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Molecular and biochemical aspects of carnitine biosynthesis

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Chapter *1*

Introduction

Introduction

Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a small water-soluble molecule, which is present in most, if not all animal species and in several micro-organisms and plants. Carnitine plays an indispensable role in fatty acid metabolism, where it is involved in the transport of activated fatty acids between different cellular compartments. In cells, fatty acids are present as esters with coenzyme A (CoA), acyl-CoAs. The acyl-moiety of acyl-CoAs can be transesterified to the 3-hydroxy-group of carnitine by the action of carnitine acyl-transferases, which results in the formation of acyl-carnitine esters (FIG. 1).

Fatty acids are degraded in both mitochondria and peroxisomes. Cytosolic long-chain fatty acids, which are destined for degradation via β -oxidation in the mitochondrial matrix, are transported over the inner mitochondrial membrane as carnitine-esters. In the mitochondrial matrix the acyl-carnitine is reconverted to acyl-CoA by another carnitine acyl transferase, after which the acyl-CoA can be β -oxidised. Furthermore, products of the peroxisomal β -oxidation system, including acetyl-CoA, are transported as carnitine-esters from peroxisomes to mitochondria for complete degradation to CO_2 and H_2O .

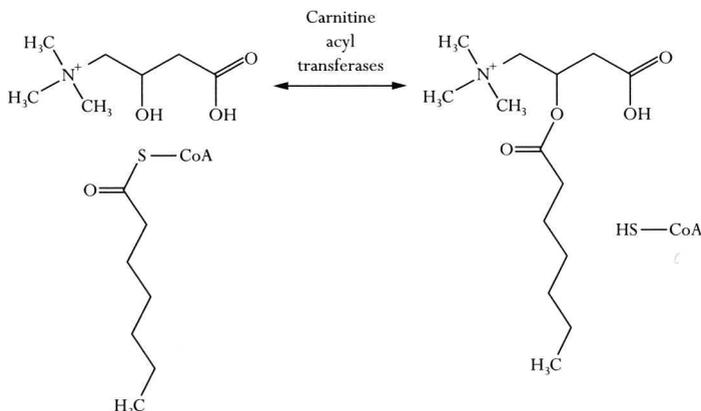


FIG. 1: The reaction catalysed by carnitine acyl-transferases; the acyl-moiety of acyl-CoA is transesterified to the 3-hydroxy-group of carnitine by the action of these enzymes, which results in the formation of an acyl-carnitine ester.

Humans obtain most of their carnitine through the diet, primarily via the consumption of meat, poultry, fish and dairy products, which contain considerable amounts of carnitine. Apart from the dietary intake, carnitine is synthesised in liver and kidney from protein-derived 6-N-trimethyllysine via 3-hydroxy-6-N-trimethyllysine, 4-N-trimethylaminobutyraldehyde and 4-N-trimethylaminobutyrate (4-N-butyrobetaine). Since in rats only the liver and in humans also the kidneys, are capable of carnitine synthesis, other cells depend on carnitine import via active uptake from the blood. This transport system is also involved in the renal tubular reabsorption and intestinal absorption of carnitine. Taken together, carnitine homeostasis is maintained by dietary intake, a modest rate of endogenous synthesis and efficient tubular reabsorption of carnitine by the kidney. At the beginning of the studies described in this thesis, there was very little information on each of these aspects of carnitine homeostasis. Indeed, although the sequence of metabolites of carnitine biosynthesis already had been established in the 1970's, none of the enzymes of this pathway had been characterised at the molecular level and relatively little was known about

the role and importance of carnitine biosynthesis in mammalian metabolism. On the other hand the crucial role of carnitine in energy homeostasis was evident from patients suffering from systemic carnitine deficiency who show excessive renal and intestinal wastage of carnitine, which results in very low plasma and tissue carnitine concentrations. This potentially lethal disorder is characterised by cardiomyopathy, myopathy, recurrent episodes of hypoketotic hypoglycaemia, hyperammonemia and failure to thrive. Studies in cells of these patients have shown that this disorder is caused by a defect in the active cellular uptake of carnitine into the cell. The gene encoding the plasma-membrane carnitine transporter, which is putatively mutated in this disorder, was not known.

A number of patients suffering from chronic fatigue syndrome have been reported to respond well to oral carnitine therapy. This disorder, which is also known as myalgic encephalomyelitis (ME), has a high incidence (~0.2% of the population) but the cause is still unknown. Chronic fatigue syndrome patients suffer from prolonged generalised fatigue, muscle weakness, myalgia, and postexertional malaise. Since carnitine therapy is beneficial to these patients, it has been suggested that a defect in carnitine metabolism might cause this syndrome. Another group of patients presenting with various pathologies in combination with low plasma carnitine concentrations has been identified by our group and by others during diagnostic screening, and it has been suggested that the underlying defect in some of these patients might be a deficiency of carnitine biosynthesis.

The study towards defects in carnitine biosynthesis, however, was hampered at that time by the lack of knowledge of the carnitine biosynthesis enzymes at the molecular level and efficient methods to analyse the metabolites of this pathway in body fluids.

In order to study carnitine metabolism in these groups of patients, complete resolution and understanding of the regulation of the carnitine biosynthesis pathway is required. Therefore, it was the purpose of these studies to shed more light on the identity of the genes coding for the enzymes of carnitine biosynthesis and to develop a method to determine the concentration of the carnitine biosynthesis metabolites in body fluids.

A review of the studies conducted on carnitine biosynthesis and transport including the data described in the experimental chapters 3-7 is presented in chapter 2. Chapters 3, 4 and 5 describe the identification and characterisation of three of the four enzymes of carnitine biosynthesis at the molecular level. Chapter 6 presents a novel HPLC tandem MS method to measure the concentration of the carnitine biosynthesis metabolites in urine. In chapter 7 the underlying molecular defect in three systemic carnitine deficiency patients is described.

