Carnitine Deficiency Disorders in Children

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ABSTRACT: Mitochondrial oxidation of long-chain fatty acids provides an important source of energy for the heart as well as for skeletal muscle during prolonged aerobic work and for hepatic ketogenesis during long-term fasting. The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of β-oxidation. The shuttle consists of three enzymes (carnitine palmitoyltransferase 1, carnitine acylcarnitine translocase, carnitine palmitoyltransferase 2) and a small, soluble molecule, carnitine, to transport fatty acids as their long-chain fatty acylcarnitine esters. Carnitine is provided in the diet (animal protein) and also synthesized at low rates from trimethyl-lysine residues generated during protein catabolism. Carnitine turnover rates (300–500 μmol/day) are <1% of body stores; 98% of carnitine stores are intracellular (total carnitine levels are 40–50 μM in plasma vs. 2–3 mM in tissue). Carnitine is removed by urinary excretion after reabsorption of 98% of the filtered load; the renal carnitine threshold determines plasma concentrations and total body carnitine stores. Because of its key role in fatty acid oxidation, there has long been interest in the possibility that carnitine might be of benefit in genetic or acquired disorders of energy production to improve fatty acid oxidation, to remove accumulated toxic fatty acyl-CoA metabolites, or to restore the balance between free and acyl-CoA. Two disorders have been described in children where the supply of carnitine becomes limiting for fatty acid oxidation: (1) A recessive defect of the muscle/kidney sodium-dependent, plasma membrane carnitine symporter, which presents in infancy with cardiomyopathy or hypoketotic hypoglycemia; treatment with oral carnitine is required for survival. (2) Chronic administration of pivalate-conjugated antibiotics in which excretion of pivaloyl-carnitine can lead to carnitine depletion; tissue levels may become low enough to limit fatty acid oxidation, although no cases of illness due to carnitine deficiency have been described. There is speculation that carnitine supplements might be beneficial in other settings (such as genetic acyl-CoA oxidation defects—"secondary carnitine deficiency", chronic ischemia, hyperalimentation, nutritional carnitine deficiency), but efficacy has not been documented. The formation of abnormal acylcarnitines has been helpful in expanded newborn screening programs using tandem mass-spectrometry of blood spot acylcarnitine profiles to detect genetic fatty acid oxidation defects in neonates. Carnitine-deficient diets (vegetarian) do not have much effect on carnitine pools in adults. A modest 50% reduction in carnitine levels is associated with hyperalimentation in newborn infants, but is of doubtful significance. The above considerations indicate that carnitine does not become rate-limiting

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unless extremely low; testing the benefits of nutritional supplements may require invasive endurance studies of fasting ketogenesis or muscle and cardiovascular work.

KEYWORDS: acyl-CoA; carnitine; children; deficiency; disorder; fatty acids; metabolism; pivalate; supplementation

INTRODUCTION

Carnitine is a small, water-soluble molecule that plays a key role in mitochondrial oxidation of fatty acids by serving as a cofactor for shuttling long-chain fatty acids from the cytoplasm across the barrier of the inner mitochondrial membrane and into the mitochondrial matrix. Since the 1960s, there has been great interest in the possibility that carnitine deficiency could cause human disease by impairing fatty acid oxidation and that nutritional or pharmacologic supplements of carnitine might be beneficial in some disorders. Despite this interest, over the past 35 years, there have been only two clear examples of disorders that are directly due to a deficiency of carnitine and that show unequivocal therapeutic benefit from carnitine treatment. The goal of this chapter is to briefly review these two examples of human carnitine deficiency diseases: carnitine deficiency due to a recessive genetic defect of the plasma membrane carnitine transporter, OCTN2; and acquired carnitine deficiency due to treatment with drugs containing pivalate, a nonmetabolizable branched-chain fatty acid. Brief comments are included in a section on other disorders associated with alterations in carnitine concentrations due to genetic defects of fatty acid β-oxidation enzymes and organic acid oxidation defects. More extensive discussion of these issues has been provided in a previously published review in *Advances in Pediatrics* in 1995.[1]

CRITERIA FOR SYMPTOMATIC CARNITINE DEFICIENCY

Over the past four decades, there has been considerable confusion about the definition of carnitine deficiency because the underlying disorders had not been identified. Initially, the terms "systemic carnitine deficiency" and "muscle carnitine deficiency" were introduced to denote patients with reduced carnitine in liver and muscle who usually presented with attacks of illness resembling Reye's syndrome as opposed to patients with reduced carnitine only in muscle who presented with weakness. Subsequently, the terms "primary carnitine deficiency" and "secondary carnitine deficiency" were introduced to denote patients whose disease was thought to be directly caused by carnitine deficiency as opposed to patients with enzymatic defects in fatty acid or organic acid oxidation that were accompanied by carnitine deficiency. Unfortunately, the cases labeled "systemic carnitine deficiency" were more heterogeneous than suspected and have turned out to include many patients with underlying enzyme defects in fatty acid oxidation (i.e., "secondary carnitine deficiency"), as well as a few examples of patients with mutations of the carnitine transporter, OCTN2 (i.e., "primary carnitine deficiency"). For this reason, it is probably best to discard these ill-defined terms in favor of more specific terms linking carnitine deficiency to the specific underlying disorder (e.g., carnitine deficiency

due to OCTN2 defect or carnitine deficiency associated with isovaleric acidemia). Additional issues then include whether the carnitine deficiency is the cause, rather than a consequence, of the disorder and whether there are demonstrable benefits from treatment with carnitine. The author has suggested the following criteria should be considered in deciding whether a particular clinical disorder (e.g., impaired fatty acid oxidation) is due to deficiency of carnitine:[1]

(1) The tissue concentration of free carnitine must be low enough to limit flux through the pathway (e.g., of mitochondrial fatty acid oxidation). For long-chain fatty acid oxidation, this means values below the K_m for carnitine of carnitine palmitoyltransferase-1 of about 50–200 μM. Since tissue concentrations of free carnitine in muscle and liver are normally 1–3 mM, this means that carnitine levels probably have to be less than 10% of normal before they become a limiting factor.
(2) There should be direct evidence that the pathway is limited. For example, in the case of hepatic fatty acid oxidation, it might be shown that the ketogenic response to prolonged fasting is impaired.
(3) There should be direct evidence that the pathway limitation is relieved by treatment with carnitine. For the example of hepatic fatty acid oxidation, carnitine treatment should be demonstrated to completely correct the defect in fasting ketogenesis.
(4) The underlying mechanism of the carnitine deficiency should be identified.

The following sections will briefly review examples of "carnitine deficiency", focusing on the two disorders that meet these criteria.

OVERVIEW OF CARNITINE METABOLISM

Carnitine is a trimethyl-amino-3-hydroxy acid that can be provided both from the diet and by endogenous synthesis from trimethyl-lysine. The rate-limiting step in synthesis is the slow rate at which posttranslationally methylated lysine residues are released during turnover of body proteins. Dietary sources are limited to animal products, such as meats and milk. Carnitine is not metabolized and is excreted, mostly as free carnitine, in the urine.[1]

FIGURE 1 outlines the body pools and daily turnover of carnitine. Over 99% of body carnitine is intracellular. Tissue concentrations are very high: 2–3 mmol/kg in muscle and 800–1500 mmol/kg in liver. Circulating carnitine accounts for only about 0.5% of body carnitine, and plasma levels are very low, 40–60 μmol/L. Daily turnover of body carnitine is similar in size to the plasma pool, with daily urinary carnitine excretion equal to the sum of dietary absorption and endogenous synthesis. Dietary sources account for about 75% of carnitine turnover; however, carnitine-restricted diets have little impact on total body carnitine because of efficient renal conservation of carnitine.

The most important factor controlling body pools of carnitine is the plasma membrane sodium-dependent carnitine transporter (see FIG. 1). This transporter, OCTN2, is encoded by the SLC22A5 gene on 5q.[2,3] It is expressed on the plasma membrane of cultured skin fibroblasts and is responsible for maintaining the large tissue gradient for carnitine in muscle (but possibly not in liver) and for setting the renal

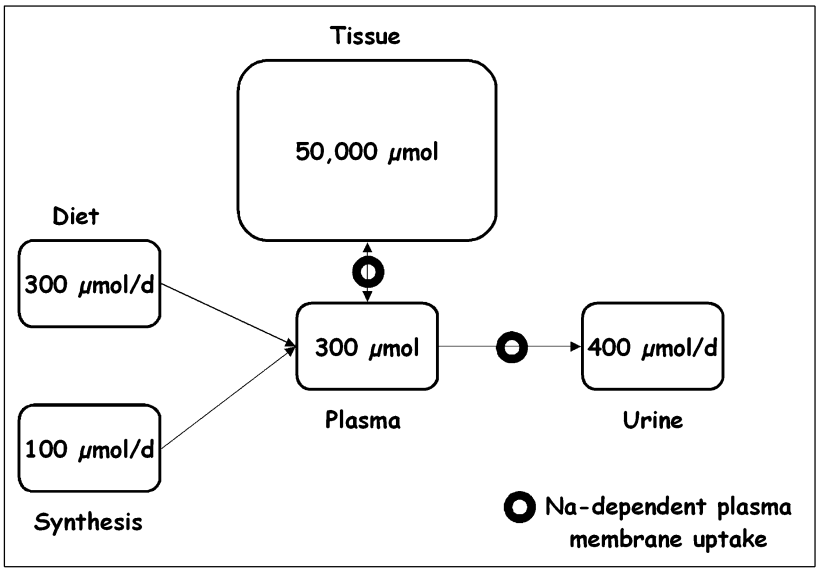

FIGURE 1. Body stores of carnitine and rates of carnitine turnover from diet, endogenous synthesis, and urinary excretion.

threshold for carnitine excretion at ~50 μmol/L (equal to normal plasma carnitine concentration). Within tissues, such as muscle and liver, carnitine concentrations are 20–50 times higher than plasma levels.

As shown in FIGURE 2, carnitine shuttles fatty acids across the mitochondrial inner membrane via the carnitine cycle. Fatty acyl-CoA esters exchange with carnitine to form acylcarnitine esters via an outer mitochondrial CPT-1; the acyl-carnitine esters are carried across the inner mitochondrial membrane by CACT (SLC25A20 on 3p); the acylcarnitine esters are converted back to acyl-CoA esters by CPT-2 to enter the β-oxidation cycle; and the free carnitine is recycled.

CARNITINE DEFICIENCY DUE TO RECESSIVE MUTATIONS OF THE PLASMA MEMBRANE SODIUM-DEPENDENT CARNITINE TRANSPORTER (OCTN2 OR SLC22A5)

This disorder was first defined in 1988 in an infant who presented with fasting hypoketotic hypoglycemic coma and fatty hepatomegaly associated with extremely low concentrations of carnitine in plasma, liver, and skeletal muscle.[4] Treatment with oral carnitine at pharmacologic levels led to complete correction of fasting ketogenesis, indicating both that the carnitine deficiency was responsible for the impairment in hepatic fatty acid oxidation and that the defect was correctable with carnitine supplementation. Since the child developed the illness while on a carnitine-deficient soy formula, the possibility of nutritional deficiency was entertained. However, *in vitro* studies demonstrated a nearly complete absence of sodium-gradient

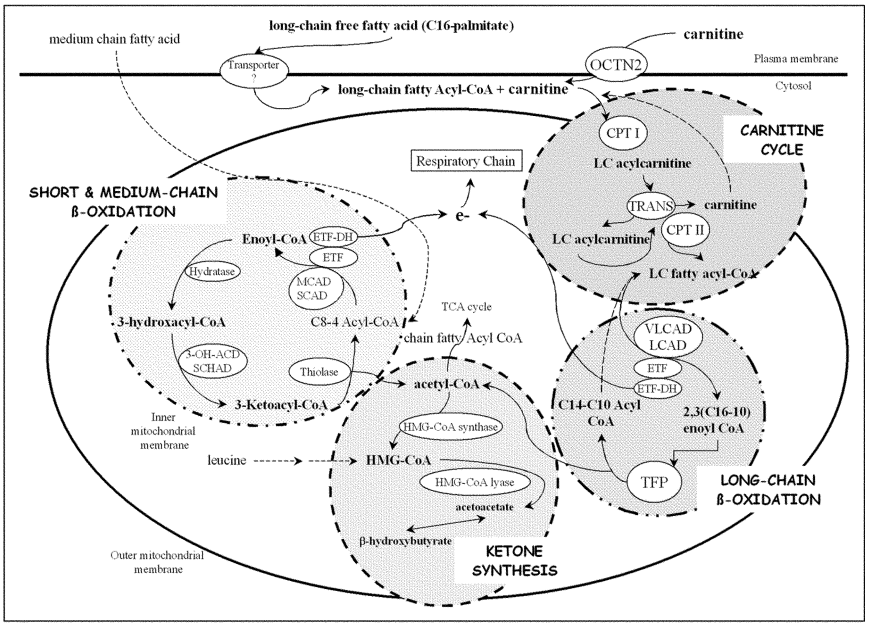

FIGURE 2. Carnitine and the pathway of mitochondrial fatty acid oxidation. Abbreviations: OCTN2, plasma membrane sodium-dependent carnitine transporter; CPT-I, carnitine palmitoyltransferase-1; TRANS, carnitine-acylcarnitine translocase; CPT-II, carnitine palmitoyltransferase-2; VLCAD, very long chain acyl-CoA dehydrogenase; ETF, electron transfer flavoprotein; ETF-DH, ETF dehydrogenase; TFP, trifunctional protein (long-chain hydroxyacyl-CoA dehydrogenase); MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; SCHAD, short-chain 3-hydroxy-acyl-CoA dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

dependent carnitine uptake by cultured skin fibroblasts, as well as intermediate plasma carnitine levels and fibroblast carnitine uptake activity in both of the child's parents. Subsequent genetic studies have shown that this carnitine transporter defect is due to recessively inherited mutations of the OCTN2 (SLC22A5) gene on 5q.[2,3] Studies of the mechanism of the carnitine deficiency indicated that the plasma carnitine deficiency was due to a failure of renal conservation of filtered carnitine, with a renal threshold of nearly 0, compared to the normal value of 40–60 μmol/L. Thus, this disorder fulfills all of the criteria spelled out above for defining clinical carnitine deficiency.

A majority of the children who have been subsequently identified with this OCTN2 deficiency have presented not with attacks of fasting hypoglycemic coma, but with progressive cardiomyopathy and skeletal muscle weakness.[5] The average age of presentation of cardiomyopathy has been 2–4 years of age, indicating that it takes a long period of time for manifestations of severe carnitine deficiency in heart and skeletal muscle to appear. Several instances of death from cardiac failure in patients prior to diagnosis have been seen, suggesting that OCTN2 deficiency is eventually fatal if not treated. Treatment with oral carnitine at pharmacologic levels

is quite effective in correcting the cardiomyopathy and muscle weakness in these children. Several children have survived into their third decade and appear to be doing well, even though carnitine treatment raises muscle carnitine concentrations to only 5–10% of normal. At least one case has completed college-level education. Two patients have died suddenly of cardiac arrest in their second and third decade when carnitine supplementation was discontinued, suggesting that continued treatment is essential.[6]

Data available on a small number of cases indicate that pharmacological doses of carnitine can raise plasma levels nearly to normal, but that muscle concentrations reach no higher than 5–10% of normal.[1,5] This poor response is consistent with defective functioning of the muscle plasma membrane carnitine transporter; that is, carnitine uptake by muscle is limited to passive diffusion from plasma, giving concentrations of not much more than 30–60 μmol/L (vs. normal levels of 2000–3000 μmol/L). Since these very modest tissue concentrations of carnitine seem to be sufficient in affected patients, the tissue levels at which carnitine becomes limiting for cardiac and skeletal muscle function must be less than 5% of values seen in normal individuals.

CARNITINE DEPLETION DUE TO CHRONIC ADMINISTRATION OF PIVALATE-CONJUGATED ANTIBIOTICS

The second of the two disorders that meet the criteria noted above for carnitine deficiency is an acquired carnitine depletion associated with chronic administration of antibiotics conjugated with pivalate, a highly branched-chain fatty acid.[1,7] In humans, pivalate is almost exclusively metabolized through formation of its acyl-CoA ester, which is then transesterified with carnitine to form pivaloyl-carnitine. The latter compound is excreted in urine in stoichiometric amounts equal to ingested pivalate. Since excretion of pivaloyl-carnitine can exceed the sum of normal daily intake and synthesis of carnitine by 10-fold, there is a net loss of total body carnitine, which eventually reduces plasma and tissue levels to values similar to those seen in patients with OCTN2 deficiency.[7]

Reports of pivalate-induced carnitine depletion have come almost entirely from Sweden, where pivalate-containing antibiotics were being used for long-term prophylaxis of urinary tract infections in children and adults. Although no cases of symptomatic carnitine deficiency were reported in treated patients (e.g., attacks of fasting hypoketotic hypoglycemia or clinically apparent cardiomyopathy and weakness that are associated with OCTN2 deficiency), formal fasting tests in a half-dozen pivalate-treated patients demonstrated clear evidence of impaired ketogenesis, which, in a few, was corrected after carnitine repletion. Subclinical manifestations may have occurred in some children on long-term treatment with pivalate conjugates; the parents of some cases reported noticing reduced stamina that improved after discontinuing pivalate or supplementation with oral carnitine.

Studies of pivalate administration to normal adults for a week showed that there was a rapid, 10-fold increase in urinary carnitine loss, all in the form of pivaloyl-carnitine, accompanied by a 50% drop in plasma total carnitine and a marked rise in plasma esterified carnitine (presumably pivaloyl-carnitine). It was estimated that a few months on usual doses of pivalate-conjugated antibiotics would be required to deplete total body carnitine stores.

TABLE 1. Plasma carnitine alterations in fatty acid oxidation disorders in children

Defect	Total plasma carnitine (μmol/L)	Plasma acylcarnitine (% of total)
OCTN2	<5	<30
CPT-1	60–100	<20
TRANS	5–30	80–100
CPT-2	10–20	40–80
VLCAD	10–30	30–60
MCAD	10–30	30–60
SCAD	10–30	30–60
LCHAD	10–30	30–60
ETF	10–30	30–60
EFT-DH	10–30	30–60
HMG-synthase	40–60	<30
HMG-lyase	10–30	30–60
Normals	40–60	<30

NOTE: Abbreviations given in the caption to FIGURE 1. Modified from reference 1.

Pivalate-induced carnitine depletion clearly fulfills the criteria for being a disorder caused by carnitine deficiency, although there do not appear to have been any spontaneous cases of morbidity or mortality. The latter observation is not surprising since, as noted above, it seems to take several years for cardiac or muscle manifestations to develop in children with carnitine deficiency associated with OCTN2 deficiency.

ABNORMALITIES IN PLASMA AND TISSUE CARNITINE LEVELS ASSOCIATED WITH GENETIC FATTY ACID OXIDATION ENZYME DEFECTS

Eleven recessively inherited enzymatic defects have been identified in the pathway of mitochondrial fatty acid oxidation, most of which are associated with abnormalities of plasma carnitine levels (see TABLE 1).[1] Many of these are defects that create blocks at the level of an acyl-CoA intermediate (such as the common MCAD deficiency) and are associated with reduction in plasma carnitine concentrations to 25–50% of normal. Notable exceptions are the mitochondrial HMG-CoA synthase deficiency (normal plasma carnitine) and CPT-1 deficiency (elevated plasma carnitine). In contrast to OCTN2 deficiency, the carnitine abnormalities in these disorders are the consequence, rather than the cause, of the impairment in fatty acid oxidation.

The mechanism of the carnitine abnormalities in those fatty acid oxidation enzyme defects associated with reduced plasma carnitine levels is different from the acylcarnitine depletion process that is discussed above for pivalate-induced carnitine

TABLE 2. Apparent renal threshold for free carnitine in fatty acid oxidation enzyme deficiencies and organic acidemias

Defect	*n*	Renal free carnitine threshold (μmol/L)
OCTN2	2	<2
CPT-1	1	>90
TRANS	1	<10
VLCAD	2	43–52
MCAD	2	13–25
Isovaleric acidemia	3	16–18
Propionic acidemia	1	14–23
Normal controls	3	50–60

NOTE: Abbreviations given in the caption to FIGURE 1. See reference 10.

TABLE 3. Half-maximal acylcarnitine inhibitory concentrations for free carnitine uptake by fibroblasts

Carnitine ester	ID_{50} (μmol/L)
Free carnitine	2.7 ± 0.6
Acetyl (C2)	4.6 ± 0.5
Octanoyl (C8)	2.9 ± 0.4
Myristoyl (C12)	0.16 ± 0.02
Palmitoyl (C16)	0.37 ± 0.06

NOTE: See reference 5.

deficiency. As shown in TABLE 1, these defects are associated with only a partial reduction in plasma carnitine levels that remains quite stable over time, rather than progressing to the complete depletion of carnitine pools as occurs with pivalate administration. The underlying mechanism of carnitine deficiency in these acyl-CoA oxidation enzyme defects appears to involve an impairment in transport of free carnitine since studies of renal handling of carnitine in a number of these disorders show a reduction of the renal threshold for free carnitine from values seen in normal controls of 40–60 μmol/L to values ranging from 15 to 40 μmol/L (see TABLE 2).[1] A likely explanation for this alteration in free carnitine conservation is that the acyl-carnitines, which are associated with the enzymatic blocks in fatty acid oxidation, particularly the long-chain acylcarnitine species, are potent inhibitors of free carnitine transport by OCTN2 (see TABLE 3).[5] This explanation is consistent with the observation that CPT-1 deficiency, which impairs the generation of long-chain fatty acylcarnitines, is associated with an increase in plasma levels of carnitine and of the renal threshold for free carnitine.[1,5]

The above considerations about the mechanism of carnitine deficiency associated with the fatty acid oxidation enzyme defects raise the need for skepticism about the utility of carnitine supplements in these disorders. Clearly, carnitine treatment would

not be expected to correct the impairment in fatty acid oxidation in these disorders as it does in patients with deficiency of OCTN2 or carnitine depletion associated with pivalate administration. Based on the examples of patients with these latter two forms of carnitine deficiency, the degree of carnitine deficiency in patients with enzymatic defects of fatty acid oxidation is not severe enough to limit the acylcarnitine transferase reactions. Oral carnitine can increase urinary excretion of the abnormal acylcarnitine ester in many of these disorders, for example, propionyl-carnitine in propionic acidemia.[8] It is unclear whether carnitine administration would help in "detoxifying" potentially harmful acyl-CoA products associated with these defects since there is not the stoichiometric relation between acyl-CoA formation and acylcarnitine excretion that applies in the case of pivalate administration. Oral administration of carnitine might increase urinary excretion of acylcarnitines (and free carnitine) in these patients to as much as 2–3 mmol/day, but this is very much less than the capacity for generating intermediates. For example, assuming that basal fatty acid oxidation accounts for 10% of basal oxygen consumption, an adult would be expected to generate 50–80 mmol/day of fatty acid intermediates, that is, 10–100 times the maximum urinary excretion of acylcarnitines. Whether carnitine therapy is helpful in these disorders remains a matter of controversy.

Other disorders associated with reduced plasma carnitine concentrations include patients with renal Fanconi's syndrome (impaired renal tubular conservation of filtered carnitine), carnitine-free feedings (e.g., neonates on intravenous alimentation), and children treated with valproate for seizures. In all of these situations, plasma and tissue carnitine levels appear to be much higher than the threshold for impairing fatty acid oxidation suggested by the data on children with the OCTN2 deficiency. In these disorders, also, the evidence of benefits from carnitine treatment remains uncertain.[1,9]

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