Monatshefte für Chemie 136, 1255–1268 (2005)
DOI 10.1007/s00706-005-0339-2

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

L-Carnitine: New Aspects of a Known Compound – A Brief Survey

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Received February 4, 2005; accepted February 16, 2005
Published online July 18, 2005

Summary. This review gives a brief overview on the importance of *L*-carnitine for cell function. A well established role of carnitine is the transport of fatty acids (FA) into the mitochondrial matrix. There exist analogous carnitine dependent transport systems for FA into microsomes and peroxisomes. Different membrane transporters control the intracellular homeostasis of carnitine in particular the organic cation transporter OCTN2. Beyond that carnitine is involved in the regulation of gene transcription, inhibits platelet aggregation, stimulates erythropoiesis, acts as a radical scavenger, improves the age-associated decline of learning and memory as well as age associated changes in oxidative metabolism, and inhibits apoptosis of blood cells. These newly described functions of carnitine demonstrate that a given metabolite can be involved in a great number of different processes and cell functions.

Keywords. Carnitine; Carnitine esters; Carnitine functions; Carnitine deficiency; Carnitine mediated.

Introduction

Carnitine (γ-trimethylamino-β-hydroxybutyric acid) is a small molecule to be found in almost all cells. Carnitine-dependent metabolic pathways, together with glycolysis, belong to the oldest in evolution. Carnitine can be found in archea, where for some species it is the only source of carbon and nitrogen [1]. For other bacteria, carnitine is an essential osmoprotective agent [1–3]. Also plants possess carnitine and carnitine-acyltransferase activities, but the proteins are not yet identified [4, 5]. In plants carnitine most likely facilitates the transport of activated fatty acids during desaturation, elongation, and lipid synthesis needed during periods of rapid membrane synthesis or lipid mobilization and transport to the glyoxysomes. Also in the metabolism of insects carnitine plays an important role [6, 7]. In all eukaryotes – from yeast to man – carnitine is an integral and essential factor in intermediary metabolism [8–10].

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Animal tissues contain amounts of carnitine varying between 0.2 and 6 μmol/g with the highest concentration in heart and skeletal muscle [10, 11]. This is maintained either by endogenous synthesis or through uptake from nutrients, but exogenous carnitine does not act as a regulator for carnitine biosynthesis. Carnitine is mostly obtained from red meat, fish, and dairy products and becomes deficient in rare conditions such as extreme vegetarian diet, premature infants, pregnancy, hemodialysis patients, and in some chronic diseases [10, 12–14]. The level of carnitine in blood is regulated mainly by the kidneys and is age and sex dependent. Excretion of carnitine and carnitine derivatives occurs *via* urine and bile [15, 16].

The intracellular level of carnitine influences the transcription rate of certain enzymes. In cases of carnitine deficiency transcription of mitochondrial CPT1 and carnitine acetyltransferase (CRAT) is reduced. Addition of carnitine results in a dose-dependent normalization [17–19]. Carnitine and carnitine acyltransferases, modulating the availability of fatty acids and/or acyl-CoAs, also may influence the transcriptional regulation of a wide variety of genes related to the control of oxidative metabolism, adipogenesis, glycolysis, and gluconeogenesis. An increasing number of receptors and genes is being detected, whose transcription seems to be directly or indirectly regulated by these metabolites.

Other important functions of carnitine also have been reported as will be shown in this issue of ''100 Years Research on Carnitine''.

Uptake of Carnitine from Nutrients

In omnivorous humans about 75% of the carnitine comes from diet and only 25% is synthesized *de novo* [16, 12]. The efficiency of the absorption is about 65 to 75% [20] – less than that of protein-derived amino acids. In the large intestine microorganisms degrade most of the unabsorbed carnitine and only a small amount is excreted in the feces.

Gudjonsson et al. [21, 22] could demonstrate for rats *in vivo* that carnitine is taken up by the small intestinal mucosa in a saturable and structure specific process, but appears in the circulation with a delay.

Cellular Uptake and Release of Carnitine

L-Carnitine transport has been extensively studied using animals and human tissue samples, and Na^+-dependent and -independent systems have been identified. They are also classified as high affinity and low affinity transport mechanism, and both types may function in parallel [10, 23–25].

Different membrane transporters control the intracellular homeostasis of carnitine. The organic cation transporters (OCTNs), in particular OCTN2, physiologically the most important one, operate on the intestinal absorption and renal reabsorption of *L*-carnitine and play a major role in tissue distribution and variations in transport rates. Inborn or acquired defects on this carnitine transport mechanism lead to primary or secondary systemic carnitine deficiency. The OCTN2 mRNA content of cells is reduced in aging [26] and by oxygen radicals [27] and the OCTN2 protein is directly inhibited by several agents and substances known to induce systemic carnitine deficiency [28].

OCTN1 is a multispecific, bidirectional, *pH* dependent organic cation transporter widely expressed in various tissues [29, 30]. OCTN1 and OCTN3 contribute to reabsorption of carnitine in kidney but may also have additional functions due to low activity of OCTN1 and the highly specific expression of OCTN3 in testis [30–32].

In human testis the novel carnitine transporter (CT2) has been discovered recently. The primary structure of CT2 revealed that it is physiologically located between the organic cation transporter (OCT/OCTN) and the anion transporter (OAT) families [33]. Furthermore in mouse colon, an unrelated amino acid transporter $ATB^{0,+}$ which has a high K_m for carnitine has been identified [34]. $ATB^{0,+}$ could therefore, like OCTN1, represent the low affinity sodium independent carnitine transporter [25, 30, 35].

Experiments with perfused liver suggest a protein-mediated release of carnitine from hepatocytes. Starvation decreases carnitine output from perfused liver, suggesting decreased activity of the protein [36]. Such a protein could be regulated hormonally because glucagon increases the carnitine content of the liver, as does starvation, but not that of the heart [36].

OCTN1 transporter could also modulate intracellular carnitine, providing a *pH* regulated path of efflux of carnitine from cells. Presumably, this export, down a concentration gradient, could be due to passive diffusion. A volume activated amino acid channel may also contribute to carnitine efflux from swollen cells. Several

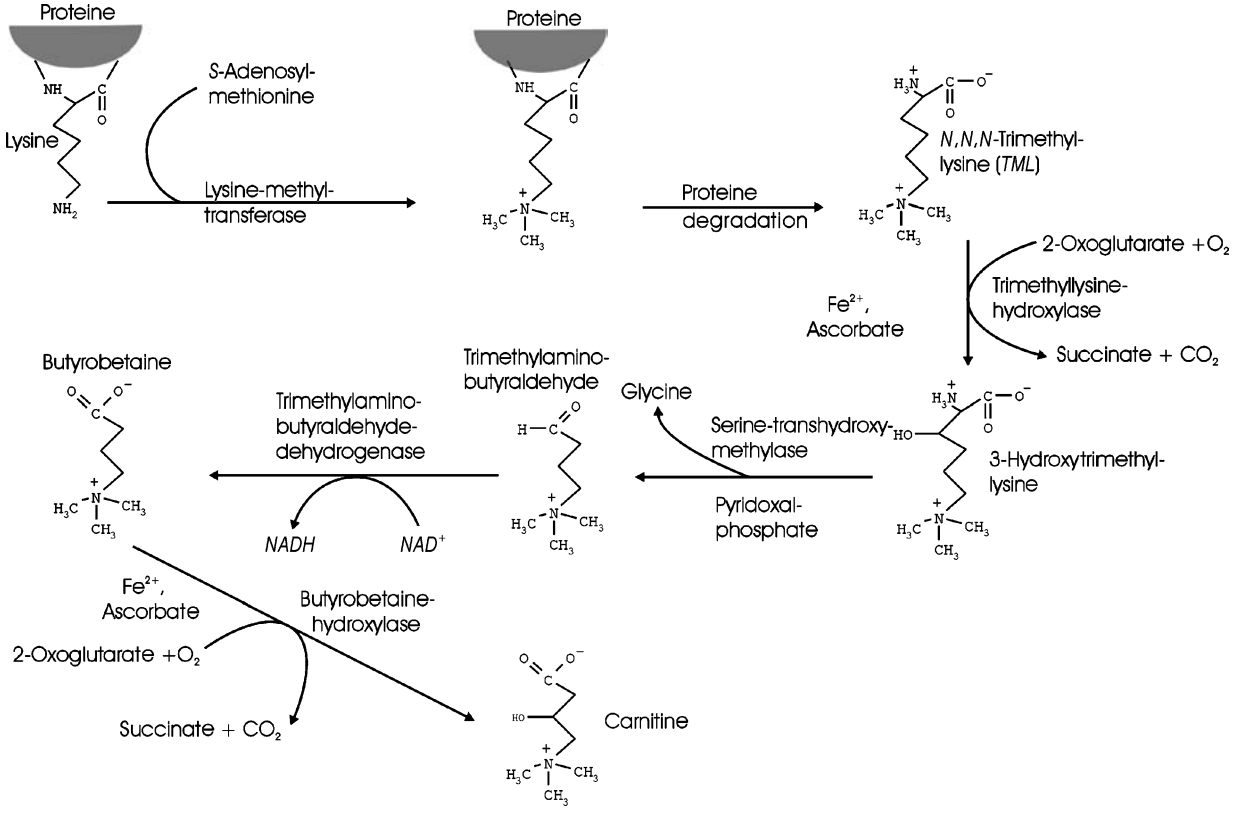

Fig. 1. Biosynthesis of carnitine

observations suggest a local regulation, because palmitoyl-CoA and *ATP* can alter the number of carnitine binding sites [37]. Also the membrane potential affects the transport of *L*-carnitine [38].

Endogenous Synthesis and Regulation of Carnitine Biosynthesis

Carnitine is synthesized in mammals from the essential amino acids lysine and methionine [10, 16, 39] (see Fig. 1). Availability of the intermediate trimethyllysine limits carnitine biosynthesis, and most of the trimethyllysine body stores are located in skeletal muscle protein. Consequently skeletal muscle protein turnover is considered to be the rate-limiting step in carnitine biosynthesis [40]. The last step, the hydroxylation of butyrobetaine to carnitine, is limited to liver, kidney, and brain, other tissues depend on active uptake of carnitine from the circulation [41]. The enzymes involved in carnitine biosynthesis as well as their cofactors and subcellular localization have been reviewed extensively by *Vaz* and *Wanders* [16].

In rats carnitine biosynthesis could be greatly increased by feeding them γ-butyrobetaine. Similarly, children and adults receiving a diet enriched with γ-butyrobetaine excreted an up to 30-fold increase in carnitine [42]. High doses of exogenous carnitine reduce the activity of γ-butyrobetaine dioxygenase by about

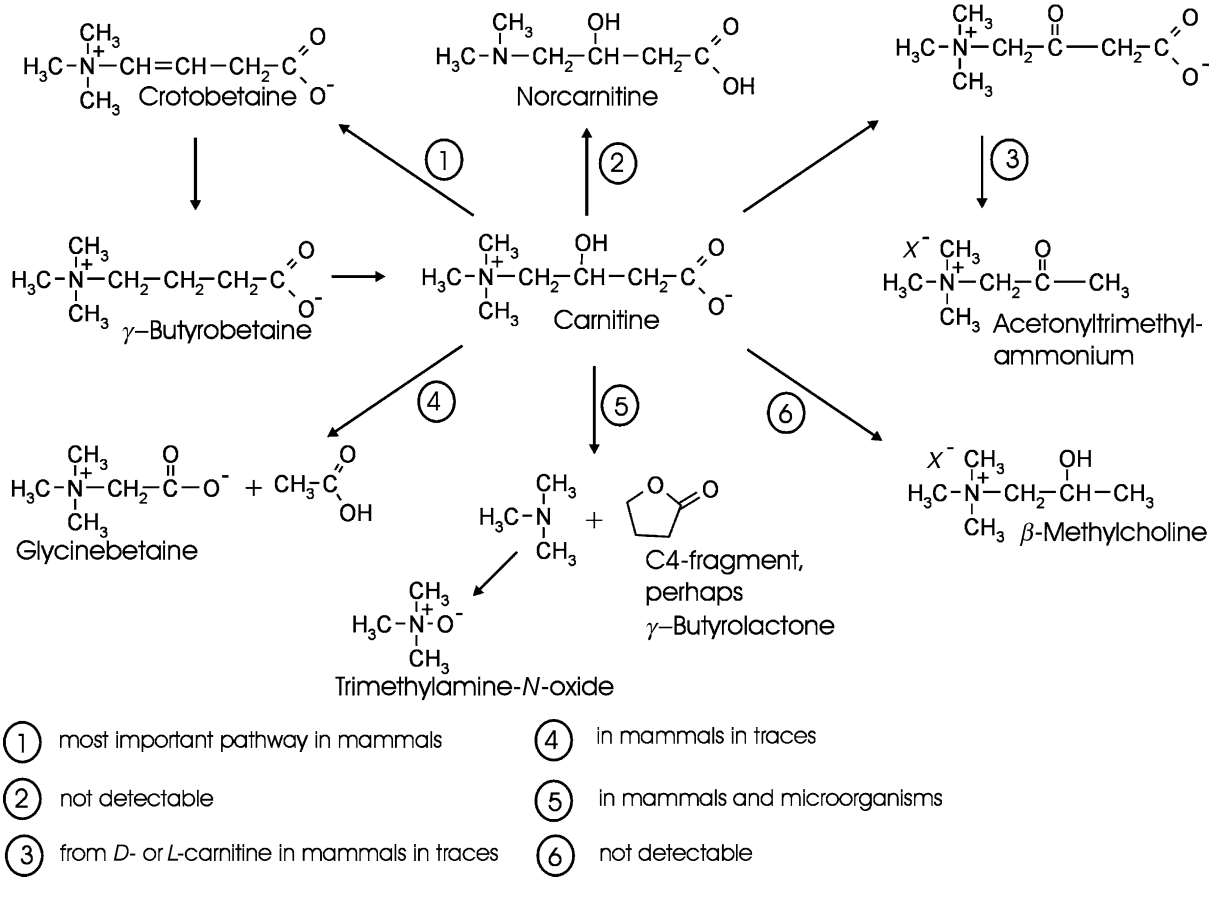

Fig. 2. Carnitine catabolism

20% but the capacity of this enzyme is obviously up to 30-fold higher than necessary to meet the rate of endogenous carnitine biosynthesis [16].

Breakdown of Carnitine

The breakdown of carnitine was first demonstrated in animal experiments using pharmacological doses of carnitine [43] and it has also been determined in man for conditions of normal food intake [44]. By the use of germ-free animals it could be shown that the breakdown of carnitine does not take place in the tissues of mammals but as a result of microbial action in animals and man. In addition to *Escherichia coli*, *Proteus vulgaris*, and *Salmonella typhimurium*, many other species of bacteria are known to be able to break down carnitine [20, 45–47]. Possible degradation products are shown in Fig. 2.

Renal Excretion

Kidneys play an important role in the homeostasis of carnitine levels in the blood [16, 37, 48, 49]. In perfused rat kidney more than 95% of free carnitine is reabsorbed in the ultrafiltrate and also acetylcarnitine is reabsorbed in significant amounts in contrast to longer chain carnitine esters. In the rat about 7% of the body pool is excreted in urine each day [50].

OCTNs are also expressed in the kidneys and favour the transport of carnitine. OCTN1, OCTN2, and OCTN3 are membrane-potential-driven transporters and are localized to the basolateral membrane of renal tubules [35, 50].

In cases of reduced carnitine uptake from the food, excretion by the kidneys is also reduced [51]. It is known that various physiological and pathophysiological factors as well as diet influence the rate of carnitine excretion and reabsorption [52]. In the case of a fat-rich diet, the rate of carnitine excretion is increased compared to that of a diet rich in carbohydrates [53, 54]. Also diet rich in proteins results in an increased rate of carnitine excretion due to a rise in the glomerular filtration rate [53, 54].

Since 1978 it is known that in cases of metabolism with increased lipolysis, fasting, or ACTH injection, a dramatic increase in carnitine excretion occurs – in particular as carnitine esters – which cannot be compensated by synthesizing new endogenous carnitine [55].

Carnitine Loss in Bile

Carnitine derivatives are excreted *via* the urine and in bile, where it was demonstrated that long chain acyl derivatives accumulate [28].

The fact that considerable amounts of carnitine enter the enterohepatic circulation in the bile was hardly ever considered in calculating carnitine balances. Carnitine is reabsorbed but is subject to bacterial breakdown in the gut as described [20, 45–47]. This breakdown presents an important alternative mechanism for carnitine loss. More precise investigations by *Hamilton* and *Hahn* [56] showed that the bile of fed rats contained 80% acylcarnitine esters, a third of which was long chain acylcarnitine. Fasting does not lead to a change in the rate of secretion

of acylcarnitine in bile although the secretion of long chain acylcarnitine doubles. These results indicate a connection between the carnitine pool in bile and the hepatic metabolism of carnitine and confirm the presence of carnitine and carnitine esters in the intestinal lumen in man [51, 56].

Carnitine Acyltransferases

Carnitine acyltransferases (CATs) are important enzymes for energy homeostasis and fat metabolism through their modulation of the pools of acetyl-CoA and long chain acyl-CoA in distinct compartments. These enzymes belong to the family of carnitine choline acyltransferases and have different properties with respect to intracellular location, substrate specificity, kinetics, and physiological function. CPT1 (carnitine palmitoyl transferase) and COT (carnitine octanoyl transferase) transesterify long and medium fatty acyl chains, whereas CRAT (carnitine acetyl transferase) transesterifies short chain fatty acids. These enzymes and their functions are described in detail in other articles of this issue.

Role of Carnitine in Regulation of Gene Transcription

Carnitine itself has direct effects in regulation of gene expression and may additionally exert effects *via* fatty acids.

L-Carnitine stimulates the transcriptional activity of the glucocorticoid receptor (GRα). This effect is solely dependent on the presence of glucocorticoid-responsive elements on the promoter and on the expression of functional GRα by the cell [57].

Glucocorticoids interact with intracellular receptors expressed in almost every tissue. In the absence of related ligands, GRα is cytoplasmatic and transcriptionally inactive, because it is associated with several proteins [58, 59]. Binding of glucocorticoids to their receptor induces dissociation of receptor-associated proteins, with subsequent GRα activation and translocation into the cell nucleus [60]. Homodimers of the activated receptor modulate the transcription of various (one to two hundred) responsive genes by binding to specific DNA associated glucocorticoid responsive elements (GREs). Pharmacological doses of *L*-carnitine activate GRα thus regulating glucocorticoid-responsive genes, potentially sharing some of the biological and therapeutic properties of glucocorticoids [57]. In general, the modulatory effects of *L*-carnitine on glucocorticoid receptor (GRα) functions may be tissue- and/or gene-specific, influenced by receptor abundance and distribution, and/or by transcription of regulatory or co-regulatory molecules, such as transcription factors, co-activators, and/or corepressors. *L*-Carnitine may act similarly to other tissue/gene-selective GR modulators but has side effects weaker than those of dexamethasone or natural glucocorticoids [61]. *In vitro L*-carnitine stimulates the GRα mediated transactivation of known viral and synthetic glucocorticoid-responsive promoters, triggers nuclear translocation of the GRα in the absence of dexamethasone, and competes with this molecule for the binding to GRα. This finding might indicate the function of *L*-carnitine as an allosteric regulator of GRα [62].

Additive effects of *L*-carnitine and betamethasone have been shown in fetal lung maturation in cases of premature delivery [63, 64]. Similar relationships of the effects of carnitine and thyroxine have also been reported [65]. *L*-Carnitine is a peripheral antagonist of thyroid hormone action. It inhibits the entry of triiodo

thyronine and thyroxine into the cell nuclei. It could be shown by *Benvenga et al.* [66] that 2–4 g of *L*-carnitine per day could reverse hyperthyroid symptoms even in the most serious form of hyperthyroidism: thyroid storm.

It has been suggested that the nuclear carnitine acyltransferase(s) and carnitine could conceivably contribute to the transcriptional regulation of distinct genes by modulating the availability of fatty acids and/or of acyl-CoAs [67]. It appears that acetyl-*L*-carnitine can modulate gene expression through regulation of histone acetylation [68]. Long-chain acyl-CoA esters bind to nuclear factors and affect transcription-factor binding to DNA [69]. Furthermore long-chain fatty acids suppress the induction of the gene by dexamethasone (*i.e.* carbamoylphosphate synthetase) in primary cultured rat hepatocytes with simultaneous activation of activator protein-1 (AP-1) DNA binding activity [70, 71].

Increased DNA binding activity of AP-1 and also increased mRNA levels of the oncogene c-jun and c-fos were detected in the liver of JVS mice [70, 71]. The proto-oncogenes, c-jun and c-fos, coding for nuclear proteins regulate cell growth and differentiation by controlling gene transcription through AP-1 enhancer element [72, 73]. The cis-element for AP-1, found in both mouse and rat, outside the minimum essential enhancer fragment of the rat CPS (carbamyl-phosphate synthetase) gene is probably responsible for the suppression of the CPS gene in the liver of carnitine-deficient JVS (juvenile visceral steatosis) mice, although the glucocorticoid signal transduction pathway was found to be activated [70, 71] and serum cortisol was higher than in controls [74, 75]. After carnitine treatment, all the symptoms disappear [76].

Fatty acids are known to modulate not only the enzymes and certain other proteins involved in their own metabolism but also a wide variety of genes related to the control of adipogenesis, glycolysis, and gluconeogenesis [77, 78]. An increasing number of genes have been detected so far whose transcriptions are very likely influenced by these metabolites either directly or by some other mechanism [67, 79–81].

Immunomodulatory Effects and Differentiation

In rodents, treatment with *L*-carnitine (50–100 mg/kg body weight) markedly suppressed the lipopolysaccharide (LPS)-induced cytokine production, improving their survival during cachexia and septic shock [82, 83]. Similarly, *L*-carnitine reduced the *ex vivo* release of tumor necrosis factor-α (TNFα) in *S. aureus*-stimulated human polymorphonuclear leucocytes [84]; moreover, decreased serum TNFα levels have been reported after *L*-carnitine supplementation in surgical patients (8 g *i.v.* at the end of surgery and 24 h afterwards) and AIDS patients (6 g/day for 2 wk) [85, 86].

Millimolar concentrations of *L*-carnitine were also shown to increase osteoblast activity *in vitro* [87, 88]. In a study with a small group of patients it has been shown that high doses of *L*-carnitine might share the immunomodulatory properties of glucocorticoids but not their deleterious effects on the bone [66].

Inhibition of Apoptosis in Immune Cells

Signal transduction *via* the surface glycoprotein Fas, which is also known as CD95, is considered as the most important pathway for the regulation of programmed cell death (apoptosis). *L*-Carnitine inhibits apoptosis by interaction with

the FasL (=Fas-ligand)–FasR (=Fas-receptor) system [89, 90]. Signal-transduction *via* the Fas-receptor activates acid sphingomyelinase (in lysosomes) and consequently a breakdown of sphingomyelin and a release of ceramide occur. Immediate inhibition of acid sphingomyelinase has already been shown *in vivo* and *in vitro* [91]. In addition, an inhibition of caspases 3, 7, and 8 and an inhibition of the so-called "mitochondrial permeability transition" could be induced by *L*-carnitine addition [92, 93]. Another anti-apoptotic mechanism of carnitine was detected in T-lymphocytes, where addition of *L*-carnitine and consequently a reduction of ceramide stimulated the level of insulin-like growth factor (=IGF-1) in serum [94, 95]. IGF-1 is known to inhibit dimerisation of apoptosis regulating proteins BCL-2-BAX in the mitochondrial membrane [96]. It also inhibits activation of transcription involving the BCL-2 promoters [97]. A review on the effect of carnitine on sepsis by *Eaton et al.* is given in this issue.

Uptake of *L*-Carnitine into Blood Cells

Uptake of *L*-carnitine into granulocytes has been documented in inflammatory disorders subsequent to multiple trauma and bacterial infections, whereas in patients with *Crohn*'s disease (chronic inflammatory bowel disease) the carnitine concentrations were increased in T-lymphocytes [82, 85, 86, 98]. *De Simone* showed that peripheral blood mononuclear cells (PBMC) from AIDS patients with normal serum carnitine levels are depleted in intracellular carnitine, which indicates that serum carnitines do not strictly reflect cellular concentrations. This fact has also to be considered with other phenomena cited in this review.

In AIDS patients treated for 2 weeks with high-dose *L*-carnitine (6 g/d) a significant trend towards the restoration of appropriate intracellular carnitine levels was found [85, 86]. A review on this topic by *Alesci et al.* can be found in this issue.

Preliminary observations suggest that *L*-carnitine-preloading also protected peripheral blood lymphocytes from aged donors when such cells were exposed to an oxidative stress [99]. Considering an age-associated downregulation of carnitine acyltransferases, nutritional supplemention of *L*-carnitine may be especially considered for aged individuals [26].

Stimulation of Hematopoiesis

Matsumura et al. [100] reported a relationship between *L*-carnitine and stem cells as well as progenitor cells of the hematopoietic system, and carnitine was found to stimulate erythropoiesis. The study confirmed also *in vivo* investigations on patients with renal anemia in that the effect of erythropoietin could be further enhanced by *L*-carnitine [101].

Carnitine, Arachidonic Acid, Platelet Function, and Free Radical Scavenging

Among fatty acids arachidonic acid has a key role in the activation of platelets, being converted to the vasoconstrictor and aggregating agent thromboxane A_2

[102]. Incubation of platelets with carnitine inhibits dose-dependently collagen induced platelet aggregation, thromboxane A_2 formation [103], and consequently reduces the risk of arteriosclerosis.

Evidence is growing that radicals are produced by platelets, leukocytes, and endothelial cells, where they may exert different functions. They are intermediate metabolites of several enzymatic reactions involved in the post-translational protein turnover and play a role on the control of signal transduction [103–105].

Carnitine (10–50 μM) is able to inhibit dose-dependently arachidonic acid incorporation into platelet phospholipids and agonist induced arachidonic acid release. Incubation of platelets with carnitine inhibits dose dependently collagen-induced platelet aggregation, thromboxane A_2 formation, and Ca^{2+} mobilization without affecting phospholipids A_2 activation. A role for the *NADP*H oxidase in arachidonic acid-mediated $O_2^{\bullet -}$ production has been suggested. Incubation of platelets with carnitine reduced significantly arachidonic acid-mediated *NADP*H oxidase activation. Moreover, the activation of protein kinase C was inhibited by 50 μM carnitine [103].

Ischemia in endothelial cells results in release of carnitine, increased oxidative stress, and compromised blood flow regulation, which can be overcome by intravascular carnitine administration [106, 107].

Volek et al. [106] reported recently that oral application of *L*-carnitine-*L*-tartrate significantly alters exercise induced increases in plasma markers of purine catabolism (hypoxanthine, xanthine oxidase), serum uric acid, circulating cytosolic proteins/myoglobin, fatty acid binding protein, and creatine kinase.

Age Associated Changes in Oxidative Metabolism

Aging is characterized by a general decline in physiological functions that affects many tissues. The aging process is often associated with altered mitochondrial function and decreased energy production during oxidative metabolism.

The free radical theory as important factor in aging is now generally accepted and mitochondria are considered as the main superoxide radical endogenous source. In the scheme of mitochondrial respiration electron transport involves a coordinated four-electron transfer, thus reducing O_2 to H_2O. Actually the electron flow is nonstoichiometric. Thus also formation of $O_2^{\bullet -}$ radicals occur. This so-called leakage of oxidants from the electron transport chain appears to be unavoidable [108–110] and radical generation amounted up to 1–2% of total electron flow [108].

A second source of oxygen radicals is traced to peroxisomal β-oxidation of fatty acids generating H_2O_2 as a by-product. Also oxidant leakage from peroxisomes may be enhanced during rapid cell proliferation [111].

Production of free radicals by macrophages, endothelial cells, and platelets is important in favoring low density lipoprotein (LDL) oxidation and in turn, LDL accumulation within the vessel wall (initiation and progression of arteriosclerosis) [104].

Beyond that numerous other enzymes capable of generating radicals under normal pathological conditions exist [112]. Consequently, mitochondrial decay appears to be one of the principal underlying causes that lead to cellular decline

in the aging process [113] and preservation of mitochondrial function is important for maintaining overall health during aging.

In aged rats, with the exception of liver there is a significant decrease of total carnitine levels in tissues [114]. *L*-Carnitine substitution induced increase in carnitine levels in older animals results in a revision of liver and heart mitochondria to a more youthful state, both structurally and functionally [115–121] and improves mitochondrial FA oxidation in the tissues studied [116, 117, 122–126]. Moreover, it has been shown that carnitine reverses the age associated decline in cardiolipin levels, the activity of various mitochondrial translocases, and cytochrome c oxidase, indicating that there exist many other carnitine functions besides its role in mitochondrial FA metabolism [127].

In accordance we showed that *L*-carnitine increased the mRNA content and the activity of carnitine acyltransferases in the livers of old rats compared to livers of untreated animals [128].

The central nervous system, which controls other organs by sending impulses through neurons, is most affected by aging. This organ is highly susceptible to free radical attack, more than any other organ [108]. The neurons show multiple changes, *i.e.*, yellowish brown lipid lipofusin, loss of essential myelin (fatty materials around axons), and general shrinkage and neuronal changes are a more decisive hallmark of age than widespread death of neurons. The connections between neurons, not just the neurons themselves, change with age. There is a reduction in the branching of dendrites (fibers on which axons of other neurons terminate) and a decline in the number of properly functioning connections between neurons.

Feeding old rats *L*-carnitine or acetyl-*L*-carnitine restores brain tissue levels of free and acylcarnitines to that found in plasma and brain tissues of younger animals [129]. Administration of carnitine over several weeks improves the age-associated decline of learning and memory [128, 130]. Clinical trials with acetylcarnitine showed some improvements in *Alzheimer* disease or dementia-associated cognitive dysfunction [131–134] and application of *L*-carnitine with antioxidant showed a synergistic effect on reversing the decay of spatial memory in old animals [109, 110, 135].

The administration of *L*-carnitine or acetyl-*L*-carnitine (ALC) increases the ambulatory activity in old rats similarly, but ALC is more effective on lipid peroxidation in the brain of old rats [136].

Concluding Remarks

The state of art in carnitine research – an already 100 years lasting story: at the beginning it took nearly 50 years until the absolute configuration of the physiological *L*-carnitine was established together with its main physiological functions. And now 50 years later: an outburst of data, many unexpected news, and the conclusion that carnitine is much more important for human beings and all other creatures on this planet than expected. Almost one paper a day at present shows an overwhelming interest in carnitine research which certainly will go on in near future. Looking beyond the borders of the present state of art we may get the feeling that we will have to rewrite the successful carnitine story already very soon ....

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