normalized to the red blood cell carnitine data of cord blood from full-term neonates. The units for the original carnitine concentration data are nmol/mg hemoglobin. The full-term neonates received human milk (FT HM), and the preterm neonates received either total parenteral nutrition containing no carnitine (PT CF TPN) or total parenteral nutrition containing carnitine at 50 μmol/mL and then 100 μmol/mL (PT CS TPN). Figure is adapted from data reported by Borum et al.[16]

SYMPTOMS OF CARNITINE DEFICIENCY IN THE NEONATE

If delivery of the needed substrates (long-chain fatty acids) to the mitochondrial matrix were the only function of, or even the main function of carnitine, evaluation of the carnitine conditional essentiality hypothesis would be relatively straightforward. Neonates who receive inadequate dietary carnitine would have biochemical and clinical symptoms associated with impaired β-oxidation of long-chain fatty acids. Biochemical symptoms such as an altered intravenous fat tolerance test, decreased oxidation of exogenous fatty acids, and decreased production of ketone bodies would be expected. The expected clinical symptoms would be intolerance of intravenous lipid emulsions, failure to thrive, and impaired function of organs such as cardiac muscle and skeletal muscle that are highly dependent on fatty acid oxidation for fuel. Several studies have shown that preterm neonates maintained on carnitine-free total parenteral nutrition always have decreased plasma carnitine concentrations. Although there is some inconsistency, many of the studies show an impaired ability to use long-chain fatty acids infused intravenously as a commercially available lipid emulsion.[23–28]

In the past, it has often been assumed that the need for carnitine in the neonatal diet is directly related to the amount of long-chain fatty acids in the diet. Many formulas designed for preterm neonates contain higher concentrations of medium-chain fatty acids than are found in formula designed for full-term neonates. Unfortunately, some of the formulas containing high concentrations of medium-chain fatty acids have been promoted as containing fat that is oxidized independently of carnitine. The medium-chain fatty acids in formulas that contain small percentages of medium-chain triglycerides are probably

oxidized in the liver where carnitine would not be required. However, when a formula contains 50% or more of the fat as medium-chain triglyceride, tissues such as muscle are expected to use the medium-chain fatty acids as a source of calories, and thus carnitine is required for oxidation.[29] When 13 growing preterm infants (mean birth weight, 1.42 kg) received 46% of their dietary triglyceride as medium-chain fatty acids, they had higher concentrations of urinary octanoate, sebacate, suberate, adipate, 7-hydroxyoctanoate, and 5-hydroxyhexanoate than when they received only 4% of their dietary triglyceride as medium-chain fatty acids.[30]

The interrelationship of carnitine metabolism and medium-chain fatty acid metabolism has also been shown in term infants. Term infants fed formula with predominately medium-chain fatty acids excreted a higher concentration of acylcarnitine than when they were fed a formula with long-chain fatty acids.[31] In addition, when infants were fed medium-chain fatty acid formula, they excreted more medium-chain dicarboxylic acids than when they consumed long-chain fatty acids and more than infants fed the same medium-chain fatty acid formula supplemented with carnitine.[31] In another study, normal male full-term neonates fed soy formula without carnitine from 6 to 9 days to 112 days of life had lower serum carnitine, higher serum free fatty acids, and higher excretion of all their medium-chain fatty dicarboxylic acids than the infants receiving the soy formula supplemented with carnitine.[32]

Carnitine also plays an important role in nitrogen metabolism in neonates. Preterm infants were fed human milk or human milk supplemented with carnitine, 300 nmol/mL, for 7 days. At day 7, approximately 50% of the supplement was being excreted in the urine, indicating that some of the supplement was contributing to the tissue accretion of carnitine. The infants receiving the carnitine-supplemented human milk showed increased plasma carnitine, increased β-hydroxybutyrate, lower plasma concentrations of amino acids alanine and glutamine, decreased plasma urea, and decreased nitrogen excretion. The authors reported a trend of decreased excretion of 3-methylhistidine with carnitine supplementation, suggesting a reduced protein catabolism.[33,34] Consistent with a role of carnitine in nitrogen metabolism, supplementation of total parenteral nutrition with carnitine has been reported to improve nitrogen balance and to improve growth of preterm infants.[24] Data from a recent report are consistent with earlier reports that carnitine supplementation of total parenteral nutrition of preterm neonates increases plasma carnitine concentrations and increases tolerance to intravenous fat emulsions, with enhanced ketogenesis.[35] Other investigators have found lower plasma free carnitine associated with higher concentrations of blood ammonium in low-birth-weight infants. They suggest that carnitine status may regulate blood ammonium levels.[36]

In addition to symptoms that are consistent with altered fatty acid metabolism or altered nitrogen metabolism, many case studies have reported carnitine deficiency associated with a wide variety of symptoms in neonates. For example, one group of investigators have suggested that carnitine deficiency is a possible cause of gastrointestinal dysmotility. They studied one infant with a diet containing low concentrations of carnitine until 3 years of age who had gastrointestinal dysmotility manifested by postprandial vomiting, oral drooling, delayed gastric emptying, and infrequent bowel movements. At 3 years of age, the patient had low serum carnitine concentrations, and a muscle biopsy showed deposition of lipid between myofibrils and unusually shaped mitochondria. He was changed to a meat-based diet high in carnitine and showed a dramatic clinical recovery, with disappearance of chronic drooling, improved gastric motility, and improved muscle strength.[37]

CARNITINE REQUIREMENTS OF NEONATES WITH METABOLIC DISEASE

Children with a variety of metabolic diseases have been shown to have altered plasma carnitine concentrations. Some of the metabolic errors involve the acylcarnitine transferases or the translocase.[38,39] During the past 20 years, many patients have been described with muscle carnitine deficiency and a lipid storage myopathy.[40,41] Recently, numerous patients have been identified with medium-chain acyl-CoA dehydrogenase deficiency who have a secondary carnitine deficiency.[42–44] Several types of organic acidemia such as propionic acidemia[45] are often accompanied by a secondary carnitine deficiency. In addition, some medications such as valproic acid cause a secondary carnitine deficiency in some patients.[46,47] Many of the secondary carnitine deficiencies appear to be the result of excessive excretion of carnitine as the ester of the accumulating metabolite. Although the detoxification role of carnitine is useful in the effort to maintain normal metabolism, the increased requirement for carnitine exceeds normal biosynthetic capability and typical dietary intake. The role of carnitine in these metabolic diseases are discussed in greater detail in other articles in this supplement.

Children with inborn errors not typically associated with carnitine supplementation may benefit from increased intake. For example, children with cystic fibrosis have abnormally low total, free, short-chain, and long-chain carnitine in plasma.[48] Dietary carnitine increased the total and free carnitine concentrations to that of the reference population, but short-chain and long-chain carnitine concentrations remained low.[48]

Some neonates being evaluated for incompletely defined syndromes such as sudden infant death syndrome may benefit from carnitine supplementation. It has been recommended that all neonates who are determined to be at risk for sudden infant death syndrome have blood carnitine analysis included in the work-up.[49]

DIETARY SUPPLEMENTATION VERSUS PHARMACOLOGIC TREATMENT

Carnitine is both a nutrient and a drug. The breast-fed neonate appears to benefit greatly from dietary carnitine but receives less than 5 mg/kg daily. In contrast, children with primary and secondary carnitine deficiency are routinely treated with daily doses of 100 mg/kg, and some children receive even higher doses. There is a growing body of evidence that patients such as those with renal disease being treated with hemodialysis benefit from carnitine supplementation at low dosages but may lose that benefit or even experience adverse effects from higher doses. Adverse effects are extremely rare in the many investigations evaluating carnitine supplementation even at oral doses of 100 mg/kg daily.

There is one report of intravenous supplementation at 10 to 30 times the usual oral carnitine intake that resulted in impaired growth. Low-birth-weight infants were given total parenteral nutrition supplemented with 48 mg/kg of carnitine daily for days 4 through 7 of life. Free and total plasma carnitine concentrations increased approximately 10-fold. The supplemented group took 9 days to regain birth weight, whereas the unsupplemented group took 7 days. Fat oxidation was increased in the supplemented group, but protein oxidation as measured by nitrogen excretion in the urine was increased in the supplemented group. The conclusion of this investigation was that the infants should not be supplemented intravenously with such high doses.[50]

PRUDENT APPROACH TO DIETARY INTAKE OF CARNITINE IN NEONATES

A nutrient can be designated essential after it is demonstrated that decreased intake causes decreased body stores accompanied by pathophysiology and that both the decreased stores and the pathophysiology can be reversed or prevented with adequate intake of the nutrient. It is clear that lack of carnitine intake by the neonate alters circulating carnitine and tissue carnitine concentrations. Data concerning pathophysiology accompanying the lack of carnitine intake that can be consistently prevented or reversed with carnitine intake are much more difficult to obtain in a consistent manner. The neonates who are receiving a carnitine-free diet in the United States are usually very immature and very sick, with a broad spectrum of pathophysiologies of multifactorial origin. For both technical and ethical reasons, it has been very difficult to design studies that would tease out the symptoms due to carnitine deficiency. As investigators, we continue to perform studies showing that when compared to preterm neonates receiving exogenous carnitine, preterm neonates not receiving exogenous carnitine (1) have lower blood total and free carnitine; (2) may have reduced tolerance of dietary fat, with reduced blood concentrations of ketone bodies; (3) may have reduced tolerance of dietary protein, with increased blood ammonia concentrations; and (4) may have slightly reduced growth rate. Today we have the analytic chemistry techniques and the neonatal animal models that will allow us to address the issues listed in Table 1 and therefore bring us closer to either proving or disproving the essentiality of exogenous carnitine for the neonate.

Table 1. Questions Concerning Carnitine Supplementation of Preterm Neonates That Need to Be Addressed in the Human Neonate or in the Neonatal Animal Model

1. How does carnitine supplementation affect the profile of individual acylcarnitines (from C2 through C24) in different tissues, in different components of circulating blood, and in excreted carnitine?
2. What are the pharmacokinetics and tissue metabolic compartmentation of different levels of carnitine supplementation?
3. Which readily accessible carnitine parameters are the best assessment indicators of carnitine status?
4. Does carnitine supplementation of preterm neonates alter any or all of the following?
   - Oxidation of long-chain and medium-chain fatty acids in specific tissues
   - Glucose production and utilization in specific tissues
   - Protein synthesis and degradation in specific tissues
   - Excretion of exogenous toxic acyl-CoA compounds and endogenous metabolites that accumulate to toxic concentrations
   - Brain growth and myelination
   - Surfactant synthesis
   - Anemia of prematurity
   - Antioxidant defenses
   - Prostaglandin synthesis and degradation
   - Cardiac function
   - Skeletal muscle function
   - Renal function
   - Hepatic function
   - Immunologic defenses

When caring for neonates, one cannot delay feeding the neonates until the carnitine conditional essentiality hypothesis is either proved or disproved. At this point in the mid 1990s, there are several reports of benefit and no reports of adverse effects when neonates receive carnitine supplementation at approximately 2 to 10 mg/kg daily. The quality of the carnitine being used for supplementation is very important. Many of the non-pharmaceutical-grade products are of very poor quality and should never be administered to neonates.[51]

Reports concerning the role of carnitine in metabolism and its usefulness in patient care are appearing in the literature with great rapidity. It is the opinion of this reviewer that in the future, with improved techniques for assessing carnitine status of the individual neonate and with improved techniques for tracking the metabolism of administered carnitine, carnitine supplementation will play an even more important role in care of neonates than it does today.

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Original Article

Carnitine Deficiency in Epilepsy: Risk Factors and Treatment

David L. Coulter, MD

ABSTRACT

Numerous studies have shown that plasma carnitine levels are significantly lower in patients taking valproate than in controls. Free carnitine deficiency is not uncommon in these patients and also occurs in newborns with seizures and in patients taking other anticonvulsant drugs. Carnitine deficiency in epilepsy results from a variety of etiologic factors including underlying metabolic diseases, nutritional inadequacy, and specific drug effects. The relationship between carnitine deficiency and valproate-induced hepatotoxicity is unclear. Carnitine treatment does not always prevent the emergence of serious hepatotoxicity, but it does alleviate valproate-induced hyperammonemia. These studies suggest that specific risk factors for carnitine deficiency can be identified. Preliminary data suggest that carnitine treatment may benefit high-risk, symptomatic patients and those with free carnitine deficiency. Carnitine treatment is not likely to benefit low-risk, asymptomatic patients and those with normal carnitine levels. (*J Child Neurol* 1995;10(Suppl):2S32–2S39).

Controversy persists about the role of carnitine in epilepsy, despite research on the subject that accelerated from the initial reports in 1982 to the present time and now includes many published studies. Clinicians still need answers to the questions of when to measure carnitine levels and when to provide treatment with carnitine. The purpose of this review is to summarize available information as it relates to these clinical questions. In particular, this review will seek to identify risk factors that might help clinicians to anticipate carnitine deficiency and to predict the response to treatment.

CARNITINE LEVELS

Table 1 summarizes data from some of the articles that investigated the occurrence of carnitine deficiency in patients with epilepsy. In general, mean plasma levels of total carnitine and free carnitine were lower in patients taking valproate than in controls. These differences were statistically significant in most studies[2–12,14–17] but not in a few others.[13,18] Two studies that examined carnitine levels longitudinally in patients before and after starting valproate showed that levels were significantly lower after taking valproate.[10,12] When examined separately, carnitine levels were lower in patients taking valproate plus other anticonvulsant drugs than in patients taking valproate alone.[3,6–8] In several studies, carnitine levels in patients taking valproate alone did not differ significantly from those of controls.[7,13,14] Most studies showing significantly lower carnitine levels in valproate-treated patients have investigated children. One study showed that carnitine levels in valproate-treated younger children (1 to 10 years old) were significantly lower compared to older children (10 to 18 years old).[19] Free carnitine deficiency was found in 13 of 18 newborn infants with seizures.[20] Significantly lower carnitine levels have also been reported in valproate-treated adults.[6,7]

Significantly lower carnitine levels have been reported in patients not taking valproate who were taking other anticonvulsant drugs, such as carbamazepine, phenytoin, or phenobarbital.[6,8] Other studies have found no difference, however.[7,13,14]

Several questions arise regarding the significance of these data. One is the validity of plasma carnitine levels as a measure of total body carnitine status. In some valproate-treated patients with normal plasma carnitine levels, muscle carnitine levels were significantly lower than in controls not treated with valproate.[21] These data (though unconfirmed) are not surprising, because 90% of total body carnitine is in muscle tissue, and the concentration of carnitine in muscle is as much as 10 times higher than in blood.[22] If blood carnitine levels fall late in the course of total body carnitine depletion, then it would make sense that muscle carnitine levels might be low while blood levels were still normal. Later in the course,

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Table 1. Carnitine Levels With Valproate Therapy*

Source	Subjects Taking VPA: Description	n	TC	FC	AC	Controls: Description	n	TC	FC	AC
Ohtani et al[2]	Age 3–21 yr	14	32.6†‡	28.6‡	—	Epilepsy, other drugs	11	48.6	43.0	—
						Healthy	27	49.0	44.2	—
Laub et al[3]	VPA alone; age 3–21 yr	14	46.4	34.7	11.7	Epilepsy, other drugs	21	47.0	39.9	7.0
	VPA plus other drugs	7	36.4	28.9	7.5	Healthy	21	49.5	41.2	8.0
Morita et al[4]	Age 1–30 yr	12	33.4‡	21.5‡	12.0	*Epilepsy, other drugs*	13	43.6	31.5	12.0
						Healthy	32	60.3	51.7	9.7
Melegh et al[5]	Children	11	24.3‡	16.8‡	—	*Healthy*	11	34.9	26.5	—
Rodriguez-Segade et al[6]	Age 17–65 yr	34	36.9	26.4	10.5	Epilepsy, other drugs	149	48.1	41.2	6.9
						Healthy	49	53.3	47.1	6.2
Beghi et al[7]	VPA alone; age 1–39 yr	54	49.4	36.2	13.1	Epilepsy, other drugs	51	46.9	37.0	10.1
	VPA plus other drugs	55	44.2	33.0	11.2	Epilepsy, no drugs	53	50.8	41.4	9.2
Hug et al[8]	VPA alone	53	35.6‡	27.0‡	8.6‡	Phenobarbital	119	32.7‡	24.6‡	8.1‡
	VPA plus other drugs	18	30.1‡	23.2‡	6.9‡	*Healthy*	32	57.8	42.5	15.3
Opala et al[9]	VPA alone	43	40.8	29.9‡	10.9	Epilepsy, other drugs	43	48.1	36.7	8.9
	VPA plus other drugs	91	29.3‡	21.4‡	8.0	*Healthy*	89	44.2	36.8	8.9
Riva et al[10]	Taking VPA for 45 days	22	50.0‡	35.0‡	15.0‡	*Same patients before VPA*	22	60.0	49.0	11.0
Toksoy et al[11]	Children	24	—	33.5‡	—	*Healthy age/sex matched*	24	—	50.8	—
Zelnik et al[12]	Taking VPA	14	—	29.1‡	—	*Same patients before VPA*	14	—	37.6	—
Murphy et al[13]	Under age 10 yr	13	39.6	—	—	Not stated	—	50.0	—	—

VPA = valproate; TC = total carnitine; FC = free carnitine; AC = acylcarnitine.
*Adapted from Coulter.[1]
† All carnitine values are mean levels in μmol/L.
‡ Value is significantly different ($P < .05$) from respective value of the control group shown in italics in the same study.

blood levels might also fall. This argument predicts that when blood carnitine levels are low, muscle carnitine levels should also be low. Thus, total body carnitine depletion would be expected when blood levels are significantly low and might also occur even when blood levels are normal.[21] This argument is fairly speculative and needs to be confirmed.

Another question arises regarding the relevance of data showing significant differences in group means between valproate-treated patients and controls, as shown in Table 1. How many patients actually had plasma carnitine deficiency, defined as a free carnitine level more than two standard deviations below the mean for the controls? Using this definition, plasma carnitine deficiency was found in 4% to 76% of patients taking valproate[2,6–8,13,16] and in 8% to 36% of patients taking other anticonvulsant drugs.[6,8] The reasons for this wide variation in prevalence of plasma carnitine deficiency are unclear but presumably reflect the composition of the study group. Plasma carnitine deficiency appears to be more common in young patients with multiple disabilities[2,5,16] than in relatively healthy adults.[7] Studies that specifically examined the correlation of plasma carnitine deficiency with clinical symptoms found no relationship.[2,7,13,16]

In summary, plasma carnitine levels are decreased in many patients with epilepsy. They are lowest in patients taking valproate plus other anticonvulsant drugs but may also be decreased in patients taking valproate alone and in patients not taking valproate but taking other anticonvulsants, such as phenobarbital, phenytoin, or carbamazepine. Actual carnitine deficiency (free carnitine level more than two standard deviations below the mean) is also fairly common. Low carnitine levels in the blood probably reflect low levels in muscle tissue, but plasma levels may be normal even when tissue levels are low. The published studies suggest that risk factors for carnitine deficiency include young age, multiple disabilities, and presence of valproate plus other anticonvulsant drugs (valproate polypharmacy).

ETIOLOGY

The etiology of carnitine deficiency in patients with epilepsy may be related to nutritional factors, inborn errors of metabolism, or the effects of drugs and other diseases. In some patients, it may reflect the combined effect of several factors.

Carnitine deficiency has been reported in patients with seizures who have a variety of underlying metabolic disorders. These include defects in fatty acid metabolism,[24–27] mitochondrial disorders such as mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) or Leigh disease,[28–32] glutaric aciduria type 2[32] and other organic acidurias,[33] carnitine palmitoyltransferase deficiency,[34,35] and urea cycle disorders. Indeed, a patient with one of these inborn errors of metabolism may decompensate or become comatose when given a drug such as valproate that alters carnitine metabolism.[23,24,36]

Dietary carnitine is found in highest concentration in products derived from red meat and milk, so patients whose diets are deficient in these products may be at risk for nutritional carnitine deficiency. Most intravenous hyperalimentation solutions do not contain carnitine, so patients on total parenteral nutrition for long periods of time are also at risk for nutritional carnitine deficiency. Indeed, carnitine levels were lowest in premature infants with seizures who were on intravenous hyperalimentation.[20] Products used in tube feedings may or may not include carnitine, so clinicians need to check the label of whatever product is being used in order to prevent nutri-

tional carnitine deficiency in patients who rely on these products for most or all of their nutrition. Institutionalized and severely handicapped patients with multiple disabilities, whose dietary intake of carnitine may be deficient for any or all of these reasons, may be particularly likely to have carnitine deficiency. One study found that serum carnitine levels in these patients were significantly correlated with arm circumference, a measure of lean body mass reflecting nutritional status.[4] The etiology of carnitine deficiency in these patients may be multifactorial, including nutritional factors, underlying inborn metabolic errors causing multiple disabilities, and anticonvulsant drug therapy including valproate.

Valproate is a fatty acid and so would be expected to have effects on fatty acid metabolism. The weight gain sometimes associated with valproate therapy has been attributed to inhibition of fatty acid metabolism.[37] In developing mice, administration of valproate interfered with fatty acid oxidation and decreased levels of free coenzyme A in the liver,[38] an effect that was largely prevented by coadministration of carnitine and pantothenic acid.[39,40] Evidence of impaired fatty acid metabolism was also found in humans treated with valproate.[41,42] These metabolic effects were reversed by carnitine treatment in one study[42] but not in another.[41]

As a fatty acid, valproate combines with carnitine to form a valproylcarnitine ester.[43] This and other acylcarnitines are excreted in the urine. Total urinary excretion of acylcarnitines was increased in valproate-treated patients with elevated ammonia levels.[44,45] Acylcarnitine excretion was also increased in relatively asymptomatic patients in one study[10] but not in other studies.[5,46] This increased urinary excretion of acylcarnitine might also interfere with renal reabsorption of free carnitine.[44] In mice, valproate increased urinary excretion of acylcarnitine, whereas other anticonvulsant drugs (phenobarbital, phenytoin, and carbamazepine) increased urinary excretion of free carnitine.[47] These results suggest that the etiology of carnitine deficiency in valproate-treated patients might be related in part to increased renal excretion of carnitine. A similar mechanism might also account for the cases of carnitine deficiency observed in patients treated with other anticonvulsant drugs.

A previous review noted the absence of studies investigating the effects of valproate on carnitine absorption, transport, biosynthesis, and tissue uptake.[1] Tein et al subsequently studied the effect of valproate on carnitine uptake in cultured human skin fibroblasts. They found that valproate induced a reversible, dose-dependent reduction of carnitine uptake by as much as 50%[48,49] and noted that this effect might also account for the observation by others of reduced renal reabsorption of free carnitine. This valproate-induced impairment of tissue carnitine uptake could be an important mechanism causing carnitine deficiency in patients treated with valproate.

In summary, these published studies regarding the etiology of carnitine deficiency suggest that additional risk factors include nutritional inadequacy, reliance on tube feedings or intravenous hyperalimentation, and the presence of an underlying inborn error of metabolism.

VALPROATE-RELATED HEPATOTOXICITY

Several clinical patterns of hepatotoxicity exist in patients treated with valproate[50] and have been associated with carnitine deficiency. Transient elevation of transaminase levels often occurs when patients are first started on valproate. This is usually asymptomatic and typically reverses whether the dose is decreased or not.[51-53] Although this phenomenon has not been associated directly with carnitine deficiency, a possible relationship was suggested by in vitro studies of isolated rat hepatocytes. Valproate exposure induced leakage of transaminases into the culture medium, an effect that could be prevented by cotreatment with carnitine.[54]

A much more serious pattern of hepatic failure occurs rarely but may be irreversible and fatal. More than 100 patients have died from valproate-induced hepatic failure.[55] The risk varies from one in 500 for very young children to one in 120,000 for adults on monotherapy.[53,56] Although the principal risk factors are young age, presence of concomitant neurologic disabilities, therapy with multiple anticonvulsant drugs, and the recent addition (in the past 6 months) of valproate therapy,[53] these risk factors may be absent in as many as one third of fatal cases.[55] Symptoms and signs include anorexia, nausea, vomiting, lethargy, edema, fever, coma, and seizures.[50] Magnetic resonance imaging may show diffuse high signal intensity on T_2-weighted images.[57]

The role of carnitine deficiency in valproate-induced severe hepatotoxicity is unclear. Carnitine deficiency was found in several cases of valproate-induced hepatotoxicity[19] and in some cases of a Reye syndrome–like illness associated with valproate therapy[13,44,58] but not in another case.[3] Carnitine levels may be normal before the onset of hepatic failure, and pretreatment with carnitine may not prevent the onset of symptoms.[59,60] When symptoms developed, treatment with up to 100 mg/kg daily of L-carnitine in several cases did not reverse the symptoms or prevent death.[3,60,61] No controlled clinical trials have been reported of carnitine pretreatment to prevent valproate-associated hepatotoxicity or of acute high-dose carnitine therapy for patients with symptoms of valproate-induced hepatic failure, however. Thus, the absence of reported cases in which carnitine therapy was successful does not necessarily mean this therapy is useless, and further studies are needed. Until controlled studies demonstrate that a defined protocol for carnitine treatment does in fact protect against valproate-induced hepatotoxicity, clinicians should not rely on carnitine treatment to prevent this complication.

Anecdotal evidence suggests that unsuspected underlying inborn errors of metabolism may be a significant risk factor for valproate-induced coma or hepatic failure. Disorders of fatty acid oxidation[23,24] and urea cycle disorders[62] have been reported in patients who survived these

complications of valproate therapy. The author is aware of several unreported sibships in which one sibling died of valproate-induced hepatic failure before another sibling was found to have an inborn error of metabolism. These metabolic disorders may be associated with carnitine deficiency or insufficiency.[63] Preexisting metabolic disease may not account for all cases of valproate-induced hepatic failure, but it appears prudent to avoid giving valproate to patients who may have an inborn metabolic error. This means that in patients whose etiology of seizures is unknown, screening for metabolic disease should precede therapy with valproate.[64] This screening includes measurement of urinary organic acids, blood gases, and blood levels of lactate, pyruvate, carnitine, and ammonia.

Not all cases of valproate-associated hepatotoxicity are fatal, but no consistent differences have been found between those who survive and those who do not.[50] A protocol that included valproate monotherapy and carnitine supplementation was effective in preventing recurrence of hepatotoxicity and controlling seizures in several patients who had survived a previous hepatotoxic episode while on valproate polypharmacy and who had no evidence of underlying metabolic disease.[65] These patients were treated before the newer anticonvulsants such as felbamate or lamotrigine became available, however. This protocol must be considered high risk and warranted only when no reasonable alternatives exist for patients with serious refractory seizures.

The author is not aware of any studies that have examined carnitine levels in patients with other adverse effects of valproate such as pancreatitis or thrombocytopenia. Also, apparently no studies have examined carnitine levels in patients with adverse effects of other anticonvulsant drugs.

VALPROATE-RELATED HYPERAMMONEMIA

Hyperammonemia is a common occurrence in valproate-treated patients who do not have any evidence of hepatic failure. The initial reports emphasized the correlation of hyperammonemia with symptoms of lethargy and hypotonia.[66,67] Similar symptoms were noted in several subsequent reports of valproate-related hyperammonemia[68–71] but not in other studies.[2,7,15,72,73] Ammonia levels were not reported in two patients who developed asterixis on valproate.[74] Although hyperammonemia without hepatic failure is almost always reversible, one unreported case with a fatal outcome is known to the author. As a result of these studies, the clinical significance of hyperammonemia is controversial, and some authors consider it to be of little significance.[50,73] Hyperammonemia is surely not good, but it is unclear whether it is bad or always warrants a change in therapy. For example, the author does not change therapy if the patient is asymptomatic and the ammonia level is less than twice the normal level.

The mechanism of valproate-related hyperammonemia appears to be related to inhibition of carbamoyl phosphate synthesis and urea formation,[72,75] as well as to increased renal ammonia production.[76,77] It appears to be more common in patients on multiple anticonvulsant drugs including valproate than in patients on valproate alone.[1,78] Hyperammonemia was correlated with carnitine deficiency in some studies[3,14,15] but not in others.[3,7,15,17,46] Animal studies showed that carnitine treatment prevented or reversed valproate-related hyperammonemia.[79–84] Carnitine treatment also reversed hyperammonemia in several human studies.[2,15]

These data suggest that a reasonable clinical approach is to measure ammonia levels in patients taking valproate who develop symptoms of altered consciousness, hypotonia, asterixis, or ataxia. If the ammonia level is increased and alternative anticonvulsant therapy is available, valproate should probably be discontinued. If the ammonia level is increased and continued valproate therapy is indicated, withdrawal of other anticonvulsant drugs (valproate monotherapy) could be attempted first. If hyperammonemia persists, treatment with carnitine could then be provided.

CARNITINE TREATMENT

Carnitine treatment does not appear to be indicated for every patient taking valproate, so a risk-screening strategy seems warranted to identify patients most likely to benefit from treatment with carnitine.[85] Table 2 identifies risk factors that might be expected to predict carnitine deficiency, based on the data reviewed above. Young children (less than 10 years old) appear to have a greater risk than older children and adults. Patients with mental retardation who have multiple neurologic disabilities, such as cerebral palsy, microcephaly, or blindness, and who are nonambulatory are more likely to have carnitine deficiency than are patients with mental retardation who are robust and healthy. Patients who are malnourished or underweight or whose diets are low in carnitine-containing products, including those on intravenous hyperalimentation or on tube feedings not supplemented with carnitine, are at risk for nutritional carnitine deficiency. Numerous studies have shown that carnitine levels are lowest in patients taking multiple anticonvulsant drugs including valproate, so these patients are at risk for carnitine deficiency. The risk appears to be less in those on valproate monotherapy or on other anticonvulsant drugs,

Table 2. Risk Factors for Carnitine Deficiency

Young age (less than 10 years old)
Multiple neurologic disabilities (mental retardation, cerebral palsy, blindness, microcephaly)
Nonambulatory status
Underweight (decreased weight for height)
Diet low in meat and dairy products
On tube feeding or intravenous hyperalimentation
Taking multiple anticonvulsant drugs including valproate
Hyperammonemia
Hypoglycemia
Metabolic acidosis

but carnitine deficiency can occur in these patients as well. Hyperammonemia was correlated with carnitine deficiency in several studies. Hyperammonemia, hypoglycemia, and metabolic acidosis are risk factors for carnitine deficiency because they may indicate the presence of an underlying inborn error of metabolism.

When the risk factors shown in Table 2 were assessed in 38 children with epilepsy who had plasma carnitine levels measured, there was a significant inverse correlation between the number of risk factors present and the carnitine level. Carnitine levels were lowest in those with the greatest number of risk factors present. All children with less than two risk factors had normal carnitine levels, whereas all children with five or more risk factors had carnitine deficiency.[86]

Table 3 shows the effects of carnitine treatment on symptoms and signs in children with two or more of the risk factors shown in Table 2.[86] Clinical improvement was noted in more than 50% of children with symptoms of apathy, lethargy, listlessness, anorexia, constipation, nausea, and vomiting. Improvement was also noted in some children with symptoms of weakness and hypotonia, and some children had fewer seizures. Carnitine levels were not measured in all children before treatment, so correlation of the clinical response with preexisting carnitine status was not possible. This was an unblinded, uncontrolled study using varying doses of carnitine, and the response was measured clinically, so the results should be interpreted with caution. They do provide some preliminary support for further controlled trials of carnitine therapy in patients at risk for carnitine deficiency. The risk factors shown in Table 2 could be used to identify appropriate candidates for such trials.

Kelley noted the paucity of good scientific data on the effects of carnitine therapy in patients with epilepsy and called for more carefully controlled clinical trials to examine the benefits of this therapy.[87] Only one such study has been reported so far. This study examined the effects of carnitine therapy in 47 children with epilepsy. Seventeen were taking valproate alone, five were taking valproate plus other anticonvulsant drugs, and 15 were taking carbamazepine alone. All had normal carnitine levels, all were asymptomatic, and their baseline well-being was rated by their parents as above average on a scale devised by the investigators. Using a double-blind, placebo-controlled crossover design, the investigators showed no significant improvement in well-being when the children were taking carnitine.[88] These results underscore the importance of a risk factor approach to identifying patients for carnitine treatment; the subjects in this trial were asymptomatic and would not be considered at risk for carnitine deficiency or expected to benefit from carnitine therapy. Although the number of subjects in this study was small, the results provide no support for carnitine treatment of asymptomatic children taking valproate. The results also suggest that carnitine treatment is not likely to benefit patients with normal carnitine levels or patients with minimal risk of carnitine deficiency. Given the design of the study, however, these results should not be misinterpreted to predict that carnitine treatment would be equally ineffective in symptomatic patients and in patients who are at risk for carnitine deficiency. More controlled clinical trials of carnitine treatment are needed that explicitly target these substantially different populations.

Table 3. Results of Carnitine Therapy in 20 Children With Epilepsy Who Had Two or More Risk Factors for Carnitine Deficiency

Symptom or Sign Examined	*Present Before Treatment, no.*	*Improved After Treatment, no. (%)**
Muscle weakness	13	2 (15)
Muscle pain or tenderness	0	— (—)
Hypotonia	7	2 (29)
Incoordination	12	2 (17)
Frequent seizures	16	5 (31)
Lethargy	11	7 (64)
Listlessness	11	6 (55)
Apathy	13	7 (54)
Poor concentration	7	1 (14)
Headaches	0	— (—)
Loss of appetite	8	7 (88)
Nausea or vomiting	6	5 (83)
Constipation	4	3 (75)

*One child was worse because of irritability and agitation.

Most studies have reported that carnitine treatment had no effect on blood levels of valproate,[15,89] but one study reported a decrease in the valproate half-life after carnitine treatment.[90] Carnitine is usually well tolerated but occasional adverse effects include a "fishy" body odor, nausea, and diarrhea. Indeed, some parents elect to continue carnitine treatment for their children primarily because of the apparent improvement in gut motility and reduction in constipation. The benefits and adverse effects of very large doses of carnitine in patients with metabolic disease remain controversial.[87]

CONCLUSIONS

The purpose of this review was to summarize the available data in order to help clinicians answer the questions of when to measure carnitine levels and when to provide treatment with carnitine. Two conclusions are immediately available: carnitine deficiency is not uncommon in patients with epilepsy, and some patients appear to benefit from carnitine treatment. Faced with a particular patient, however, clinicians need to know whether that patient is likely to have carnitine deficiency and whether that patient is likely to benefit from carnitine treatment.

The data reviewed suggest that it may be possible to identify risk factors for carnitine deficiency in patients with epilepsy. These risk factors include young age, inadequate nutrition, multiple neurologic disabilities, therapy with multiple anticonvulsant drugs including valproate, hyperammonemia, and evidence of an underlying inborn error of metabolism (Table 2). Thus, measurement of carnitine levels is not necessary in all patients. When these risk factors are absent, carnitine deficiency appears to be unlikely, and measurement of carnitine levels is generally unnecessary. When several of these risk factors are pre-

sent, the likelihood of carnitine deficiency is increased and measurement of carnitine levels is worth considering. Mildly reduced carnitine levels that are still within the normal range may be of no clinical significance, but careful attention is warranted when plasma carnitine deficiency is present (free carnitine level more than two standard deviations below the mean for healthy controls). Muscle carnitine deficiency may exist even when blood carnitine levels are normal, but muscle biopsy for measurement of carnitine levels is ordinarily not indicated in patients with epilepsy.

Carnitine treatment will probably be ineffective and is not indicated if the patient is asymptomatic, has few or none of the risk factors listed in Table 2, or has carnitine levels in the normal range. Carnitine treatment is worth considering if the patient has free carnitine deficiency, hyperammonemia, or symptoms of apathy, lethargy, listlessness, anorexia, constipation, nausea, vomiting, weakness, or hypotonia. The patient's clinical response to carnitine therapy should be monitored carefully, and carnitine may be discontinued if there is no evidence of improvement. Routine administration of carnitine to young children taking valproate in order to prevent hepatic failure does not appear to be warranted, because there is no evidence that it is protective and hepatic failure has occurred in children taking carnitine. Acute administration of carnitine to patients with valproate-induced hepatic failure is generally recommended, however.

Clearly, more studies are needed to investigate the role of carnitine deficiency and its treatment in patients with epilepsy. These studies should be well designed and should include carefully controlled clinical trials in appropriately selected patients.

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Original Article

Carnitine in Human Immunodeficiency Virus Type 1 Infection/Acquired Immune Deficiency Syndrome

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ABSTRACT

There is an increasing body of evidence that subgroups of patients infected with human immunodeficiency virus type 1 possess carnitine deficiency. Secondary carnitine deficiencies in these individuals may result from nutritional deficiencies, gastrointestinal disturbances, renal losses, or shifts in metabolic pathways. However, tissue depletion precipitated by drug toxicities, particularly zidovudine, is a major etiology and concern. Carnitine deficiency may impact on energy and lipid metabolism, causing mitochondrial and immune dysfunction. There are convincing laboratory data showing the in vitro ameliorative effects of L-carnitine supplementation on zidovudine-induced myopathies and lymphocyte function. Studies measuring the impact of L-carnitine supplementation on clinical characteristics are ongoing. (*J Child Neurol* 1995;10(Suppl):2S40–2S44).

Human immunodeficiency virus type 1 (HIV-1) can lead to severe and multisystem abnormalities. Both adults and children with acquired immune deficiency syndrome (AIDS) eventually suffer from systemic immunodeficiency, but many patients also manifest multiorgan failure, including central and peripheral nervous system disease, cardiac dysfunction, and a "wasting" syndrome.[1] The pathogenic mechanisms for many of these syndromes have not been fully elucidated. Although the predominant hypotheses focus on direct virulence of HIV-1, opportunistic infections, or secondary cytotoxic processes instigated by immune dysregulation, many of the HIV-1–associated syndromes may possess a metabolic or nutritional component.[1-7] Further aspects of drug toxicity and nutritional deficiency may precipitate, enhance, or exacerbate underlying pathogenic metabolic mechanisms.

There is evidence that patients with HIV-1 infection have an alteration of lipid and fatty acid metabolism, possibly resulting from cytokine dysregulation.[2,4,8-13] This may contribute to impaired immune function, either by altering the membranes of HIV-1–infected cells to a syncytial-forming type or through up-regulation of cytokines, and it can be hypothesized that a secondary L-carnitine deficiency could further exacerbate such a compromised situation.[13] Thus, it has been a valid pursuit to search for evidence of secondary L-carnitine deficiency and differentiate the adverse effects of HIV-1 from the other secondary complications of nutritional embarrassment, immune dysregulation, opportunistic infections, and drug toxicities. Unfortunately, there is only a small body of data concerning the effect of in vivo L-carnitine supplementation on laboratory variables and little accompanying clinical correlation.[13,14]

L-CARNITINE DEFICIENCY IN HIV/AIDS

HIV-1–infected patients, both adults and children, can have impaired nutritional profiles secondary to HIV-1–associated gastrointestinal complications, particularly resulting from malabsorption syndromes, opportunistic infections, reduction of nutritional intake, or complications of drug therapies.[7,15] Micronutrient deficiencies in HIV-infected individuals are common, and it can be expected that there may exist concomitant L-carnitine deficiency.[16,17] However, in the HIV-1–infected population, there have been very few studies directed at determining the extent of L-carnitine deficiency, either primary or secondary. DeSimone et al investigated 29 adult patients with AIDS, adequate nutrition, and no evidence of myopathy or cardiac dysfunction.[17] They found that

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72% of the patients had reduced plasma total and free L-carnitine levels compared to controls. However, all patients were receiving the nucleoside analogue zidovudine (see below), although the separate effects of zidovudine, opportunistic infections, and other drugs were not distinguishable in their study. Interestingly, 14% of the patients had levels of total L-carnitine higher than controls. Similarly, in a series of 30 adult patients, Bogden et al found 37% of patients with levels of total plasma L-carnitine above the upper limit of the normal laboratory range.[18] However, many of these patients self-supplemented their diet with vitamin preparations, and the extent of supplemented L-carnitine was not clear. In the study of Tomaka et al, no L-carnitine deficiency was noted in screening HIV-1–positive patients from a private practice, outpatient setting for various vitamins and minerals; however, there was no correlation reported to zidovudine or other nucleoside status.[16] In very preliminary findings, we have found deficiencies of free and total L-carnitine in 25% of children with HIV-1 infection who have received zidovudine for longer than 6 months (Mintz, unpublished data, 1994).

Studies of L-carnitine serum levels that have appeared in the literature must be scrutinized according to whether there is a differentiation of total versus free; short-, medium-, and long-chain acylcarnitine; or if the carnitine is tissue associated, because there may be a difference between cellular and serum levels.[13,14,19–21] It is important to determine whether individuals are self-administering carnitine supplementations, which would obviously render results inaccurate. Further, in addition to potential "risk" factors of carnitine deficiency, such as poor nutritional intake, cachexia, cardiac disease, or muscle weakness, the concomitant use of various drugs, particularly zidovudine or pyrimethamine/sulfadiazine, appears to enhance the finding of L-carnitine deficiency.[17,22]

CARNITINE AND IMMUNE FUNCTION

It has been suggested that L-carnitine may play a role in immunomodulation, particularly if impaired lipid metabolism exists.[23–25] It was known since the early years of the HIV-1 epidemic that lymphoid cells from HIV-1–infected individuals are poorly responsive to mitogenic stimuli in vitro and in vivo. In a series of 21 AIDS patients, DeSimone et al have observed that the percent of peripheral blood mononuclear cells in the S and G_2-M phases that are hyporesponsive (< 20%) to phytohemagglutinin mitogenic stimulation can be significantly augmented if the culture is pretreated with L-carnitine in doses of 100 to 200 μg/mL.[26] In patients who possessed normoresponsive peripheral blood mononuclear cells (> 20% of cells in the S and G_2-M phases), L-carnitine did not enhance proliferative responses. Additionally, no correlations with these proliferative lymphocyte findings and in vivo serum carnitine levels could be detected.

The same investigational group assessed 20 adult male patients with advanced AIDS and normal serum levels of total, free, and short-chain carnitine, low CD4 counts (22×10^6 to 109×10^6/L), receiving zidovudine (600 mg/day) and pyrimethamine/sulfadiazine, but no supplemental nutritional support, and without signs of muscle or cardiac disease.[13] They found significantly lower concentrations of total L-carnitine in the HIV-1–infected patients' peripheral blood mononuclear cells compared with controls.[13] When these patients' diets were supplemented with high-dose (6 g/day) oral L-carnitine for 2 weeks, there was a significant trend toward restoration of peripheral blood mononuclear cell L-carnitine concentrations, accompanied by an increase in serum levels. When peripheral blood mononuclear cells were isolated from patients who were exposed to high-dose L-carnitine, there was an enhancement of in vitro mitogenic responses to phytohemagglutinin stimulation, again measured as a function of the percent of lymphocytes entering the S and G_2-M phases. However, there was no change in CD4 counts before and after treatment, and thus, it can be inferred that L-carnitine supplementation for 2 weeks did not cause an expansion of the CD4 lymphocyte pool despite the peripheral blood mononuclear cell mitogenic enhancement. On the other hand, enhancement of proliferative lymphocytic responses raises the concern of increased HIV-1 replication, but markers of HIV-1 replication, such as p24 antigen, did not rise in the L-carnitine–treated group. Of interest was the significant reduction of triglycerides in L-carnitine–treated patients—an important marker of immune activation and possibly cytokine production.[4,27–29] Although there was no clinical correlation in this study, there were a number of participants who reported subjective improvements in "energy levels," "well-being," and weight gain. These results were similar to those in an earlier study showing that adult AIDS patients receiving high-dose L-carnitine supplementation for 2 weeks showed statistically significant reductions in triglyceride levels and β_2-microglobulin (a potential marker for AIDS and AIDS dementia complex), and a trend in the lowering of tumor necrosis factor levels (a potential causative factor in HIV-1–associated central nervous system disease), as well as enhancement of in vitro lymphocyte proliferation responses, but no significant effect on CD4 counts.[1,2,14,30–32] Effects of L-carnitine administration for periods longer than 2 weeks are presently being investigated by this group and others.

CARNITINE AND HIV-1–ASSOCIATED NEUROMUSCULAR DISEASE

Patients with HIV-1 infection can experience a multitude of peripheral nervous system complications, resulting from HIV-1 infection, secondary opportunistic infections, or toxicities of various drugs, particularly the nucleoside analogues.[33–35] Painful neuropathies and debilitating myopathies have been extensively delineated.[33] Additionally, there is an extensive literature on the effects of mitochondrial abnormalities inducing myopathic processes (reviewed elsewhere in this supplement), which proffers

the hypothesis of mitochondrial and carnitine involvement in HIV-1–associated or drug-induced myopathies in patients with AIDS.[36–40]

Dalakas and colleagues have reported extensive studies concerning the effects of zidovudine on muscle.[35–38,41,42] In well-nourished HIV-1–infected patients with varying degrees of myopathic signs and symptoms (fatigue, myalgia, weakness, and increased serum creatinine phosphokinase) who received zidovudine at standard dosages for more than 9 months and did not have major systemic AIDS complications, muscle specimens revealed extensive depletion of mitochondrial DNA, as well as carnitine deficiency, with associated lipid storage; many of these patients manifested clinical and laboratory symptomatology of a zidovudine-induced myopathy.[35,37,38] The severity of histologic pathology in the muscle ("zidovudine fibers," which are muscle fibers displaying ragged red–like features, red-rimmed or empty cracks, granular degeneration, and rods, which are histologic markers of muscle mitochondria proliferation or destruction) correlated with the extent of carnitine depletion and lipid accumulation.[38] Interestingly, six of the 21 patients in this study had normal histologic and muscle carnitine findings, but complained subjectively of fatigue and myalgia.[38] Furthermore, the cumulative dose and duration of therapy of zidovudine did not correlate with the severity of muscle fiber abnormalities or carnitine levels. Weakness did not correlate with the histologic or biochemical abnormalities.

Many hypotheses can be considered, but zidovudine-induced myopathy may result from impairment of the cytochrome system and inefficient oxidative phosphorylation, which may in turn lead to a deficiency of muscle carnitine.[43] Alternatively, the uptake of L-carnitine may be reduced if the mitochondria are dysfunctional, or free carnitine may be esterified and exported out of the mitochondria if dysfunctional mitochondria cause a shift toward the glycolytic pathway.[38] The end result of whatever mechanism is at play likely is a substantial reduction of available energy resources in muscle fibers, which is expressed clinically as myopathic symptoms.[38]

The role of carnitine in zidovudine myopathy is further supported by the observations of in vitro myotube cultures supplemented with L-carnitine. Supplementation of myotube cultures reversed many of the destructive effects of zidovudine including a preservation of the structure and volume of mitochondria and prevention of lipid droplet accumulation.[41,42] These findings are provocative and lend additional support for the study of L-carnitine in HIV-1–infected individuals receiving zidovudine, both in the asymptomatic stages and particularly if they are experiencing myopathic symptoms, but as yet there exist no in vivo data on the protective or preventive effects on myopathy. Ongoing clinical trials will soon shed some light on the clinical use of L-carnitine in HIV-1–infected patients for the prevention or treatment of zidovudine-induced myopathies. Results may somewhat extrapolate to other nucleoside analogues.[44]

CARNITINE AND THE CENTRAL NERVOUS SYSTEM

An HIV-1–associated neurologic syndrome has been well defined in adults and children, termed AIDS dementia complex and HIV-1–associated progressive encephalopathy, respectively. The exact neuropathogenic mechanisms have not been fully elucidated, but there has been speculation surrounding direct neurovirulence of HIV-1; secondary cytotoxic mediators produced from macrophages, arachidonic acid metabolites, and activated neural elements; and dysregulation of calcium channels.[1,2,31,32,45–47] Possibly, unrecognized metabolic mechanisms are contributing.[3,5,6]

Cytokines, particularly tumor necrosis factor and arachidonic acid metabolites, likely play an important role in the development of AIDS dementia complex/progressive encephalopathy, as well as in the up-regulation of HIV-1 replication.[2,32,46,48] In addition to antiretroviral therapies, specific ways in which cytokine activity may be reduced may provide important future adjunctive therapies in the treatment or prevention of AIDS dementia complex/progressive encephalopathy.[1] There has been some anecdotal evidence of anti-inflammatory therapy being beneficial in progressive encephalopathy, but other recent studies investigating tumor necrosis factor inhibitors have been disappointing.[49,50] In studies of inflammation, there is evidence that carnitine may modulate cytokine production.[13,14,25] This suggests a potential pathogenic or therapeutic role of L-carnitine in AIDS dementia complex/progressive encephalopathy. However, there is no direct evidence to date correlating plasma or tissue L-carnitine status with AIDS dementia complex/progressive encephalopathy, and as well there are no data concerning L-carnitine supplementation affecting AIDS dementia complex/progressive encephalopathy.

DISCUSSION

There is a growing body of evidence that HIV-1–infected individuals, particularly those receiving the nucleoside analogue zidovudine, may possess a carnitine deficiency syndrome. Such a deficiency can be measured in serum, peripheral blood mononuclear cells, or muscle tissue. Many mechanisms producing carnitine deficiency may be involved, but the effect of zidovudine on mitochondrial function appears to be a predominant mechanism. Alternatively, HIV-1–infected patients are prone to nutritional deficiencies, through an inadequate diet, malabsorption, chronic diarrhea, or opportunistic infections, which may create a secondary carnitine deficiency. Renal disease or a shift toward glycolytic pathways may add excessive urinary excretion as an additional mechanism of carnitine loss. Patients who supplement their diet with vitamins may be receiving exogenous L-carnitine, obscuring the true extent of carnitine deficiency. Likewise, the overwhelming use of nucleoside analogues in AIDS patients makes it very difficult to distinguish the natural history of carnitine deficiency from the effects of drug toxicity. Additionally, studies in the literature require more consis-

tency in measuring L-carnitine in the free, short- or long-chain, or acetyl forms, and in various tissues or peripheral blood mononuclear cells.

When L-carnitine deficiency has been identified in peripheral blood mononuclear cells or muscle tissue in patients receiving zidovudine, the in vitro supplementation of culture media with L-carnitine has effected a reversal of the identifiable carnitine deficiency; immune enhancement as measured by lymphocyte mitogenic responses to phytohemagglutinin; and amelioration of abnormal muscle histology and biochemical findings. However, sufficient data concerning beneficial effects on clinical features has been lacking, although there is a suggestion of subjective improvement in some studies.

Carnitine deficiency in HIV-infected patients places them at risk for alterations in fatty acid oxidation and potential mitochondrial dysfunction. Laboratory findings suggesting that carnitine supplementation may be beneficial in energy utilization provide hypothetical evidence that carnitine supplementation may assist in avoiding or reversing the antimitochondrial effects of zidovudine or other nucleoside analogues. Clinical trials of L-carnitine supplementation involving clinical correlation with laboratory findings in HIV-1–infected patients receiving zidovudine, particularly those with myopathic symptoms, are ongoing, but no data are presently forthcoming. However, many difficulties in measuring outcomes—amelioration of clinical or laboratory myopathic symptomatology, enhancement of immune function, effects on the central nervous system—will be encountered and may require large cohorts to reach statistical significance. Additionally, the lack of patients with AIDS who are zidovudine or nucleoside naive are presently rare, and garnering appropriate control groups may present additional difficulties. Nevertheless, with the data reported to date and the low toxicity profile of L-carnitine, the pursuit of further clinical investigations of the effects of L-carnitine supplementation in HIV-1–infected individuals is warranted. In addition to addressing issues of efficacy, studies may assist in providing guidelines for dosing and the timing of commencing L-carnitine therapy. Only a small number of laboratories and investigators have reported data concerning L-carnitine and HIV-1 infection or AIDS, and reproducible findings in additional centers would be desirable.

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Original Article

The Role of L-Carnitine in Pediatric Cardiomyopathy

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ABSTRACT

Metabolic and genetic factors underlie some forms of cardiomyopathy in childhood. A variety of inborn errors of metabolism can impair mitochondrial energy production, or β-oxidation, in the heart and lead to myocardial dysfunction. L-Carnitine, an essential element of β-oxidation, transports fatty acids across the mitochondrial membrane for energy production. L-Carnitine deficiency syndromes are now well described as secondary to a variety of inborn errors of metabolism and often include cardiomyopathy in the clinical picture. Despite traditional therapies for cardiomyopathy, mortality for this disorder remains at well over 50%. Review of reports of L-carnitine supplementation studies and results from our own trial underscore the importance of its role in cardiac function and demonstrates that there is likely a subpopulation of patients with cardiomyopathy responsive to L-carnitine treatment. (*J Child Neurol* 1995;10(Suppl):2S45–2S51).

There has been much interest in elucidating the determinants of cardiomyopathy in the pediatric population. Efforts directed at investigating the metabolic basis for myocardial dysfunction have been particularly active. Some forms of cardiomyopathy, known for their high mortality and resistance to intervention, are now being better understood as outcomes of genetic defects and metabolic disturbances. One area that has yielded significant insights into the pathogenesis of pediatric cardiomyopathy has been the examination of defects in mitochondrial energy production; impairments of the mechanism of the myocardium's primary source of energy, the oxidation of fatty acids, may eventuate into these life-threatening clinical syndromes. Here, we review the clinical background and course of cardiomyopathy, its incidence and prognosis, and the role that L-carnitine, the primary transport molecule of mitochondrial energy production, plays in the pathogenesis of this disease. In addition, we provide case studies that exemplify a variety of circumstances in which genetic and metabolic defects can occur and outline their characteristic clinical sequelae. We also provide our own findings from a two-center trial investigating the effect of carnitine supplementation in this group of patients.

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FEATURES OF CARDIOMYOPATHY

Cardiomyopathy refers to a group of disorders in children and adults characterized by primary involvement of the ventricular myocardium. The World Health Organization/International Society and Federation of Cardiologists task force on the classification of cardiomyopathies has defined this condition as a heart muscle disease of unknown cause that is not secondary to an acquired or congenital heart disease.[1,2] It is an important cause of morbidity and mortality; a total of 43,000 patients were hospitalized in 1990 for this disorder in the United States during the 1st year of life, and the incidence is approximately one in 10,000 live births.[3] The survival of patients with cardiomyopathy is dismal, in the range of 50% to 60% after 2 years.[4]

The three pathophysiologic classifications of cardiomyopathy currently recognized are hypertrophic, restrictive, and dilated. Hypertrophic cardiomyopathy is hypertrophy of the ventricular myocardium without an identifiable cause. Dilated cardiomyopathy, accounting for over 90% of all reported cardiomyopathies and by far the most common cardiomyopathy in individuals less than 19 years old, is a primary disease of the ventricular myocardium characterized by increased left ventricular or biventricular volumes without an appropriate increase in ventricular septal or free wall thickness. The essential

physiologic derangement is decreased myocardial contractility (systolic dysfunction) accompanied by a variable degree of impairment of diastolic function.

Clinical Features

Pediatric cardiomyopathy typically presents with normal prenatal and postnatal health followed by a sudden onset of respiratory distress, decreased appetite, lethargy, and occasionally, vomiting, irritability, or fever. The illness often begins during or after a mild upper respiratory tract infection. Children are generally pale with a rapid pulse and may have signs of shock. The liver is enlarged, and peripheral cyanosis is frequent. Cardiac murmurs may be present. Marked cardiomegaly is evident on radiograph, with pulmonary venous congestion. The electrocardiogram often shows extreme left ventricular hypertrophy but may reveal low voltage in some cases. The echocardiogram reveals depressed systolic function with decreased fractional shortening and ejection fraction. Left atrial enlargement is noted, and global left ventricular dysfunction without major regional wall abnormalities may be seen. Intracavitary thrombus may also be detected.[5,6]

Histopathology

Typical ultrastructural features of dilated cardiomyopathy include myocyte hypertrophy and degeneration, including mitochondrial hyperplasia, abnormal Z bands, dilated and disorganized sarcoplasmic and transverse tubular systems, loss of myofibrils, increased lipid droplets, myelin figures, increased phagolysosomes, and increased glycogen. A study of five pairs of twins with dilated cardiomyopathy revealed abnormal mitochondria with circular and stacked cristae. This particular pathology has also been described in several inborn errors of mitochondrial fat metabolism with resultant secondary carnitine deficiency or carnitine insufficiency.[7]

Etiology

Multiple etiologies for cardiomyopathy have been identified,[8–12] but in the majority of cases, the etiology remains obscure. It has been estimated that 20% of cardiomyopathies may be inherited. In addition, the molecular basis of these disorders as they relate to muscle function provide valuable clues to the pathophysiology of cardiomyopathy and to potential therapeutic strategies.[4] Cardiomyopathy has been described as secondary to inborn errors of metabolism, primary and secondary carnitine deficiency or carnitine insufficiency, vitamin deficiencies, electrolyte disturbances, endocrine disease, drug toxicities, collagen vascular diseases, trace mineral deficiency or toxicities, single gene or mitochondrial genetic diseases, and anoxic damage.[13] Idiopathic cardiomyopathy, cardiomyopathy with no identifiable cause, may in part be a consequence of a poorly understood and as-yet-undiagnosable metabolic defect or a defect in mitochondrial functioning.

Treatment and Prognosis

The cornerstones of traditional pharmacologic therapy for the congestive heart failure associated with progressive cardiomyopathy have been digitalis, diuretics, and afterload reducing agents. Although review of the literature reveals somewhat discrepant mortality statistics (possibly due to the effects of maternal disease in early infancy*), despite traditional therapies, mortality has been reported as high as 58% at 1 year and 80% at 5 years in patients with dilated congestive cardiomyopathy, the most common cardiomyopathy in pediatric patients.[34–44] Cardiac transplantation remains the only alternative for patients with nonresponding disease.

L-CARNITINE, METABOLISM, AND CARDIOMYOPATHY

Levocarnitine (L-carnitine), 3-hydroxy-4-*N*-trimethylaminobutyric acid, is a quaternary ammonium compound present in all tissues and is an essential cofactor in the system that transports long-chain fatty acids across the inner mitochondrial membrane, where they undergo energy production. Carnitine is obtained from the diet, the major sources being red meat and dairy products. Carnitine is also synthesized from lysine and methionine, the final synthetic step occurring in the liver. It is not metabolically altered by the body and is excreted in the urine as either free carnitine or acylcarnitine. Free carnitine is largely reabsorbed in the renal tubule, and acylcarnitine is excreted.[45]

Once absorbed from the diet, carnitine enters the circulation and actively crosses muscle membrane. The same transport mechanism in muscle membrane is proposed for the renal transport of carnitine.[11]

Carnitine plays a central role in the shuttle of fatty acids across the inner mitochondrial membrane, and defects in carnitine metabolism can lead to disturbances in mitochondrial energy metabolism. Disturbances include poor dietary intake or malabsorption of carnitine, excessive loss of carnitine in the urine from impaired renal tubular function, and inborn errors of metabolism.[46]

Clearly, defects in many of the steps of mitochondrial fat metabolism result in decreased adenosine triphosphate (ATP) production; these defects have been and are continuing to be described. Impairments of energy production have foremost consequences for myocardial functioning. The heart relies chiefly on the aerobic breakdown of fat for its energy supply. Known as β-oxidation, this complex, multiple-enzyme process occurs in the mitochondria. Each enzyme-controlled step is consequently vulnerable to genetic defects that may partially or completely impair subsequent metabolic steps.

Normally, long-chain fatty acids are released by lipolysis and cross into the mitochondria in a series of steps. The initial step is the activation of the fatty acid to coenzyme A (CoA) in the cytosol to form fatty acyl-CoA. Once activated, the fatty acyl-CoA cannot cross into the mitochondria until transferred by carnitine. This step occurs via the enzyme carnitine acyltransferase I. Once the fatty

*In February 1993, a consensus of the National Pediatric Cardiology Study Group meeting at Jackson Hole, WY.

acylcarnitine molecule is formed, the molecule is transported across the mitochondrial membrane via carnitine acyltranslocase. Once the fatty acylcarnitine molecule reaches the mitochondrial matrix, it is again transferred to CoA via carnitine acyltransferase II. The fatty acyl-CoA molecule that is formed is then ready to undergo β-oxidation. Coupling of β-oxidation with the electron transport pathway within the mitochondria leads to the formation of chemical energy, ATP.[47]

The carnitine released in the matrix of the mitochondria can return to the cytosol via carnitine acyltranslocase. In addition, carnitine may also form an ester with an acyl-CoA molecule within the mitochondria via the enzyme carnitine acyltransferase II, and this acylcarnitine molecule may then leave the mitochondria via the translocase. This mechanism provides a route for removal of acyl derivatives that accumulate during normal metabolism and for large amounts occurring during states of abnormal metabolism. Thus, carnitine can also play a detoxifying or scavenging role.[47]

GENETIC DEFECTS AND INBORN ERRORS OF METABOLISM

Treem et al[11] described an autosomal recessive disorder of carnitine membrane transport resulting in low muscle carnitine levels, as well as low plasma levels due to altered renal reabsorption. Patients can develop cardiomyopathy with this disorder, which was found to be reversible with carnitine supplementation.[11] Carnitine acyltransferase I and II and translocase defects have also been reported. All are inherited as autosomal recessive disorders and can have an associated cardiomyopathy.[4]

Defects of fatty acid oxidation, such as long-chain fatty acyl-CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, have been reported associated with cardiomyopathy. Carnitine supplementation in these disorders remains controversial, but restriction of long-chain fats in the diet has been reported to be helpful.[4,48,49]

Electron transport defects result in decreased ATP production and both skeletal myopathy and cardiomyopathy. Historically, these disorders have been named according to their symptom complexes, such as MELAS for myoclonic epilepsy, lactic acidosis, strokelike syndrome and MERRF for myoclonic epilepsy, ragged red fiber disease. More recently, these disorders are being differentiated via the molecular defects in maternally inherited mitochondrial DNA. Many of these disorders are associated with cardiomyopathy.[4,48–50]

Secondary carnitine deficiency states have now been well described in association with inborn errors of metabolism in which acyl-CoA derivatives accumulate within the mitochondria. These disorders result in low levels of free carnitine and increased acylcarnitines. Such disorders include many of the organic acidurias: propionic aciduria, methylmalonic aciduria, isovaleric aciduria, and glutaric aciduria II are examples.[48,51–53]

In addition, in several inherited disorders of fatty acid metabolism, dicarboxylic acids accumulate, such as deficiencies of medium-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, and very long chain acyl-CoA dehydrogenase.[49] The alteration of tissue carnitine in these disorders can result in general muscle weakness and cardiomyopathy.

Cardiomyopathy can also be a symptom in multiple acyl-CoA dehydrogenase deficiency (glutaric acidemia type II),[53] which is characterized as a deficiency of cofactors for several different enzymes, with resulting accumulation of carboxylic acids derived from the breakdown of fatty acids and amino acids, as well as an accumulation of dicarboxylic acid.[51,53] The literature reports that carnitine can promote the excretion of excess organic or fatty acids in patients with defects in fatty acid metabolism or specific organic acidopathies that bioaccumulate acyl-CoA esters.[52,53] Carnitine supplementation, although not correcting the primary metabolic error, has been found to relieve many of the symptoms associated with these disorders.[46]

Carnitine deficiency has also been associated with diabetes mellitus. Dietary deficiency of carnitine was described in infants receiving unsupplemented soy formulas and those on total parenteral nutrition. Infants appear particularly susceptible to nutritional carnitine deficiency.[54] Renal loss of carnitine occurs in patients with renal tubular dysfunction disorders, such as renal tubular acidosis and renal Fanconi syndromes. Long-term renal dialysis can also lead to carnitine deficiency.[41]

REVIEW OF CARNITINE SUPPLEMENTATION STUDIES

Much of the interest in examining the role of carnitine in cardiomyopathy has centered on investigating carnitine levels in these disorders. Enough evidence has been accumulated to indicate that at least a subset of cardiomyopathies are accompanied by a carnitine deficiency[55,56] or carnitine insufficiency.[57,58] Carnitine insufficiency is described as an abnormal ratio of plasma acylcarnitine to free carnitine (greater than 0.4) and is an indicator of metabolic disturbance. Each of these reports documents cases responding with clinical improvements to carnitine supplementation and normalization of their cardiac functioning.

Similarly, cases in which a specific inherited defect has been identified have been shown to have a responsive carnitine deficiency. In 1988, Ino et al[59] reported two cases of X-linked recessive dilated cardiomyopathy and three cases with an enzymatic defect of fatty acid oxidation. These patients had low free and elevated esterified carnitine and showed improvement in cardiac function (by echocardiography) with combined therapies of digitalis, diuretics, and carnitine supplementation. Also, two reports describe two patients with Duchenne muscular dystrophy who also had low muscle carnitine levels, suggesting that carnitine deficiency might play a role in the cardiomyopathy that develops consequent to Duchenne muscular dystrophy.[60]

Pierpont[44] has described two cases of carnitine transport defect–associated cardiomyopathy in siblings. One

ient, in spite of 2 years of therapy with digoxin, diuret- and vasodilators, experienced a steady deterioration of cardiac function and was considered a candidate for cardiac transplantation. Skeletal muscle biopsy on both patients revealed a lipid storage myopathy, and examination of plasma and muscle carnitine levels revealed a severe deficiency; carnitine therapy was subsequently initiated in the symptomatic sibling. The resultant improvement in cardiac functioning permitted removal from intravenous medication within 2 days and discharge from the hospital in 1 week. Cardiac function and size returned to normal after 6 months of carnitine therapy.

The asymptomatic younger sibling was found to have mitral regurgitation and a cardiac condition similar to her brother's, with a low plasma level of free carnitine. Supplementation was thought to be life saving in both of these patients. The transport of carnitine from supplementation in these transport defect cases was attributed to passive diffusion of the carnitine across the mitochondrial membrane.

Waber et al[61] reported a similar case of lipid myopathy in a boy who presented at age 3 plus years with cardiomegaly, distinctive electrocardiogram, and history of a brother dying of cardiomyopathy. Muscle and plasma carnitine were reduced to 2% to 10% of normal. After a year of carnitine supplementation, the cardiac disease resolved, and muscle strength became normal. Although the plasma carnitine concentration was in the low-normal range, the urinary concentration of carnitine was 30 times normal, suggesting a distinct form of deficiency; defective renal or gastrointestinal carnitine transport was the likely cause of this patient's disorder.

Tein et al[62] described four unrelated children with primary carnitine-responsive cardiomyopathy demonstrated by carnitine uptake fibroblast cultures. Each child was noted to have negligible uptake of carnitine (2% of control values), and their parents showed intermediate uptake rates. Serum carnitine levels were low in all four children before carnitine supplementation. Cardiac dysfunction improved within 1 month of therapy. Left ventricular parameters showed marked positive changes, accompanied by improved clinical outcome in their failure to thrive, school performance, and motor function. It was thought that carnitine played an important role in sequestering the toxic long-chain acyl-CoA metabolites that had accumulated and promoted sarcolemmal membrane damage and arrhythmias.[63]

Bohles et al[63] reported 68 patients with myocardial ischemia undergoing aortocoronary bypass operation who were assigned to either carnitine or control therapy. Biopsy of the right atrial appendage was analyzed for carnitine fractions, ATP, and lactate. Analyses of biopsies from patients receiving carnitine treatment showed relatively higher ATP concentration and lower lactate concentrations than control-treated patients. Carnitine-treated patients also needed less inotropic medication postoperatively.

Patients undergoing long-term hemodialysis develop cardiomyopathy and cardiac disease as one of the most important causes of death. Hemodialysis results in a progressive and substantial loss of carnitine from muscle, and the ratio of free to total carnitine becomes abnormally low. Similarly, Kudoh et al[64] reported markedly reduced plasma carnitine levels and an inversely correlated cardiothoracic ratio in chronic hemodialysis.

In an attempt to further evaluate the role of carnitine in patients with cardiomyopathy, we conducted a retrospective review of data collected on carnitine supplemented patients from two medical centers. A total of 35 patients were selected for inclusion and evaluation.

METHODS

Thirty-five patients were identified from two centers from a 10-year period who had their cardiomyopathy treated with oral carnitine. These centers were Geisinger Medical Center in Danville, Pennsylvania, and Valley Children's Hospital in Fresno, California. The following inclusion and exclusion criteria were applied to each patient case: (1) Patients were diagnosed with cardiomyopathy, supported by echocardiography, chest radiograph, physical examination, or history. (2) Patients had received oral carnitine. (3) At least one posttherapy follow-up assessment was available with adequate data to assess efficacy. (4) Patients with an acquired metabolic disorder not due to an inborn error of metabolism were excluded from the study.

There were 21 males and 14 females with a mean age at the start of carnitine therapy of 34.5 months (SD, 65 months) and a range of birth to 23.9 years. Therapy was most commonly begun by age 2 years. The mean duration of carnitine therapy was 25.3 months, ranging from 1.5 to 84.1 months from the date carnitine was begun to the last recorded visit. The average carnitine dose given was 96 mg/kg and ranged from 14 to 455 mg/kg daily.

Cardiomyopathy type was classified as dilated (23 patients), hypertrophic (six patients), or restrictive (one patient). A metabolic etiology for cardiomyopathy was suspected in 12 patients based on organic acid analysis, skeletal muscle myopathy, or enzymatic analysis. Table 1 shows the distribution of diagnoses. Twelve of the 35 patients had a proven or suspected inborn error of metabolism. All proven or suspected metabolic disorders involved mitochondrial fat oxidation.

Data Collection

Markers of treatment efficacy included echocardiogram results (shortening fraction), plasma carnitine levels, and mortality. These data were collected both before and after the administration of carnitine, where available.

Table 1. Distribution of Diagnoses

Diagnostic Category	*Specific Diagnosis*	*Patients, n (%)*
Fatty acid defect	Skeletal muscle myopathy	6 (75.0)
	Long-chain fatty acyl-CoA dehydrogenase deficiency	2 (25.0)
	Total (% of all patients)	8 (22.9)
Organic and amino aciduria	Glutaric aciduria type 2	4 (100.0)
	Total (% of all patients)	4 (11.4)
Unknown	Unknown diagnosis	23 (100.0)
	Total (% of all patients)	23 (65.7)
Total patients	—	35 (100.0)

Table 2. Patient Deaths

Patient	Type of Cardiomyopathy	Diagnosis	Age at Death, mo	Cause of Death	Time on Carnitine, mo
1	Dilated	Muscle myopathy (FAO)	35.6	Cardiac arrest	11.6
7	Dilated	Muscle myopathy (FAO)	21.3	Arrythmia	4.0
9	Dilated	Unknown	25.0	Cardiac arrest	16.6
22	Dilated	Muscle myopathy (FAO)	197.8	Cardiac arrest	28.2
27	Hypertrophic	Unknown	76.2	Arrythmia	6.3
28	Hypertrophic	LCAD	NA	NA	NA
29	Restrictive	Muscle myopathy	292.2	CHF	5.6
33	Hypertrophic	Unknown	20.5	Cardiac arrest	8.9

FAO = fatty acid oxidation defect; LCAD = long-chain acyl-CoA dehydrogenase deficiency; NA = not available; CHF = congestive heart failure.

Analysis

The echocardiogram shortening fraction values before and after carnitine administration were compared using a paired *t*-test procedure in patients who had both values available (23 patients). In cases in which there were multiple posttreatment shortening fractions, the last echocardiogram value was used. The observed mortality rate was compared with literature values.

Carnitine Status

Carnitine deficiency was defined as a plasma free carnitine level less than 20 μmol/L, and plasma ester to free carnitine ratios of more than 0.4 were considered abnormally high. Twenty-four patients had pretreatment carnitine levels available. Of these, 10 (42%) had levels below 20 μmol/L and were considered carnitine deficient (mean, 29.59 μmol/L; SD, 18.5 μmol/L). Of 23 patients measured after therapy, all had free carnitine levels above 20 μmol/L (mean, 59.2 μmol/L; SD, 29.2 μmol/L).

RESULTS

Eight (23%) of 35 patients died from their cardiomyopathy (Table 2). Five of these had a suspected or proven inborn error of metabolism. Three of the expired patients had carnitine deficiency before therapy.

Twenty-three patients had both baseline and posttreatment echocardiograms available. Pretreatment and posttreatment echocardiogram values are presented in Table 3. The difference between the mean pretreatment fractional shortening value and the mean posttreatment fractional shortening value (0.25 versus 0.30) was statistically significant ($P = .043$).

Laboratory values, including complete blood counts, serum chemistry, and urine analysis, were collected and reviewed by one of the investigators for changes attributable to carnitine therapy. All laboratory values could be explained by the patients' underlying disorder, and no unexpected changes were noted.

DISCUSSION

Twelve of the 35 patients had either a proven or suspected inborn error of metabolism as the primary etiology for their cardiomyopathy. This was a surprisingly high proportion of patients with a metabolic disorder because metabolic etiologies of cardiomyopathy are generally thought to be rare. The metabolic disorders suspected as a cause of cardiomyopathy in these 12 patients fell into three categories: long-chain fatty acyl-CoA dehydrogenase deficiency, electron transport flavoprotein deficiency (glutaric aciduria II), and disorders of mitochondrial electron transport or metabolism. The finding of cardiomyopathy in such defects is not surprising considering the known dependency of cardiac muscle metabolism on fatty acid oxidation as an energy source. Because all three types of metabolic disorder are associated with an accumulation of acyl-CoA derivatives within the mitochondria, carnitine therapy would be expected to improve the metabolic dysfunction, with removal of acylcarnitine derivatives.

Currently, cardiologists would not expect to find a high percentage of patients with cardiomyopathy to have an underlying inborn error of metabolism as the etiology. In addition, the finding of only three categories of metabolic defects (long-chain fatty acyl-CoA dehydrogenase deficiency, glutaric aciduria II, and mitochondrial defects) as the cause is even more surprising.

Overall, eight (23%) of 35 patients died from their cardiomyopathy, and five (62.5%) of these had a suspected or proven inborn error of metabolism. Because overall there were 12 patients in this study with a suspected or proven inborn error of metabolism, their mortality rate was five (42%) of 12.

We postulate that carnitine administration results in improved excretion of toxic acyl-CoA intermediates that accumulate due to aberrant fatty acid catabolism and may have contributed to the improvement in cardiac function. Treatment with pharmacologic levels of carnitine needs to be considered in patients presenting with life-threatening cardiomyopathy. Measurement of tissue and plasma carnitine levels of both free and acylcarnitines and proper investigations into mitochondrial disorders are imperative. Studies should include evaluation for organic acidopathies, fatty acid oxidation defects,

Table 3. Mean Echocardiogram Fractional Shortening Values Before and After Carnitine Treatment

	Pretreatment	Posttreatment*	P
n	23	23	—
Mean FS	0.254	0.304	.043
SD	0.14	0.13	—
Minimum	0.080	0.10	—
Maximum	0.660	0.54	—

*Mean elapsed time from treatment start was 23.8 months (range, 3 to 68 months).
FS = fractional shortening; SD = standard deviation.

defects in mitochondrial electron transport, carnitine membrane transport defects, and defects of carnitine acyltransferases I and II and translocase. Carnitine deficiency secondary to dietary deficiency, malabsorption, increased renal loss, dialysis, or pharmacologic agents needs to be evaluated.

Studies to consider include: organic acids; amino acids; plasma, urine, or tissue carnitine levels; muscle biopsy for mitochondrial enzymes and muscle carnitine membrane transport; mitochondrial DNA studies; and assessment of renal tubular function. Carnitine treatment of some of the specific defects remains controversial, but in most of the defects, including the organic acidopathies and carnitine membrane transport defects, carnitine therapy can be life saving.

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