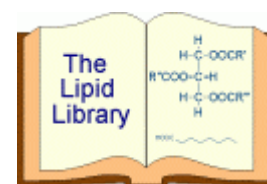
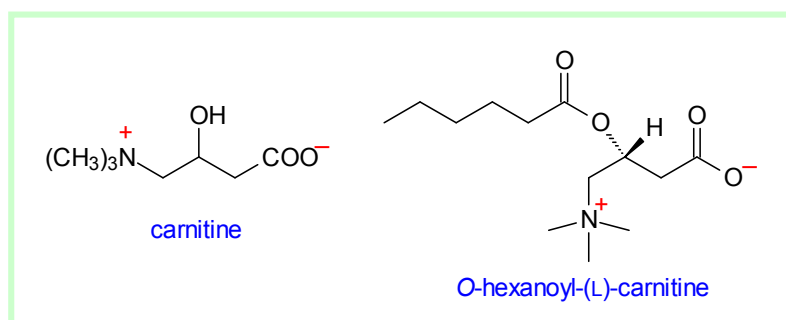


CARNITINE AND ACYLCARNITINES

STRUCTURE, OCCURRENCE, BIOLOGY AND ANALYSIS



Carnitine (L-3-hydroxy-4-aminobutyrobetaine or L-3-hydroxy-4-*N*-trimethylaminobutanoic acid), and its acyl esters (**acylcarnitines**) are essential compounds for the metabolism of fatty acids. They are present in animals, plants and some microorganisms. In animal tissues, carnitine concentrations are relatively high, typically between 0.2 and 6mmol/kg, with most in the heart and skeletal muscle.



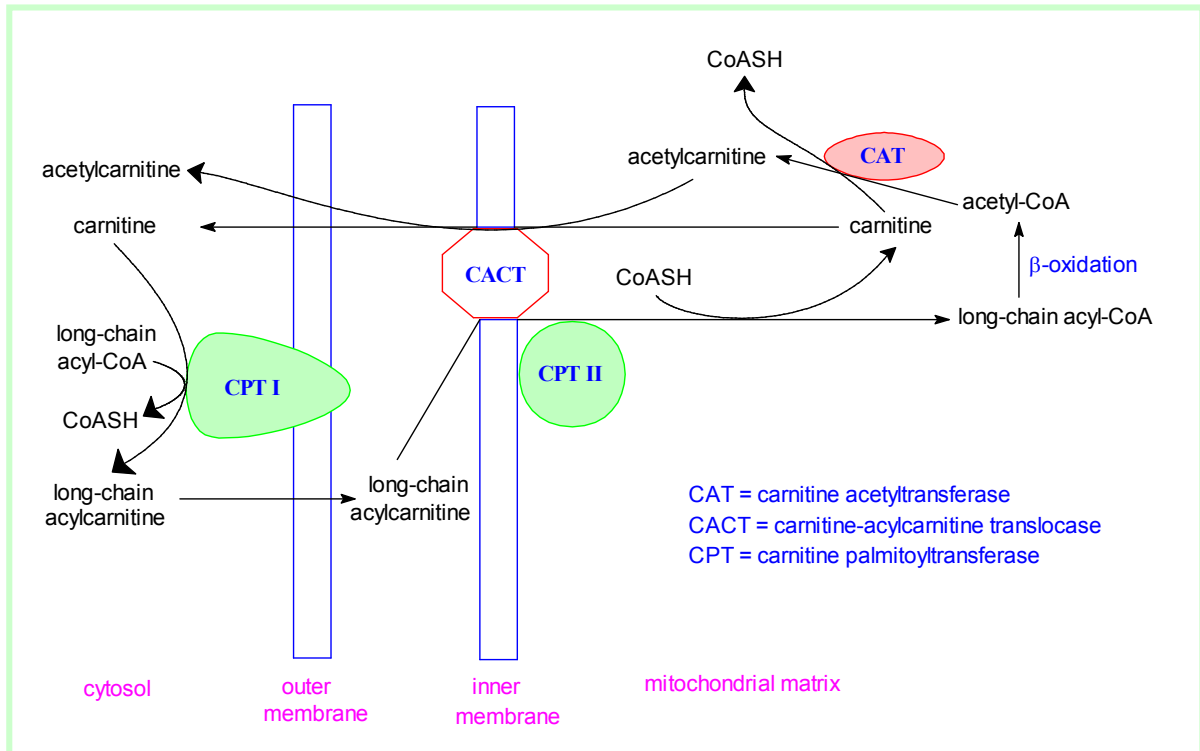
L-Carnitine can be synthesised *de novo* in animal cells by a multi-step process, with lysine and methionine, derived from protein degradation, as the primary precursors and butyrobetaine as an intermediate. However, it is believed that most comes from the diet, and plasma carnitine levels are positively correlated with the dietary intake. In humans, the major sources of carnitine are meat, fish and dairy products, which can supply 2 to 12 μmol per day per kg of body weight, as opposed to 1.2 μmol per day per kg of body weight of endogenous carnitine. The latter is synthesised in the kidney, liver and brain and is transported to other tissues in the circulation before it is taken up by active transport systems. In the kidney, carnitine and butyrobetaine are reabsorbed efficiently so urinary loss is minimized.

In mammals, carnitine functions through the reversible esterification of its 3-hydroxyl group, with subsequent translocation of the acylcarnitines produced from one cellular compartment to another. Carnitine acyltransferases are the enzymes responsible for the production of acylcarnitines, and these can have differing chain-length specificities, but covering the entire range of acyl chain lengths, depending on the cellular location and metabolic purpose.

The main function of carnitine is to assist the transport and metabolism of fatty acids in mitochondria, where they are oxidized as a major source of energy. In so doing, carnitine maintains a balance between free and esterified coenzyme A, since an excess of **acyl CoA** intermediates is potentially toxic to cells. In addition, carnitine is required to remove any surplus of acyl groups from mitochondria, and to export acetyl- and other short-chain acyl groups from peroxisomes via the action of carnitine octanoyltransferase, which as the name suggests is specific for medium-chain fatty acyl moieties. These activities influence in turn innumerable aspects of carbohydrate and lipid metabolism, including the regulation of insulin secretion by pancreatic β -cells and the determination of tissue insulin sensitivity.

Several enzymes are involved in the various processes that occur. Fatty acids are first activated by being bound to coenzyme A to form highly polar thiol esters, i.e. acyl-CoA, on the outer mitochondrial membrane. As these cannot cross the inner mitochondrial membrane, the acyl group is first transferred to carnitine with formation of acylcarnitines, which can enter the mitochondria

with the assistance of specific translocases. The transport system consists of the enzyme carnitine palmitoyltransferase I (CPT-I) present in the mitochondrial outer membrane, carnitine:acylcarnitine translocase, an integral inner membrane protein, and carnitine palmitoyltransferase II (CPT-II) located on the matrix side of the inner membrane. Then, inside the mitochondria, carnitine and acyl-CoA are regenerated, and the latter is catabolized in two-carbons units by *beta*-oxidation, with production of acetyl-CoA in normal circumstances. Finally, the acetyl groups are converted to acetylcarnitine via the action of carnitine acetyltransferase for transport out of the mitochondria.



This is a greatly simplified account of the process, and a number of enzymes are involved in the *beta*-oxidation aspect especially. In fact, at least 16 proteins are required that are organized into two functional subdomains, one associated with the inner face of the inner mitochondrial membrane and the other in the matrix. In addition, there are three isoforms of CPT I, each present in specific tissues: CPT IA in liver and kidney, CPT IB in heart and skeletal muscle and CPT IC in the brain. Malonyl-CoA binds with high affinity to each of these isoforms and is important for the regulation of the transfer of fatty acids into the mitochondrial matrix and thence their oxidation.

Deficiencies in any of these enzymes can cause an accumulation of acyl-CoA of specific chain-lengths, and these can have toxic effects if they are not removed by formation of acylcarnitines. As the acylation state of carnitine in the plasma reflects the composition of the cytosolic acylcarnitine pool, this serves as a diagnostic marker for the equilibrium between acyl-CoA and acylcarnitine species. In consequence, unusual acylcarnitines may be identified in biological fluids at unexpectedly high concentrations (they are found at low levels only in healthy individuals), and the chain lengths can be indicative of particular enzymic disorders. For example, several inherited metabolic diseases can be identified from the presence of acylcarnitines in the blood and urine of neonates, and from their chain-length profile, the point of the breakdown in the *beta*-oxidation pathway and the disease involved can be recognized. Similarly patients with peroxisomal biogenesis disorders, such as Zellweger syndrome, or with acidemias have abnormal profiles of circulating acylcarnitines. While the potential of L-carnitine and its esters as therapeutic agents is controversial, there is no doubt that it is life saving in patients with certain rare genetic disorders of carnitine metabolism. L-Carnitine deficiency is often seen in chronic hemodialysis patients, and in consequence it has been termed a "conditional vitamin".

Carnitine is also important to lipid metabolism in brain, where fatty acid oxidation is less significant though still relevant. In this tissue, acylcarnitines function in the synthesis of lipids and thence regulate membrane composition, they modify the activity of genes and proteins and they influence neurotransmission.

Although it has long been known that carnitine *per se* is present at very low levels in the tissues of many plant species, including seeds and leaves, the presence of acylcarnitines has only recently been demonstrated definitively. The role of acylcarnitines in plant metabolism has been controversial, but it is becoming apparent that they do indeed have important functions, though much remains to be learned.

Analysis

Acylcarnitines are highly polar molecules, and special precautions are required for extraction and analysis. For example, butanol saturated with water is usually recommended for extracting them from tissues. They are also zwitterionic molecules, so tend to chromatograph with phospholipids such as phosphatidylcholine in many chromatographic systems. However, many of the technical problems appear to have been solved (see the reviews cited below). Mass spectrometric methods appear to be especially suited to routine screening of large numbers of samples of biological fluids from neonates, as they permit a considerable degree of automation, both of the analytical steps and of gathering and interpretation of data.

Recommended Reading

- o Bartlett, K. and Eaton, S. Mitochondrial β -oxidation. *Eur. J. Biochem.*, **271**, 462-469 (2004).
- o Bourdin, B., Adenier, H. and Perrin, Y. Carnitine is associated with fatty acid metabolism in plants. *Plant Physiol. Biochem.*, **45**, 926-931 (2007).
- o Jones, L.L., McDonald, D.A. and Borum, P.R. Acylcarnitines: role in brain. *Prog. Lipid Res.*, **49**, 61-75 (2010).
- o Kerner, J. and Hoppel, C. Fatty acid import into mitochondria. *Biochim. Biophys. Acta*, **1486**, 1-17 (2000).
- o Minkler, P.E., Stoll, M.S.K., Ingalls, S.T., Yang, S.M., Kerner, J. and Hoppel, C.L. Quantification of carnitine and acylcarnitines in biological matrices by HPLC electrospray ionization-mass spectrometry. *Clin. Chem.*, **54**, 1451-1462 (2008).
- o Sim, K.G., Hammond, J. and Wilcken, B. Strategies for the diagnosis of mitochondrial fatty acid beta-oxidation disorders. *Clin. Chim. Acta*, **323**, 37-58 (2002).
- o Steiber, A., Kerner, J. and Hoppel, C.L. Carnitine: a nutritional, biosynthetic, and functional perspective. *Molecular Aspects Med.*, **25**, 455-473 (2004).
- o Vaz, F.M. and Wanders, R.A.J. Carnitine biosynthesis in mammals. *Biochem. J.*, **361**, 417-429 (2002).
- o Zammit, V.A., Ramsay, R.R., Bonomini, M. and Arduini, A. Carnitine, mitochondrial function and therapy. *Adv. Drug Delivery Rev.*, **61**, 1353-1362 (2009).

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