

The Carnitine System. Biochemical and Pharmacological Relevance.

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Abstract

Carnitine is required for the transport of activated acyls, namely acyl-CoAs, across the inner mitochondrial membrane. In addition, by interacting with Coenzyme A (CoA), carnitine exerts a role in any CoA-dependent process. An increase in CoASH availability or a decrease in acyl CoA levels expands the roles of carnitine to substrate choice, removal of inhibitory metabolites and modulation of key enzymatic steps. In addition, some carnitine esters appear to specifically modify cell metabolism and function. Finally, recent reports indicate antiapoptotic effects of carnitine which do not appear related to its metabolic role.

Carnitine-dependent enzymes

The reversible exchange of acyl moieties between CoA and carnitine is catalyzed by several carnitine acyl transferases (Fig. 1).

The differences among these enzymes can be described in terms of cellular localization, substrate specificity, structure and reactivity with inhibitors. Generally, these transferases are classified on the basis of their affinity for acyl CoA (1). Carnitine acetyl transferases (CAT) catalyze those reactions involving short chain acyl esters with a chain length

ranging from 2 to 10 carbon atoms. Regarding the transferases for the long chain acyl esters (>10 C), the term COT (carnitine octanoyl transferase) is used for the extramitochondrial proteins, whereas CPT (carnitine palmitoyl transferase) is generally adopted to indicate the mitochondrial enzymes. Despite the different names, these two subgroups of enzymes show a similar broad range of specificity towards medium-chain and long-chain acyl-CoA with the highest affinity for decanoyl-CoA (2).

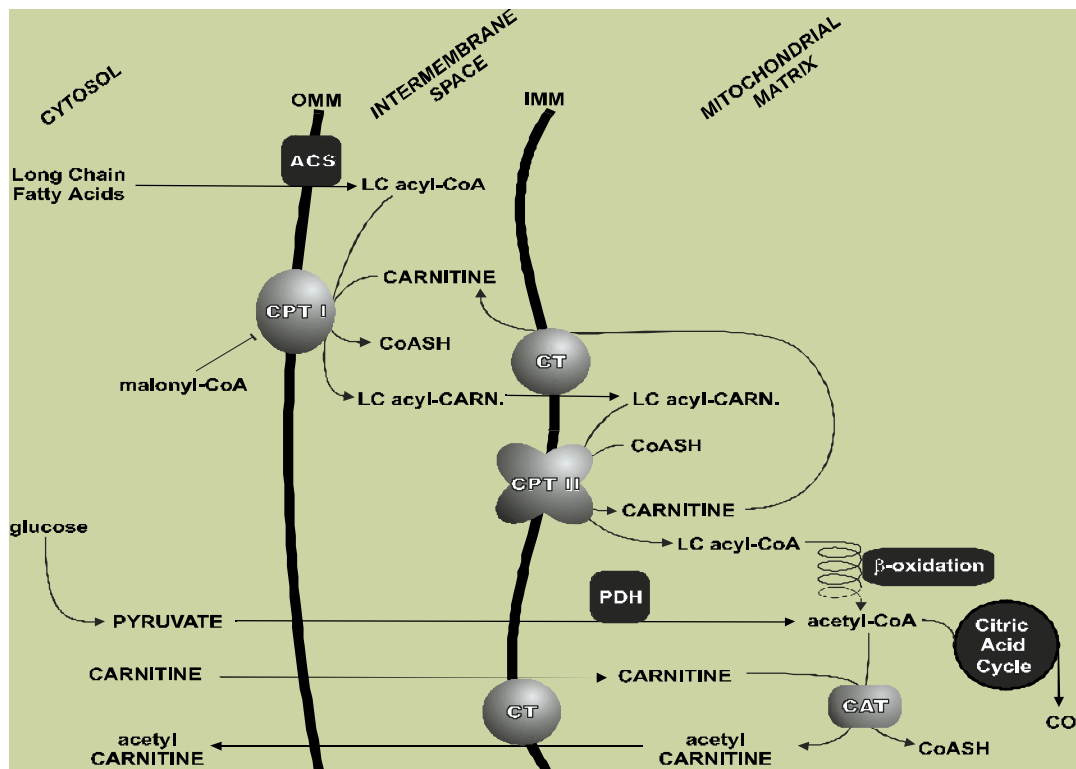


Figure 1. Carnitine-dependent enzymes and transporters involved in the mitochondrial metabolism of acyl moieties. CPT I, Carnitine Palmitoyl Transferase I; CPT II, Carnitine Palmitoyl Transferase II; CT, Carnitine Translocase; CAT, Carnitine acetyltransferase; ACS, Acyl-CoA synthase; LC, long chain; PDH, pyruvate dehydrogenase; CoASH, free Coenzyme A; Carn., carnitine; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane.

The total CPT activity of a cell results from the integrated activity of two different proteins: an oligomer made up of 6-8 subunits which is inserted in the inner mitochondrial membrane (CPT II). Another protein (CPT I) is present on the outer mitochondrial membrane (2). This latter CPT isoform is responsible for malonyl-CoA inhibition which is a crucial step in the overall control of intracellular lipid metabolism (3).

CAT is a monomer associated with the inner mitochondrial membrane. Up to this point no regulatory mechanism has been described, so CAT appears to function as a simple Michaelis-Menten enzyme. It was the first carnitine-dependent enzyme to be isolated (4). Its commercial availability made the enzymatic assay of carnitine possible thus disclosing the clinical interest for this compound and allowing the discovery of carnitine related defects (5,6).

Carnitine as a CoA buffer

The utilization of substrates for oxidative processes requires optimal activities of carnitine dependent transferases. In fact, mitochondrial carnitine palmitoyl transferases (CPT) are necessary for the *import* of activated acyls into the mitochondrial matrix where the β -oxidation machinery is located (1) (Fig. 2).

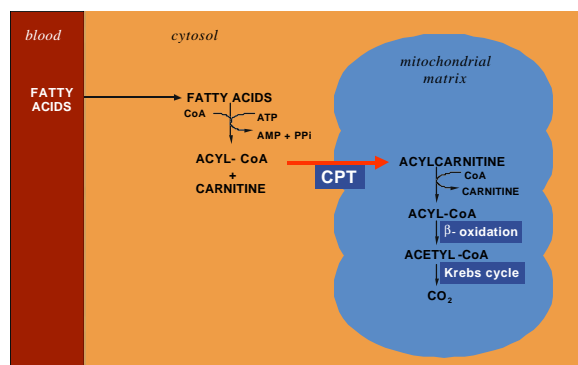


Figure 2. By means of CPT I and II carnitine directs the flow of long chain acyl moieties towards the mitochondrial matrix.

Besides this obligatory role, carnitine is involved in the intermediary metabolism as a whole by both modulating the acyl-CoA/CoA ratio and buffering the availability of CoASH in the cell. This function is mostly dependent on the formation of short-chain acylcarnitines (SCACar) catalyzed by carnitine acetyl transferase (CAT) (1,7). The CoA-carnitine relationship is pivotal for energy metabolism. CoA is required for β -oxidation, for the catabolism of several amino acids, for the detoxification of organic acids and xenobiotics, for pyruvate dehydrogenase (PDH) (8), for α -ketoglutarate dehydrogenase (9,10) and thus for the TCA (tricarboxylic acid) cycle (11) (Fig. 3). A reduced availability of carnitine induces a decrease of matrix CoASH and a parallel increase of the acyl-CoA/CoASH ratio both of which are inhibitory in the aforementioned mitochondrial dehydrogenases.

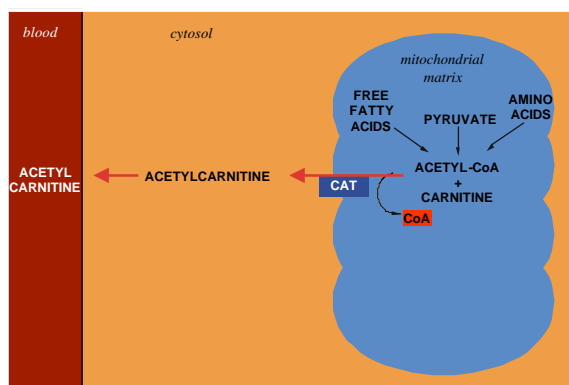


Figure 3. By means of CAT carnitine allows the exit of acetyl and short chain acyl moieties out of mitochondria. The net result is the release of free CoA which is made available for the maintenance of oxidative processes.

Consequently, not only the oxidation of fatty acids (12), but also the utilization of carbohydrates, the catabolism of several amino acids and the detoxification of organic acids and xenobiotics become impaired (13) (Fig. 4).

In the case of CAT catalyzed reactions the direction of metabolite flux appears the opposite of that catalyzed by CPT (Fig. 3). In fact, SCACar are mostly formed within the matrix space and exported into the cytosol (Fig. 3). Unlike the corresponding acyl-CoAs, acylcarnitines, especially SCACar, are capable of diffusing across cellular membranes and may be eliminated in the urine. The urinary excretion of specific acylcarnitines is relevant for the diagnosis of several inborn errors of metabolism (14) (Fig. 4).

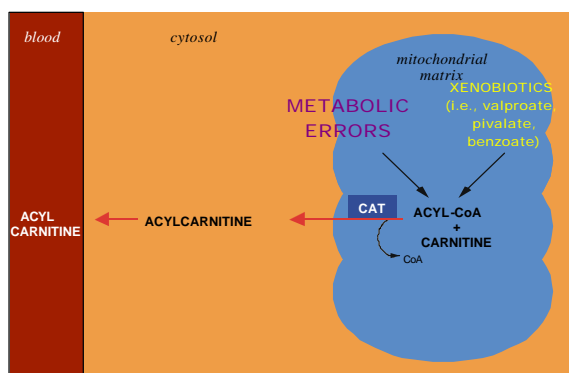
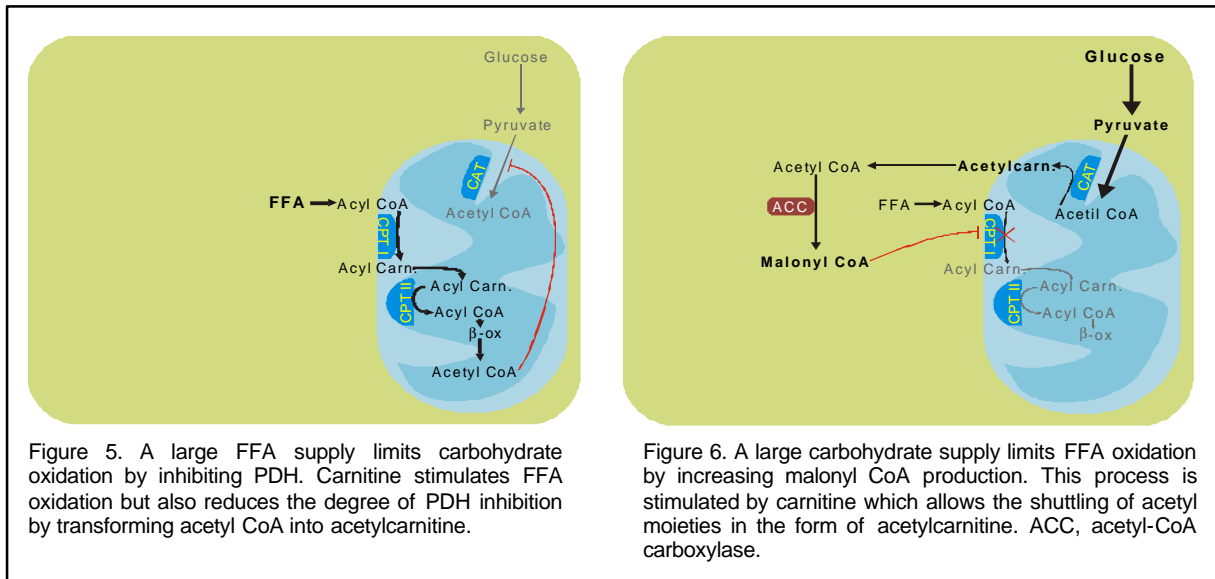


Figure 4. Detoxification properties of carnitine. Xenobiotics can be activated into acyl-CoAs which are devoid of further catabolic fates. In addition, acyl-CoAs can accumulate within mitochondrial matrix when their degradation is hampered by enzymatic deficits. Under those conditions the excess of acyl moieties or those that cannot be metabolized are eliminated by means of their transformation into the corresponding acylcarnitines.

Carnitine and substrate choice

Owing to the obligatory role of carnitine in the oxidation of long chain fatty acids, the contribution of carnitine to the oxidation of carbohydrates has been frequently overlooked. Indeed, carnitine is necessary to convert acetyl CoA into acetylcarnitine, thus removing a powerful inhibitor of pyruvate dehydrogenase (Fig. 5).



On this basis it is not surprising that in the heart a large availability of carnitine reduces the oxidation of fatty acids, while favoring that of glucose (15). Part of this stimulation is due to the formation of malonyl CoA which reduces the entry of acyl CoA into the mitochondrial matrix by inhibiting CPT I (Fig. 6) (16).

Acetylcarnitine, whose formation is stimulated by carnitine, provides the acetyl units which are carboxylated into malonyl CoA. Thus, carnitine results eventually in the decrease of fatty acid oxidation in the heart. This effect appears to be beneficial in the ischemic tissue (17). Indeed, it has been shown that the contractile recovery of the ischemic myocardium is favored high glucose oxidation rates, whereas is depressed by elevated rates of lipid oxidation (18,19). The occurrence of this latter possibility is promoted by a decreased formation of malonyl CoA owing to the inhibition of acetyl CoA carboxylase by AMP (Fig. 7) (18,19).

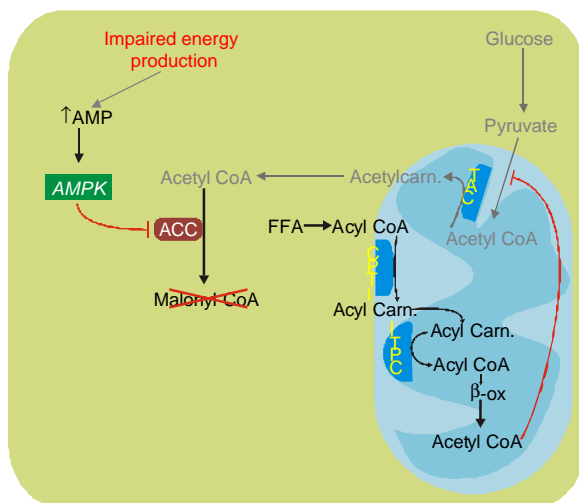


Figure 7. Disturbances in energy metabolism favor FFA oxidation and reduce carbohydrate utilization owing to decreased levels of malonyl CoA. ACC, acetyl-CoA carboxylase; AMPK, AMP-dependent protein kinase.

Carnitine and ischemia

The relevance of carnitine role in modulating acyl-CoA/CoASH ratio is exemplified by the biochemical changes occurring in anoxic tissues. Profound alterations of mitochondrial physiology are produced by long chain acyl CoA (LCACoA) (20) which accumulate during ischemia (21-23). These CoA esters are amphipathic molecules which can insert in the phospholipid bilayer altering both membrane architecture and permeability (20,24). These changes are more likely to occur at LCACoA concentrations above the CMC (critical micellar concentration) which in the case of palmitoyl CoA is $\cong 30 \mu\text{M}$. At lower concentrations, LCACoA are able to specifically affect the activity of various transport systems of the inner mitochondrial membrane without perturbing its permeability (25). Carnitine addition is able to restore mitochondrial function by changing LCACoA to LCACar which are devoid of Inhibitory effects (Fig. 8).

More recently, LCACoA have been added to the long list of promoters of the cyclosporine-sensitive membrane transition pore (MTP) (26,27). Also the abrupt changes of membrane permeability and function brought about by MTP opening can be prevented or partially restored by carnitine (27,28).

In isolated mitochondria as well as in the intact heart, LCACoA toxicity could be ascribed, at least in part, to a decreased CoASH availability. The role of CoASH/esterified-CoA and carnitine/esterified carnitine ratios in the evolution of ischemic damage has been investigated in various experimental models, especially in perfused rat hearts.

Anoxia or ischemia also result in an increase of esterified/free carnitine ratio (22). This metabolic shift, which reproduces the analogous modification of CoA status, is caused by the inhibition of mitochondrial dehydrogenases consequent to the excess of reduced flavin and pyridine coenzymes.

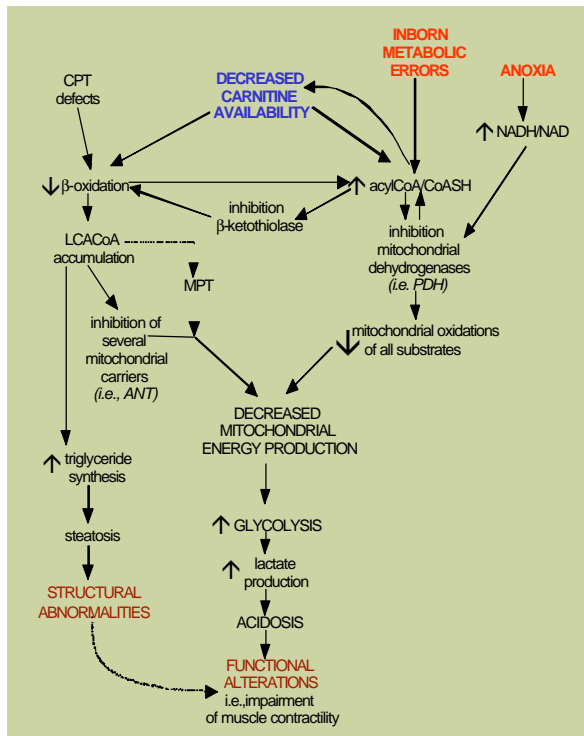


Figure 8. Metabolic derangements related to a decrease in free carnitine availability such as that caused by several metabolic errors or anoxia.

The reduced rates of β -oxidation and the TCA cycle freeze CoA in the form of LCACoA or SCACoA and available carnitine acts as a scavenger of acyl moieties in order to liberate CoA. This action of carnitine appears to be pertinent for pyruvate utilization. In fact, in hypoxic tissues pyruvate is mostly converted to lactate due to PDH inhibition. By decreasing acetyl-CoA/CoA ratio, carnitine might stimulate PDH, thus diverting pyruvate from its reduction to lactate and causing its oxidation to acetyl-CoA and then into acetylcarnitine (Fig. 9).

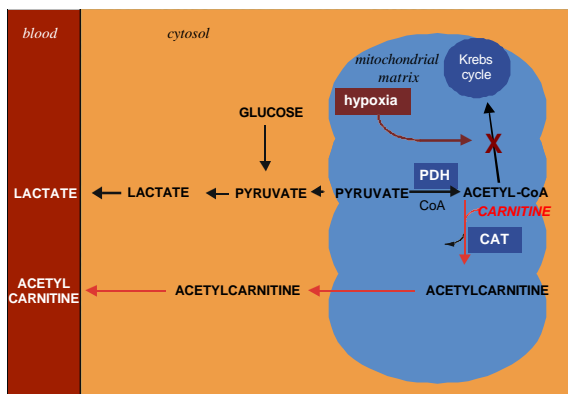


Figure 9. Mechanism of the lactate-decreasing effect of carnitine. The transformation of acetyl-CoA into acetylcarnitine favors the oxidation of pyruvate, thus decreasing its reduction into lactate.

Experimental and clinical evidence support these concepts. Ferrari and Coll. were the first to demonstrate that carnitine administration can reduce lactate formation in subjects suffering from coronary

also in patients suffering from intermittent claudication (30). In healthy individuals subjected to maximal cycle ergometer exercise, we demonstrated that plasma lactate is inversely correlated with acetylcarnitine (31).

An influence of carnitine on muscle metabolism has been argued by considering steady-state carnitine contents rather than metabolic fluxes (32). During the time of our exercise protocol muscle carnitine content would have been increased by less than 2% (32). Obviously such an increase is unlikely to modify CAT activity. However, carnitine transport across the sarcolemma seems to occur mainly by a 1:1 exchange with intracellular carnitine or carnitine esters (33). Thus when acetylcarnitine accumulates in the cytosol, a large availability of extracellular carnitine could promote the exchange and consequently the washout of carnitine esters without major changes in carnitine tissue content (34). This also appears to be the case in inborn errors of metabolism treated with carnitine. Indeed carnitine, given orally to patients with isovaleric acidemia, rapidly induced a large increase in plasma and urine isovalerylcarnitine (35,36). This increase is most likely the result of an exchange of exogenous free carnitine with tissue isovalerylcarnitine formed from the excess isovaleryl-CoA.

Beyond energy metabolism: anabolic and antiapoptotic roles of carnitine

The maintenance of an optimal acyl-CoA/CoASH ratio expands the role of carnitine in the metabolism of phospholipids adding anabolic functions to the abovementioned involvement in the oxidative catabolism of energy substrates. Indeed, membrane phospholipid fatty acid turnover and repair depend on LCACoA availability. In this respect carnitine and CPT role is two fold: provide acyl-units at no ATP-cost and buffer the harmful decrease in cellular CoASH levels (37) (Fig. 10).

Supporting this concept, CPT inhibition has been shown to result in a marked depression of the recylating capability in both human erythrocytes and neuronal cells (38,39). These experimental studies provide the biochemical rationale for both the decreased abnormalities in the deformability of erythrocytes and the increased hematocrit which has been reported in dialyzed patients treated with carnitine (40).

The functions of carnitine in both energy metabolism and phospholipid turnover indicate a general role of this compound in the maintenance of cell viability. More recent studies indicate the involvement of carnitine in the cellular defense against apoptosis by means of inhibitory effects on ceramide synthesis (41) and caspase activities (42) (Fig.11).

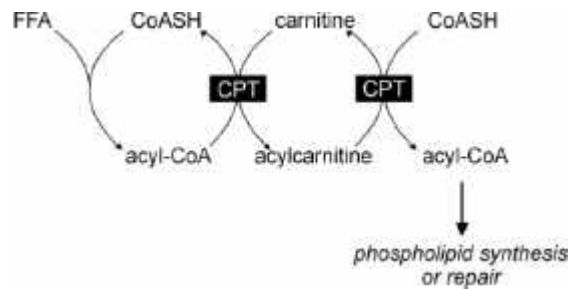


Figure 10. Long chain acylcarnitines might represent a source of acyl moieties for esterification processes, such as phospholipid synthesis and repair. Thus, besides optimizing the availability of free CoA (CoASH), carnitine adds anabolic roles to its function in the catabolic pathways of energy metabolism.

Surprisingly, the antiapoptotic role of carnitine appears to be largely independent of the actions of the various carnitine transferases. In cardiac myocytes exposed to doxorubicin, carnitine administration reduced the degree of apoptotic death in intracellular levels of ceramide, a powerful endogenous promoter of apoptosis (40,41). The inhibition on ceramide production is the result of two different actions of carnitine. In fact the subtraction of palmitoyl CoA which is diverted from ceramide synthesis to oxidative metabolism (43) is reinforced by the inhibitory effect on acid sphingomyelinase, which generates ceramide in response to a host of apoptotic stimuli (41). In addition, carnitine has been shown to inhibit the activity of caspases 3 and 8 (42), which act as initiator and executioner of apoptosis, respectively. Not only is the action of carnitine specific, but it is also reversed by palmitoylcarnitine. Since during the apoptosis of Jurkat cells carnitine levels decrease as opposed to the increase in palmitoylcarnitine, the free/esterified carnitine ratio has been suggested to play a relevant role in the cell commitment to apoptosis (42).

Clinical use of carnitine

Much of our knowledge on the biochemistry and the therapeutic efficacy of carnitine derives from the clinical data obtained from patients suffering for carnitine deficiencies or various mitochondrial pathologies. From a clinical standpoint, when carnitine availability is reduced or the activities of carnitine dependent transferases are impaired, fatty acid oxidation is prevented resulting in the most severe cases in life-threatening alterations of skeletal and cardiac muscle.

A secondary deficiency of carnitine is observed in several inborn errors of metabolism (44) where a large proportion of the available carnitine is esterified to buffer the accumulation of specific acyl CoA induced by the enzymatic defect. The transfer of the non-metabolizable acyls from CoA to carnitine has two major advantages: (i) CoASH is made available for other essential oxidative pathways; (ii) acyl CoAs are mostly compartmentalized within the mitochondrial matrix

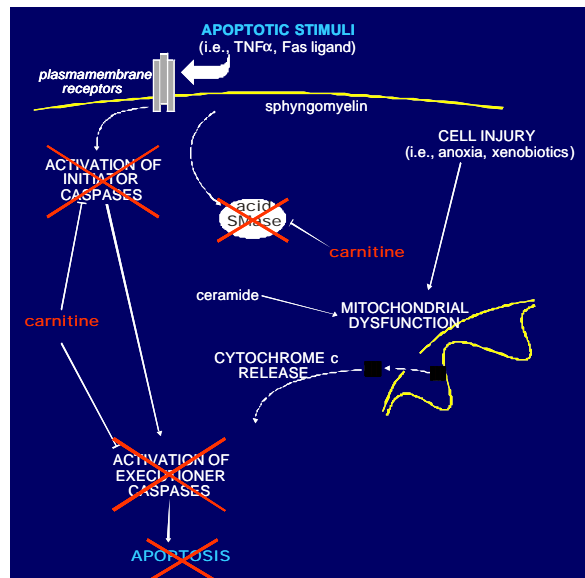


Figure 11. Mechanisms underlying the antiapoptotic efficacy of carnitine

and cannot cross membranes, whereas the corresponding carnitine esters not only can escape mitochondria, but they are also released in the blood stream and are eventually excreted in the urines. The detection of specific acylcarnitines in plasma or urine can be pathognomonic of several metabolic errors, such as isovaleric or propionic acidemia. In the most severe cases, the endogenous pool of free carnitine becomes insufficient to cope with the required acyl transfer. The resulting secondary carnitine deficiency or insufficiency mimics the metabolic alterations described for the primary carnitine-related defects.

Besides these direct links between carnitine and mitochondrial disorders, the majority of mitochondrial defects is likely to alter carnitine status thus worsening the clinical outcome. For instance, any respiratory chain defect reduces the availability of oxidized coenzymes resulting in decreased oxidation rates and accumulation of metabolic intermediates. Under these conditions a large proportion of the available carnitine is going to be esterified for the disposal of acetyl CoA no longer degradable by TCA cycle. On the other hand the reduced rate of β -oxidation is followed by the accumulation of long chain acyl CoA (LCACoA) and consequently of the corresponding carnitine esters.

Acylcarnitines as pharmacological agents

The ability of SCACar to cross cellular membranes suggests the possibility that administered SCACar could reach the mitochondrial matrix space. Here, their transformation into the corresponding acyl-CoA may contribute to a useful integration of the acyls in mitochondrial metabolic pathways. Furthermore, SCACar administration could add some degree of specificity to carnitine therapy. In fact, at least theoretically, the more a tissue is endowed with CAT the more it should benefit from SCACar administration.

ACETYLCARNITINE

Since it brings the activated acetate directly into the mitochondrial matrix where becoming acetyl CoA can be oxidized by the Krebs' cycle without further energy expenditure, acetylcarnitine represents a readily available substrate that can spark mitochondrial energy-linked processes (Fig. 12) (45).

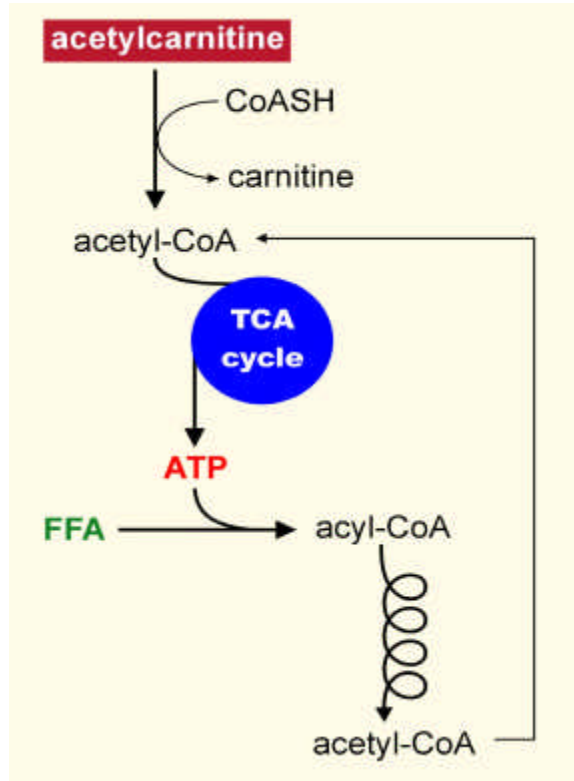


Figure 12. Sparking action of acetylcarnitine on FFA activation and oxidation

Besides being an energy substrate within the mitochondrion, the acetyl moiety esterified to carnitine is also the initial precursor for the cytosolic synthesis of structural lipids.

The absolute requirement for both optimal mitochondrial metabolism and structural integrity of the plasma membrane, upon which not only cell survival but also neurotransmission depends, renders neurons a preferred target for the metabolic actions of acetylcarnitine. This preference is reinforced by the fact that acetylcarnitine, besides being a precursor of acetylcholine, can also display cholinomimetic activity (46). Both the energy- and the acetylcholine-related properties explain the beneficial effects exerted by acetylcarnitine delaying neurodegenerative diseases associated with aging including Alzheimer disease (47). On the other hand, the clinical improvements observed in peripheral neuropathies including those caused by diabetes are likely related to the metabolic actions of acetylcarnitine (48). Of note, the effects elicited by acetylcarnitine in experimental and clinical settings were not mimicked by carnitine.

PROPIONYLCARNITINE

The biochemical rationale for propionylcarnitine (PLC) administration concerns the possibility of feeding the TCA cycle (anaplerosis) with the carbon skeleton of propionate without altering the energy metabolism (Fig. 13).

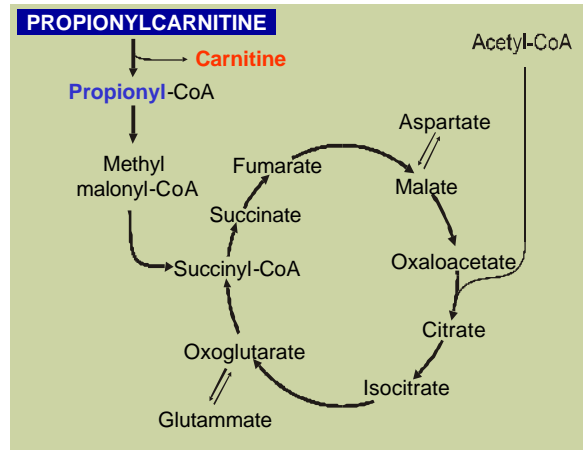


Figure 13. Propionylcarnitine combines the acyl shuttling property of carnitine with the ability of replenishing the TCA cycle with four carbon atom intermediates (anaplerosis).

A protective effect of PLC on the ischemic heart has been documented by several Authors. The protective effect of PLC may be a consequence of the anaplerotic utilization of propionate in the presence of optimal amounts of ATP, CoASH and carnitine (49). Obviously, the involvement of mitochondrial function in PLC effects does not exclude other mechanisms. It is likely that the protective action exerted by PLC might result from a positive interaction between i) improved mitochondrial function; ii) iron chelation (50); iii) preservation of vascular patency (51).

ISOVALERYLCARNITINE

Both α -ketoisocaproate and its parent amino acid, leucine, are known to inhibit lysosomal proteolysis in rat liver perfused in the absence of amino acids (52). This raises the question as to whether leucine is responsible for the inhibition per se, or via some of its catabolites. We focused our attention on isovalerylcarnitine (IVC) which proved to reproduce the leucine effect with a similar dose-dependency (53). Since only a negligible amount of added IVC is detectable within liver cells, either in perfused liver or in isolated hepatocytes, the inhibition of proteolysis could be mediated by a receptor located on the plasma membrane (54).

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