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Enzymes involved in L-carnitine biosynthesis are expressed by small intestinal enterocytes in mice: Implications for gut health

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Abstract

Background: Carnitine is essential for mitochondrial β-oxidation of long-chain fatty acids. Deficiency of carnitine leads to severe gut atrophy, ulceration and inflammation in animal models of carnitine deficiency. Genetic studies in large populations have linked mutations in the carnitine transporters OCTN1 and OCTN2 with Crohn's disease (CD), while other studies at the same time have failed to show a similar association and report normal serum carnitine levels in CD patients.
Methods: In this report, we have studied the expression of carnitine-synthesizing enzymes in intestinal epithelial cells to determine the capability of these cells to synthesize carnitine *de novo*. We studied expression of five enzymes involved in carnitine biosynthesis, namely 6-*N*-trimethyllysine dioxygenase (TMLD), 4-trimethylaminobutyraldehyde dehydrogenase (TMABADH), serine hydroxymethyltransferase 1 and 2 (SHMT1 and 2) and γ-butyrobetaine hydroxylase (BBH) by real-time PCR in mice (C3H strain). We also measured activity of γ-BBH in the intestine using an *ex vivo* assay and localized its expression by *in situ* hybridization.

Abbreviations: TMLD, 6-*N*-trimethyllysine dioxygenase; TMABA, 4-trimethylaminobutyraldehyde; HTMLA, β-hydroxy-ε-*N*-trimethyllysine aldolase; SHMT1 and 2, serine hydroxymethyltransferase 1 and 2; TMABA-DH, TMABA dehydrogenase; γ-BBH, γ-butyrobetaine hydroxylase; OCTN, organic cation transporter novel; HPRT, hypoxanthine phosphoribosyl transferase; CD, Crohn's disease; IBD, inflammatory bowel disease.

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Results: Our investigations show that mouse intestinal epithelium expresses all five enzymes required for *de novo* carnitine biosynthesis; the expression is localized mainly in villous surface epithelial cells throughout the intestine. The final rate-limiting enzyme γ-BBH is highly active in the small intestine; its activity was 9.7±3.5 pmol/mg/min, compared to 22.7±7.3 pmol/mg/min in the liver.
Conclusions: We conclude that mouse gut epithelium is able to synthesize carnitine *de novo*. This capacity to synthesize carnitine in the intestine may play an important role in gut health and can help explain lack of clinical carnitine deficiency signs in subjects with mutations with OCTN transporters.

1. Introduction

Carnitine (β-hydroxyl-γ-trimethylaminobutyric acid) acts as an obligatory cofactor for β-oxidation of fatty acids by facilitating transport of long-chain fatty acids across the inner mitochondrial membrane as acylcarnitine esters. Carnitine plays a major role in tissues (*e.g.*, heart, skeletal muscle, liver, placenta, and the intestinal tract), which depend on β-oxidation of fatty acids for energy production. Over >99% of carnitine in the body is intracellular, and the organic cation transporter OCTN2 (SLC22A5) is primarily responsible for maintaining this tissue gradient.[1] Two distinct types of carnitine deficiencies have been identified. Primary carnitine deficiency arises from defects in the plasma membrane carnitine transporter OCTN2. Patients with this disorder excrete carnitine in urine due to defective reabsorption and plasma and tissue levels of carnitine may drop below 10% of normal values.[2–6] Secondary carnitine deficiency arises from defects in any of the enzymes involved in mitochondrial fatty acid oxidation where organic acids accumulate due to block in this metabolic pathway; these organic acids then bind to carnitine and are excreted in urine in the form of acylcarnitines.[7,8] It has been estimated that roughly 75% of total carnitine in the body is obtained from dietary sources and about 25% comes from endogenous synthesis.[9]

Carnitine is biosynthesized from essential amino acids lysine and methionine (Fig. 1). ε-*N*-trimethyllysine, which is formed by methylation of protein-bound lysine and is released in free form after protein degradation, is the precursor for carnitine biosynthesis. This lysine derivative is hydoxylated by ε-*N*-trimethyllysine dioxygenase (TMLD). Subsequently, the resulting β-hydroxyl-ε-*N*-trimethyllysine is cleaved into γ-trimethylaminobutyraldehyde and glycine by β-hydroxy-ε-*N*-trimethyllysine aldolase (HTMLA), after which the aldehyde is oxidized by γ-trimethylaminobutyraldehyde dehydrogenase (TMABADH) to yield γ-butyrobetaine. The final step in this pathway involves the hydroxylation of γ-butyrobetaine by γ-butyobetaine hydroxylase (γ-BBH) to yield L-carnitine.[10,11]

While clinical consequences of carnitine deficiency (primary or secondary) in the myocardium and skeletal muscle have received much attention, the relevance of carnitine deficiency to intestinal tract, particularly during inflammation has recently been recognized and it may thus have a clinical role in gut health.[12–16] The role of carnitine in the intestinal tract has been highlighted by several publications linking mutations in genes encoding carnitine transporters OCTN1 (SLC22A4) and OCTN2 (SLC22A5) with Crohn's disease (CD).[17–23] Patients with CD have been shown to have a missense substitution 1762C→T in OCTN1 which causes amino acid substitution L503F and a G→C transversion in the promoter region of OCTN2 (−207G→C), which disrupts a heat shock binding element (HSE) in the promoter region of *OCTN2* gene. These mutations have been shown to decrease plasma membrane transport of carnitine in transiently transfected cell line studies and may thus reduce tissue content of carnitine.[17] Over the past several years, these human genetic studies have been replicated in large populations with CD, suggesting a cause-and-effect relationship between these mutations and inflammatory bowel disease. A meta-analysis of 12 of these studies with >3000 subjects in each arm showed a robust association between these mutations and CD.[24] Therefore, carnitine supplementation could potentially have a therapeutic benefit in patients with inflammatory bowel disease (IBD) in general and CD in particular. However, these studies are in sharp contrast with few other reports, which have failed to show a clear link among plasma levels of carnitine, CD and OCTN1 (1762C→T) and OCTN2 (−207G→C) mutations.[25,26] This discrepancy may be explained by the differences between plasma and tissue carnitine levels since tissue levels were not measured in human subjects with IBD. At the same time a few recent reports in animals[27,28] and humans[29] have demonstrated beneficial effects of carnitine supplementation on gut inflammatory markers and patient symptoms.

In view of this controversy regarding any potential relationship between defective carnitine transporters and IBD, we asked whether the intestinal tract is capable of synthesizing carnitine *de novo*. If this tissue does indeed possess the enzymatic machinery necessary to synthesize carnitine, it might explain the lack of gut pathology in patients with inactivating mutations in the carnitine transporters. We addressed this question by examining the expression of enzymes involved in *de novo* biosynthesis of carnitine in mouse intestinal tract.

2. Methods

2.1. Animals and sample preparation

Six normal mice (C3H strain) were used in these studies. Tissues from various parts of intestinal tract, and other viscera were thoroughly washed and freed from blood, were placed in chilled phosphate-buffered saline (PBS) and snap-frozen at −80 °C for further analysis. All experimental procedures were