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Disorders of carnitine biosynthesis and transport

Ayman W. El-Hattab [a], Fernando Scaglia [b,*]

[a] Division of Clinical Genetics and Metabolic Disorders, Department of Pediatrics, Tawam Hospital, Al-Ain, United Arab Emirates
[b] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

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ABSTRACT

Carnitine is a hydrophilic quaternary amine that plays a number of essential roles in metabolism with the main function being the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix for β-oxidation. Carnitine can be endogenously synthesized. However, only a small fraction of carnitine is obtained endogenously while the majority is obtained from diet, mainly animal products. Carnitine is not metabolized and is excreted in urine. Carnitine homeostasis is regulated by efficient renal reabsorption that maintains carnitine levels within the normal range despite variabilities in dietary intake. Diseases occurring due to primary defects in carnitine metabolism and homeostasis are comprised in two groups: disorders of carnitine biosynthesis and carnitine transport defect. While the hallmark of carnitine transport defect is profound carnitine depletion, disorders of carnitine biosynthesis do not cause carnitine deficiency due to the fact that both carnitine obtained from diet and efficient renal carnitine reabsorption can maintain normal carnitine levels with the absence of endogenously synthesized carnitine. Carnitine transport defect phenotype encompasses a broad clinical spectrum including metabolic decompensation in infancy, cardiomyopathy in childhood, fatigability in adulthood, or absence of symptoms. The phenotypes associated with the carnitine transport defect result from the unavailability of enough carnitine to perform its functions particularly in fatty acid β-oxidation. Carnitine biosynthetic defects have been recently described and the phenotypic consequences of these defects are still emerging. Although these defects do not result in carnitine deficiency, they still could be associated with pathological phenotypes due to excess or deficiency of intermediate metabolites in the carnitine biosynthetic pathway and potential carnitine deficiency in early stages of life when brain and other organs develop. In addition to these two groups of primary carnitine defects, several metabolic diseases and medical conditions can result in excessive carnitine loss leading to a secondary carnitine deficiency.

Contents

1. Introduction

Carnitine is an amino acid derivative whose name was derived from the Latin word "carnis" that means meat or flesh because it was first isolated in meat extract in 1905 [1,2]. Carnitine is obtained from both diet and endogenous biosynthesis and is not metabolized but is excreted as free carnitine in urine. It is an essential metabolite with a number of

* Corresponding author at: Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, MS BCM225, Houston, TX 77030, USA.
E-mail address: fscaglia@bcm.edu (F. Scaglia).

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indispensable roles in intermediary metabolism with the main function being the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix, where β-oxidation takes place.

Defects in carnitine metabolism were first linked to human disease in 1975 when an 11-year old boy was reported to have recurrent episodes of hepatic and cerebral dysfunction, muscle wasting and weakness, in addition to marked carnitine deficiency in skeletal muscle, plasma, and liver. This condition was named "syndrome of systemic carnitine deficiency" [3]. Several years after that initial report, a 3.5 year old boy with cardiomyopathy and muscle weakness was reported to have low carnitine in muscle and plasma. When he was treated with carnitine, his urinary carnitine dramatically increased and he was found to have increased renal clearance of carnitine. These results suggested at that time that a defect in renal transport of carnitine was a likely cause of the child's disorder [4]. In 1988 it was suggested that individuals with systemic carnitine deficiency had a defect in the carnitine transport across the plasma membrane because the carnitine concentration in fibroblasts from affected individuals was found to be very low when they were incubated in carnitine rich media. These results suggested an inability to maintain a concentration gradient of intracellular carnitine over the plasma due to a defect in a carnitine transport [5,6]. Ten years later, in 1998 the gene responsible for systemic carnitine deficiency was mapped to chromosome 5q31.1-q32 through linkage analysis [7]. In 1999, the gene encoding the carnitine transporter OCTN2 (organic cation transporter 2) was cloned. The transporter was analyzed and found to have the ability to transport carnitine in a sodium-dependent manner. Sequencing the gene encoding OCTN2, *SLC22A5*, identified mutations in patients with systemic carnitine deficiency providing the first evidence that loss of OCTN2 function causes systemic carnitine deficiency. These mutations were also shown to decrease the levels of mature OCTN2 mRNA and result in a nonfunctional transporter, confirming that the defective function in OCTN2 was responsible for primary carnitine deficiency [8,9].

Defects in carnitine biosynthesis were not described until recently. In 2011, while performing array CGH (comparative genomic hybridization) on individuals with autism spectrum disorders, a hemizygous deletion in *TMLHE* was found in a male child with autism [10]. The *TMLHE* gene is located on chromosome X and encodes the enzyme catalyzing the first step in carnitine biosynthesis. Subsequently, other mutations in the *TMLHE* gene identified in children with autism raised the possibility of a relation between carnitine biosynthetic pathway defects and autism spectrum disorders [11–13]. More recently, a homozygous deletion that contains *BBOX1* gene encoding the last enzyme in carnitine biosynthesis was first reported in a child with growth failure and speech delay [14]. The phenotypic consequences of these recently described carnitine biosynthetic pathway defects are still emerging.

In this review article we summarize carnitine function and metabolism. Then we discuss the defects in carnitine biosynthesis and transport.

2. Carnitine function, biosynthesis, and homeostasis

Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a hydrophilic quaternary amine which is an essential metabolite with a number of indispensable roles in intermediary metabolism. The main function of carnitine is the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix, where β-oxidation takes place. Carnitine is also involved in the transfer of the products of peroxisomal β-oxidation, including acetyl-CoA, to the mitochondria for oxidation via the Krebs cycle. Other functions include modulation of the acyl-CoA/CoA ratio, storage of energy as acetyl-carnitine, and modulating the toxic effects of poorly metabolized acyl groups by binding and excreting them as carnitine esters in urine [15–19].

Carnitine is present in most, if not all, animal species, and in several micro-organisms and plants. Animal tissues contain relatively high amounts of carnitine, varying between 0.2 and 6 μmol/g, with the highest concentrations present in heart and skeletal muscle [16].

Carnitine is obtained from both diet and endogenous biosynthesis and is not metabolized but is excreted as free carnitine in urine [20]. The main sources of dietary carnitine are animal products including red meat, chicken, fish, and dairy products. Carnitine is synthesized ultimately from the amino acids lysine and methionine. Lysine provides the carbon backbone of carnitine and the N-methyl groups originate from methionine. Lysine residues of certain proteins, such as calmodulin, myosin, actin, cytochrome c, and histones, undergo post-translational N-methylation. This reaction is catalyzed by methyltransferases using S-adenosylmethionine as a methyl donor and resulting in 6-N-trimethyllysine (TML) residues. Lysosomal hydrolysis of the TML-containing proteins results in the release of TML, which is the first metabolite of carnitine biosynthesis. TML is hydroxylated by TML dioxygenase (TMLD) to yield 3-hydroxy-6-N-trimethyllysine (HTML). Subsequently, HTML aldolase (HTMLA) cleaves HTML to 4-N-trimethylaminobutyraldehyde (TMABA) and glycine. Dehydrogenation of TMABA by TMABA dehydrogenase (TMABADH) results in the formation of 4-N-trimethylaminobutyrate (butyrobetaine). In the last step, butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase (BBD) to yield carnitine (3-hydroxy-4-N-trimethylaminobutyrate) (Fig. 1) [16].

The first three enzymes of carnitine synthesis TMLD, HTMLA, and TMABADH are widely distributed, however, BBD is only present in liver, kidney and brain. Therefore many tissues can synthesize butyrobetaine from TML, whereas, carnitine is synthesized in kidney, liver, and brain. BBD activity is highest in kidney and lowest in brain. In contrast to BBD enzymatic activity in kidney which is not age-dependent, the BBD activity in liver in infants is about 10% of the adult levels [21,22].

The endogenous carnitine biosynthesis is estimated to be 1.2 μmol/kg/day, whereas regular diet provides 2–12 μmol/kg/day carnitine. Therefore, in individuals consuming a regular diet about 75% of carnitine (~300 μmol daily) comes from diet and only 25% of it (~100 μmol daily) comes from endogenous synthesis. Since carnitine is present primarily in animal products, strict vegetarians (vegans) and lacto-ovo-vegetarians obtain very little carnitine from diet (<0.1 μmol/kg/day). Therefore, vegetarians obtain more than 90% of their carnitine through biosynthesis (Fig. 2) [23,24].

The carnitine pool consists of non-esterified carnitine (free carnitine) and many acylcarnitine esters, the later representing the carnitine pool bounded to different fatty acids. About 99.5% of body carnitine is intracellular (~50,000 μmol), while circulating plasma carnitine accounts for only 0.5% of total body carnitine (~200 μmol). Total plasma carnitine concentration is 30–70 μmol/L, whereas, the carnitine concentration within tissues such as muscle and liver is 25–50 times higher than plasma levels (2000–3000 μmol/L). Carnitine is not metabolized but is excreted as free carnitine in urine. Daily urinary carnitine excretion equals the sum of dietary absorption and endogenous synthesis (~400 μmol daily) (Fig. 2) [23,24].

Carnitine homeostasis is maintained by absorption from the diet, a modest rate of synthesis, and an efficient renal reabsorption. At normal circulating carnitine concentrations, renal carnitine reabsorption is highly efficient (90–99% of filtered load), but displays saturation kinetics with a renal threshold for carnitine excretion being ~50 μmol/L which is equal to the normal plasma carnitine concentration. Thus, when circulating carnitine concentration increases, efficiency of reabsorption decreases and clearance increases, resulting in rapid decline of circulating carnitine concentration to baseline. On the other hand, when dietary carnitine intake is reduced, efficiency of renal reabsorption increases and clearance decreases, resulting in maintaining the circulating carnitine concentration within the normal range. Therefore, as the dietary carnitine intake varies, urinary carnitine excretion varies to maintain the plasma carnitine level within the normal range [25].

The main factor regulating carnitine body pools is OCTN2 which is a high affinity plasma-membrane sodium-dependent carnitine