

Involvement of L-Carnitine in Cellular Metabolism: Beyond Acyl-CoA Transport.

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Abstract: Carnitine is well-known for its role in the transport of fatty acids to the mitochondrial matrix, where β -oxidation takes place. This work describes novel functions for this compound and novel data on its pharmacokinetics.

Keywords: Carnitine, acetyl-L-carnitine, free radicals, apoptosis, acetylation.

INTRODUCTION

The function of Carnitine described in ordinary Biochemistry textbooks is that it participates in the transport of fatty acids into the mitochondrion. For this, Carnitine temporarily binds to Acyl CoA to be recognized by Carnitine acyl transferase I and then by Carnitine acyl transferase II. Once inside the mitochondrion, Carnitine and Acyl CoA separate and Carnitine returns to the cytosol to start a new cycle (Fig. (1)).

Differences in L-Carnitine levels can cause various clinical symptoms that range from cramps or muscle weakness to death.

In the last years there has been a considerable increase in the data regarding other important functions of this compound.

The objective of the present review is to describe the functions associated with Carnitine in different pathways and cellular organelles.

L-Carnitine (Fig. (2)) participates not only in mitochondrial metabolism but also in: a) the protection reactions against oxidative stress; b) the regulation of the transcription of neurotrophic factors and neurohormones; and c) the decrease in the apoptosis rate in cell line cultures.

The potential action mechanisms of its acetylated derivative, acetyl L-Carnitine (ALCAR) (Fig. (3)), involve: a) acetylation of the $-NH_2$ or $-OH$ functional groups of amino acids and of the N-terminal of peptides and proteins, which produces modifications in their structure, dynamics, function and turnover; b) protection of molecules that act as molecular chaperones.

In addition, at the systemic level, in controlled double-blind studies, ALCAR has been reported to have beneficial effects in neurodegenerative diseases such as Alzheimer and Parkinson. These effects have also been observed and

studied in different model systems ranging from simple eukaryote cells to higher mammals.

In this respect, using yeast as a model, we have demonstrated in our laboratory that the presence of L-Carnitine has an effect in the mitochondrial membrane potential and protects the cell from the inhibitory effect of ferrum ions [1].

We believe that this range of effects can be related to the characteristics and functional groups present in the L-Carnitine molecule.

The effects can be analyzed either as a chain of reactions or as isolated effects. In the first case, a better use of cellular energy produces fewer free radicals, thus reducing the apoptosis rate. In the second case, L-Carnitine has different "targets" within the same cell.

We also believe that it is possible to collect, organize, and analyze all this new information, which goes beyond that provided by textbooks read during university or regular courses.

TRANSPORT OF L-CARNITINE: ROLE OF OCTN2

The transport protein OCTN2 belongs to the family of organic cation transporters (OCT). This family of transporters, which includes OCTN1, OCTN2 and OCTN3, is characterized by their ability to transport a wide variety of structurally unrelated organic cations [2].

OCTN1 and OCTN2 cDNAs have been found in humans, rats and mice, whereas OCTN3 cDNA has been found only in mice [3, 4].

OCTN2 has a unique characteristic: it can mediate the sodium-dependent transport of dipolar ions such as Carnitine and acylcarnitines. In addition, both the OCT family and the OAT (organic anion transporters) family belong to a superfamily of organic ion transporters. All these proteins are thought to contain 12 transmembrane domains.

Western blot analysis has shown that OCTN2 is expressed in kidney, skeletal muscle, heart and placenta [5, 6] but is only slightly expressed in pancreas, liver, lung, brain, small intestine, uterus, thymus and adrenal glands. A low level of expression of OCTN2 has also been observed in

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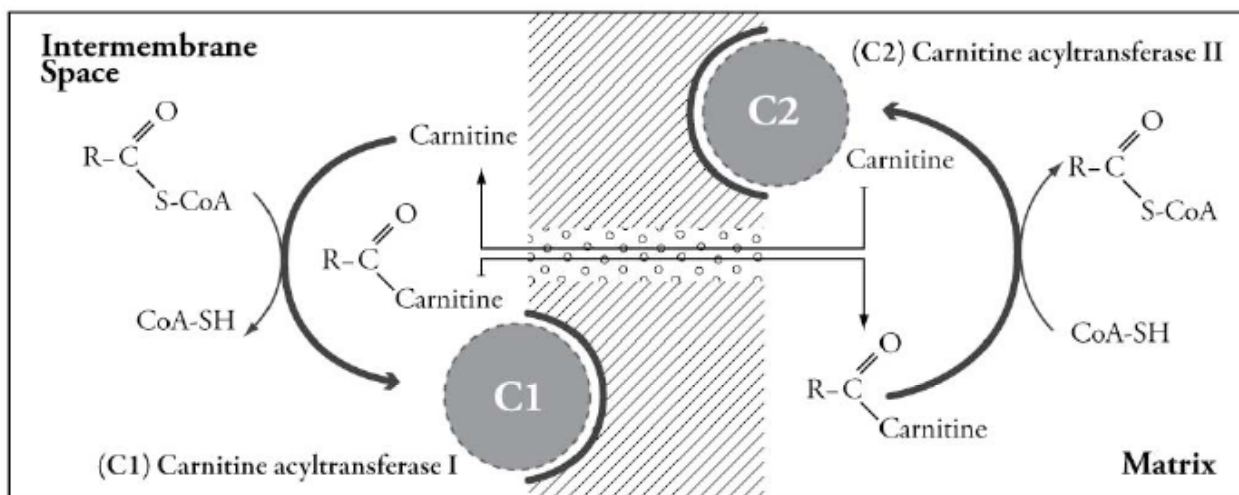


Fig. (f1). L-Carnitine transport.

fetuses. The OCTN2 cDNA encodes a 557-amino-acid protein that shares 75.8% homology with the OCTN1 transporter, which mediates the H⁺/organic cation antiport in the apical membrane of renal epithelial cells. OCTN2 contains 12 transmembrane domains, three N-glycosylation sites and six protein kinase C phosphorylation sites [5].

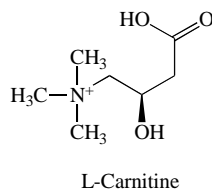


Fig. (2). Chemical structure of L-carnitine.

Peroxisome proliferator-activated receptor-alpha (PPAR α) is a nuclear receptor that plays a role in lipid metabolism and inflammatory responses. The activation of PPAR α by its ligands seems to regulate the expression of the *Octn2* gene. It has been reported that Clofibrate and Phenofibrate, two PPAR α ligands, induce the expression of the *Octn2* mRNA in cultured rat hepatocytes [7].

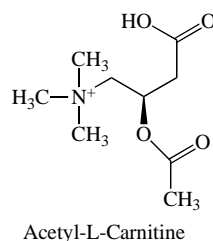


Fig. (3). Chemical structure of acetyl-L-Carnitine.

The K_m value (the substrate concentration that gives 50% of the activity) for OCTN2-mediated absorption of L-Carnitine has been estimated to be 4-20 μM [8, 5, 3, 9], which is in agreement with previous physiological studies

using membranes from kidney, skeletal muscle, heart and placenta. As mentioned above, all these tissues express high levels of OCTN2 [5]. The isomer D-Carnitine is transported with a lower K_m value (10.9 μM), which indicates that the transporter has a lower affinity for this substrate. In addition, OCTN2 transports Acetyl-Carnitine in a Na⁺-dependent manner and with high affinity ($K_m = 8.5 \mu\text{M}$). On the other hand, inhibition of transport has been observed for Acylcarnitines, which have longer fatty acid chains. These results suggest that the affinity of the OCTN2 transporter for these substrates decreases as they become more lipophilic [10].

As an OCT transporter, OCTN2 mediates Na⁺-independent transport of organic cations. However, transport of Carnitine requires Na⁺. This protein is the only OCT family member that has a high affinity for Na⁺, with an apparent K_m value of approximately 0.5 mM. Na⁺ is likely to be the driving force for transport and necessary to create a positive charge for the interaction of Carnitine with its binding site. The Hill coefficient obtained in a stoichiometric analysis was 0.926, thus suggesting that one Na⁺ ion is required to transport one molecule of Carnitine [10].

Studies carried out in mice seem to indicate that OCTN2-mediated transport of Carnitine is reabsorptive, whereas transport of fatty acids is secretory [11].

This transport mechanism is also responsible for reabsorption of filtered Carnitine in the kidney. Genetic defects that impair this transport mechanism result in primary Carnitine deficiency, which is associated with the low blood levels of Carnitine that result from the lack of reabsorption of Carnitine in the kidneys. Secondary Carnitine deficiency seems to result from competitive binding of certain drugs to the same transporter [8, 1]. In this respect, it has been observed that OCTN2 is involved in the transport of certain cephalosporins, such as cephaloridine, cephepime, cefluprenam and cefoselis. Na⁺ significantly increases the affinity of the OCTN2 transporter for cephaloridine, whereas it influences the interaction with cephepime and cefoselis only slightly. These observations explain the Carnitine deficiency observed during treatment with cephaloridine. This antibiotic

probably competes with the amino acid in the OCTN2-mediated renal reabsorption. Even if cefoselis and cephepime also compete with Carnitine, they have a lower affinity for the transporter and therefore interfere with reabsorption only slightly. It has also been observed that long-term treatment with ementine, pivalic acid and the anticonvulsant valproic acid leads to secondary Carnitine deficiency. However, valproic acid seems to affect the biosynthesis rather than the transport of the amino acid [8].

It has been demonstrated that Carnitine is absorbed by enterocytes isolated from wild-type mice. The transport process is saturable and the estimated K_m value is $20\mu\text{M}$, in agreement with the values informed above. Furthermore, analysis by immunoelectronic microscopy has shown that OCTN2 is present in the microvilli of absorptive epithelial cells of the small intestine. These observations are in line with the results published by Duran *et al.* in 2002 and 2005. That report also suggests that absorption of Carnitine in the small intestine is mainly mediated by OCTN2, although this mechanism seems not to be exclusive, since a certain level of amino acid absorption has been observed in OCTN2 knock-out mice [12].

Both in primary and secondary Carnitine deficiency, the heart is one of the most affected tissues. It is therefore interesting to investigate the role of OCTN2 in this organ. It has been observed that OCTN2 expression is higher in the ventricles than in the atria [5]. Also, healthy ventricles express higher levels of this transporter than sick ventricles [5]. OCTN2 expression does not vary with population characteristics such as age, body mass index or gender. However, pharmacological treatment is able to regulate the expression of this transporter. OCTN2 transcription is inhibited in patients treated with clopidrogel or ticlodipine as compared to control patients. Moreover, *Ocm2* mRNA levels are 40% higher in patients treated with beta blockers selective for the β_1 receptor than in control patients. In the ventricles, OCTN2 expression is highest in the cardiovascular endothelium. This tissue appears to be responsible for the uptake of blood carnitine, which is then transported down a concentration gradient to the muscle cells. carnitine concentration in this muscle is 50 times higher than in serum [5]. Another study performed by Iwata *et al.* suggests that OCTN2 localizes in the sarcolemma and intercalated discs of cardiomyocytes, whereas OCTN1 localizes in the vascular endothelium [13]. Enalapril, verapamil, spironolactone, mildronate [5] and, to a lower extent, quinidine are the drugs that interact with OCTN2 in the heart [13].

Transport of carnitine to the Central Nervous System and through the blood-brain barrier deserves special attention. The main function of carnitine is the transport of fatty acids to mitochondria, where they are oxidized in a four-reaction pathway. Since it is known that fatty acids are not the main energy source for the brain, carnitine transport to this tissue can be expected to be low or even absent. Although evidence indicates that OCTN2 is expressed in the brain [8], apparently this is not the only transporter involved. B(0,+), a transporter which is expressed together with OCTN2 in endothelial cells and has a low affinity for carnitine, seems to participate as well [14-17].

In summary, we have reviewed the structure, function and regulation of the expression of OCTN2 and other OCT family members. We have also described the role of these proteins in the regulation of L-carnitine levels in various tissues.

The activity of the transporter can be a key factor during L-carnitine deficiencies. It is worth mentioning that many molecules need to be first transported through the permeability barrier before they can participate in metabolic pathways.

METABOLISM: ABSORPTION, SYNTHESIS AND STORAGE.

Carnitine is present in most, but not all, animal species, several microorganisms and plants. It is synthesized from the amino acids lysine and methionine. The former provides the carbon skeleton and the latter provides four methyl groups. Four enzymes, one of which is mitochondrial, participate in its synthesis. Several tissues (liver, kidney, skeletal muscle, heart and brain) express all four enzymes. Trimethyllysine dioxygenase, the enzyme that catalyzes the first reaction in the synthesis has maximum activity in the kidney, whereas the other three enzymes have maximum activity in the liver, and thus carnitine is mainly synthesized in the liver [18].

Meat and fish are rich in carnitine, whereas green vegetables contain only a small amount of this amino acid. This means that people that have omnivorous occidental diets consume an average of 2-12 μmol of Carnitine per kilogram of body weight per day, whereas strict vegetarians consume less than 0.1 $\mu\text{mol}/\text{kg}/\text{day}$. Therefore, omnivores obtain 70-80% of the total available carnitine from the diet and 1/8 – 1/2 from endogenous Carnitine synthesis.

Strict vegetarians must synthesize more than 90% of the available Carnitine.

Orally administered Carnitine is absorbed in the intestine, both by a transporter-mediated process and by diffusion. However, absorption is not complete. Absorbed Carnitine accounts for 15-20% of pharmacologically administered Carnitine and for 75% of dietary Carnitine [20]. Bacteria present in the alimentary tract and specifically in the small intestine convert this amino acid into trimethylamine-N-oxide, which appears in feces, and γ -butyrobetaine, which appears in urine. The latter is absorbed and subsequently oxidized to trimethylamine-N-oxide in the liver [21].

Plasma Carnitine concentration varies with gender and age. It increases during the first year after birth and then stabilizes until puberty. During this period, plasma concentration is the same for both sexes. From puberty until adulthood, plasma Carnitine concentration in men increases and then reaches a stable value, which is lower than in women [19]. In plasma, neither L-Carnitine nor its short-chain esters are bound to proteins [20].

Animal tissues contain relatively high levels of carnitine, which range from 0.2 $\mu\text{mol}/\text{g}$ to 0.6 $\mu\text{mol}/\text{g}$. The highest concentrations are found in heart and skeletal muscle. Circulating L-Carnitine distributes between two kinetically defined compartments: one of them is large and has a low turn-

over rate (probably the muscle) and the other is relatively small and has a high turnover rate (probably the liver, kidney and other tissues) [22].

When L-Carnitine concentration is normal, renal reabsorption is highly efficient (90-99% of the filtered load; clearance, 1-3 mL/min) [20] but saturable. Therefore, when L-Carnitine concentration rises (for example, after an intravenous or oral dose), the reabsorption efficiency falls and clearance increases, which return L-Carnitine levels to normal values. The elimination kinetics for acetyl-L-carnitine is similar to that for L-carnitine [22].

PROTEIN ACETYLATION

Like other metabolic intermediates, L-carnitine has to be converted to its active form. During activation, an acetyl group is bound to the amino acid to give acetyl-L-carnitine (ALCAR).

When mice are fed ALCAR labeled in the acetyl group, a considerable amount of radioactivity is present in the liver, and lower concentrations are measured in the heart, brain, skeletal muscle and kidney. In addition, the C14 radioactive atom is found in the fatty acids of phospholipids and triacylglycerols in all these tissues [23].

Pettegrew has proposed that ALCAR is an important agent in the process of acetylation of the $-NH_2$ and $-OH$ groups of lysine, serine, threonine and tyrosin as well as of the $-NH_2$ group of other amino acids, which modifies protein structure [24].

At pH 7.0 and 38 °C, the free energy of hydrolysis of the acetyl group of several acylesters, expressed as ΔG° (Kcal/mol), is: ALCAR, -8.20; ATP, -8.20; acetylcholine, -6.99; acetyl-CoA, -8.54; and ATP (terminal PO₄), -7.60 [24].

Compared to acetylcholine or the terminal phosphate of ATP, ALCAR has a higher potential for the transfer of acetyl groups. This explains why it participates in transacetylation reactions.

In his work, Pettegrew includes three examples of potential acetylation reactions in which ALCAR may participate. The first is the acetylation of the β amyloid protein. The second is the acetylation of microtubules in PC12 cells, which results in more stable structures and reduces apoptosis, and in which histone acetylation increases. The last example suggests a role for ALCAR in acetylation of α -crystallin, a protein found in the eye lens [24]. However, he does not report whether the acetylation reaction occurs spontaneously or is enzyme-catalyzed.

NF- κ B activity can also be regulated by acetylation. The p65/RelA subunit is the target for alternative acetylation and deacetylation. These reactions are catalyzed by two acetyltransferases, p300/CBP and PCAF, and by histone deacetylase 3. Acetylated NF- κ B is not inhibited by I- κ B and can therefore bind to DNA with high affinity [25]. Ciecchio *et al.* have reported that ALCAR is the acetyl group donor. In cultured dorsal root ganglion cells, induction of p65/Rel-A acetylation increases the expression of the mGlu2 receptor mRNA. These results have been observed for ALCAR but

not for L-carnitine, thus supporting the role of ALCAR as an acetyl group donor. Although the authors of this paper point out that the mechanism by which ALCAR donates the acetyl group has not been completely elucidated, it is an interesting example of an acetylation reaction that may be enzyme-catalyzed [26].

Evidence indicates that ALCAR may also participate in histone acetylation in lymphocytes, which results in inhibition of cytogenetic expression of fragile X *in vitro* [27].

On the other hand, ALCAR seems not to be the acetyl donor for sialic acid oxidation in the Golgi apparatus, at least in the first step of the reaction [28].

It is interesting to note that studies performed in both neoplastic and untransformed cells show a relationship between CPT1 nuclear expression and histone hypoacetylation [29]. The authors of these studies show that CPT1 may be a regulatory protein in the Histone Deacetylase complex. In this sense, we can add that in tumor cells the activity of the FAS (fatty acid synthase) complex is increased, whereas that of CPT1 in the transport of fatty acids for β -oxidation is not. The higher activity of FAS probably results in elevated L-Carnitine levels that can competitively inhibit histone acetylation. Therefore, L-carnitine, rather than CPT1, may be the factor that leads to the hypoacetylation observed [29].

ROLE IN APOPTOSIS

Over the last years, many publications have suggested that L-carnitine has an antiapoptotic role. We therefore decided to analyze at which level in the apoptosis chain L-carnitine acts.

Various intracellular and extracellular stimuli, such as free radicals and TNF- α , respectively, can trigger apoptosis. The cell processes these signals and regulates the mechanism of apoptosis by means of specific regulatory proteins and the regulation of mitochondrial permeability, which is mediated by proteins that belong to the BCL-2 family. In addition, various agonists, like Ca⁺ and ROS, can affect mitochondria by causing mitochondrial permeability transitions. The formation of pores in the outer mitochondrial membrane releases cytochrome C, the apoptotic trigger, to the cytosol. The proteins of the BCL-2 family localize in the mitochondrial membrane, where they inhibit the increase in membrane permeability and stabilize proteins such as Apaf-1. These effects suppress caspase activation and apoptosis. Other BCL-2 family members can bind to BCL-2 and modulate its antiapoptotic function. For example, BCL-XL inhibits apoptosis, whereas BAX and BAD promote programmed cell death. Caspases are proteases that exist as inactive procaspases and cleave substrates at aspartic acid residues. Upstream caspases are activated by the apoptotic signal and, in turn, cleave and activate the downstream caspases. Caspase activation finally leads to the activation of DNases and DNA fragmentation.

In the cytosol, the cytochrome C that has been released from mitochondria binds to APAF-1, ATP and procaspase 9 to form the apoptosome, which activates procaspase 9. Activated caspase 9 is the upstream initiator of the apoptosis cascade [30].

We have previously described that carnitine protects the cell from free radicals that trigger apoptosis. However, this seems not to be the only level at which this molecule acts to inhibit apoptosis.

Many reports suggest that carnitine has a role at the caspase and BCL-2 levels.

GP7 is a laboratory-developed drug that induces apoptosis in a time- and dose-dependent manner. Its mechanism of action involves caspase 3 and DNA fragmentation. Pretreatment of Raji-Burkitt's lymphoma cells with L-Carnitine prevents GP7-induced activation of caspase 3, and cleavage of caspase 3 and DNA fragmentation induced by GP7 [31].

In an animal model of heart failure, an increase in the number of apoptotic nuclei in skeletal muscle, as well as an increase in caspase 3 and 9 levels and TNF- α and sphingosine serum levels was observed. Treatment of this model with L-carnitine prevented caspase activation and reduced TNF- α and sphingosine serum levels as well as the number of apoptotic nuclei in cultured muscle cells [32]. Another report indicates that high cytochrome C levels activate apoptosis in adult rat skeletal muscle fibers. *In vitro* caspase 3 assays confirmed these results. Addition of exogenous cytochrome C resulted in increased apoptosis in adult rats when compared to young rats, which suggests that cytochrome C is a limiting factor for caspase 3 activation in the cytosol. Supplementation with L-carnitine and lipoic acid reduces apoptosis in adult rats, maintains the integrity of the mitochondrial membrane and prevents cytochrome C release *in vivo* [33].

In another study, brain cortex cells, striatum cells and thalamus cells were subjected to serum deprivation for three days. The treatment reduced mitochondrial activity and the number of viable cells. The presence of dead cells with an apoptotic phenotype was also observed. Acetyl-carnitine and L-carnitine promoted cell survival and protected mitochondrial activity in a dose-dependent manner. The amino acids also inhibited DNA fragmentation, nuclear condensation, and the release of histones to the cytoplasm [34]. Furthermore, in a model of peripheral neuropathy in which a cuff was placed around the sciatic nerve in rats, apoptosis induced cytochrome C release to the cytosol, caspase 3 activation and genome fragmentation. When animals were treated with Acetyl-Carnitine, apoptosis was inhibited and reduced levels of cytosolic cytochrome C and active caspase 3 were observed. At the same time, the number of picnotic nuclei decreased [35].

All these examples show that carnitine and its acetylated derivatives regulate several steps in the apoptosis cascade, such as the release of cytochrome C to the cytosol or the activation of caspases. It is worth mentioning that all these studies were based on cells that were subjected to stress conditions. However, apoptosis also takes place in tissues that have not been exposed to noxa. At present, whether carnitine inhibits apoptosis only in the tissues and under the conditions described in the previous examples is not yet clear. Undoubtedly, these data are important steps towards the elucidation of the mechanism(s) of action of this drug. Nonetheless, in order to understand its pharmacodynamics in depth,

some elements still need to be found. We will go back to this point later in the discussion section of this review.

FREE RADICALS

At present, there is a lot of information concerning the effect of carnitine on the production of free radicals. The methods used in most of these publications to describe an increase or a decrease in the production of free radicals are measurements of DCFH fluorescence or indirect measurements of Glutathione. The activity of glutathione synthase, myeloperoxidase or superoxide dismutase and the measurement of damage through TBARS and malondialdehyde are also indicative of free radical levels.

In this way, the effect of carnitine has been studied in various organs and under different conditions that could result in free radical production.

In the nervous system, the beta-amyloid peptide, which is the main component of the senile plaques, is known to cause oxidative stress, both *in vivo* and *in vitro*. glutathione (GSH) is an important antioxidant and its levels decrease with the years. In a work carried out in rat astrocytes, ALCAR administration increased GSH levels, and the treatment of neuronal cell cultures with beta-amyloid decreased cell survival, probably due to an increased oxidation of proteins (protein carbonyl, 3-nitrotyrosine) and the formation of lipidic peroxides (4-hydroxy-2-nonenal). When these cultures were pre-treated with ALCAR, a dose-dependent decrease in cytotoxicity, protein oxidation, lipidic peroxidation and apoptosis was observed. ALCAR-treated cultures also exhibited increased GSH levels and Heat Shock Protein (HSP) expression as compared to control cultures [36,37]. Similarly, in another work, neuroblastoma SH-SY-5Y cells were treated with beta amyloid (abeta) and then cultured in either the absence or presence of ALCAR. This amino acid attenuated oxidative stress and the cell death induced by beta amyloid. Abeta also depleted ATP levels, thus suggesting that its neurotoxicity can also result from a compromised neuronal energy status. Since ALCAR prevented ATP depletion, its protective effect may be related not only to the control of oxidative stress but also to the maintenance of ATP levels [38].

In another report, four-week-old rats were fed water and 0.15% L-carnitine or ALCAR. Both drugs similarly increased carnitine levels plasma and brain. However, only ALCAR reduced lipidic peroxidation, as assessed by Malondialdehyde (MDA) measurements. These results suggest that ALCAR may be a better dietary supplement than L-carnitine [39].

As a result of forebrain ischemia, the number of intact neurons drops and ATP and GSH contents are reduced. Moreover, TBARS production increases significantly in the tissues of the hippocampus. ALCAR and propionyl-L-carnitine administration reduces attenuated neuronal damage, as evidenced by an increased number of intact neurons, higher levels of ATP and Glutathione and a decrease in TBARS in the hippocampus tissues [40].

Various drugs are known to be free radical sources. A work carried out in Wistar albino rats showed that Metrotrexate produces free radicals in different tissues.

Animals received a simple MTX dose (20 mg/kg) followed by either saline or L-carnitine (500 mg/kg). Animals were killed and samples were taken from blood, ileum, liver and kidney for MDA and GSH levels, Myeloperoxidase (MPO) activity and collagen content quantification. It was found that MTX increased MDA levels and MDO activity, but decreased collagen content and GSH level. L-carnitine reversed all these alterations. It was also observed that MTX induced a high rate of apoptosis in control rats, an effect reversed in L-carnitine-treated rats. A drop in TNF- α was also observed [41].

Adriamycin is an antineoplastic drug that has been associated with increased tissue oxidative stress. Invaginations of different sizes have been observed in the perinuclear cisternae of cardiac cells isolated from Adriamycin-treated rats but not in cells isolated from L-carnitine-treated rats. This report also suggests that L-carnitine stimulates HSP-70 response [42]. In another report, it was observed that L-carnitine inhibits hepatic damage caused by acetaminophen and that pretreatment of rats with L-carnitine reduces MDA and increases GSH in hepatocytes [43].

Irradiation is another source of oxidative stress. After treating rats with ALCAR for 5 days, an increase in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity is observed, whereas irradiated animals show low enzymatic activities. ALCAR also results in decreased total levels of nitrate/nitrite and MDA in lung and liver [44]. Gamma irradiation of the eye lens produces a significant increase in MDA levels and GSH-Px activity, as well as a decrease in SOD activity. These observations indicate the induction of oxidative stress and an early protective response. The administration of L-carnitine significantly reduces MDA levels and stimulates SOD and GSH-Px activity [45].

Oxidative stress and ALCAR effects can also be quantified at the chromosomal level. In a work carried out by Calabrese *et al.*, isolated human lymphocytes were pre-incubated with 5 mM L-carnitine and then treated with H₂O₂ and t-butyl-OOH. Chromosome aberrations were then analyzed by electrophoresis. The results showed that oxidative stress induced less simple stranded breaks and chromosome aberrations in ALCAR-treated cells [46].

Several reports also describe ALCAR-induced expression of Heat Shock Proteins [47, 36, 37].

Free radical and carnitine effects can also be evidenced through histological damage. In a study on ischemia/reoxygenation in rats, it was demonstrated that, in gastric mucosa, the damage was smaller in L-carnitine-treated animals. When TBARS were measured, the rats also presented a lower degree of lipidic peroxidation and reduced Catalase and SOD activities [49]. Similar results were obtained in rats exposed to ethanol damage, in which L-carnitine treatment reduced the size of the injuries [50].

In summary, L-carnitine has an important role in the protection from oxidative stress by modifying either the activity of key enzymes or the level of necessary protective intermediates. The mechanisms that lead to such modifications deserve further investigation.

MEDICAL APPLICATIONS

Based on the data described in this review the possible medical applications are broadly increased.

ALCAR may potentially be used in peripheral neuropathies. *In vitro* studies performed with different models of peripheral neuropathy have demonstrated that ALCAR increases the nervous conduction velocity, decreases neuronal loss and promotes neuronal regeneration [50-52]. This drug has also been shown to induce the expression of mGlu2 receptors in the central nervous system, which causes analgesy [50, 53]. Other studies suggest that analgesy produced at central level could result from the interaction with cholinergic transmission mechanisms [54].

HIV patients usually develop peripheral neuropathy, which appears to be caused by the virus itself and by pharmacological treatment with antiretroviral drugs. ALCAR treatment seems to alleviate the symptoms of neuropathy [55].

Two long-term randomized, placebo-controlled, double-blind trials evaluated ALCAR treatment in diabetic neuropathy. In the first trial, which included 333 patients, improvements in the nervous conduction velocity and in the sensory and motor amplitudes were reported [56]. In the second trial, which included 1257 patients, Diabetes Mellitus 2 patients experienced less neuropathic pain, being those with a short history of disease the ones that improved the most. Although improved vibratory perception was observed in the second trial, no improvements were found as regards the nervous conduction velocity [57].

On the other hand, ALCAR is a potential candidate for treatment of Alzheimer's disease. Several long-term trials have evaluated the clinical efficacy of this drug and analyzed changes in the clinical global impression and other psychometric tests, giving different results. Some showed improvements in the clinical global impression [58], others concluded that the drug failed to slow down the progression of the disease [59], whereas a third group suggested that ALCAR stops progression in young patients [60,61]. ALCAR has also been shown to improve certain parameters when associated with inhibitors of acetylcholinesterase [62].

Although some authors consider ALCAR as a potential therapeutic drug to slow down the progression of the disease, the previous results for treatment of Alzheimer's disease are contradictory [63,64]. Clearer results and a deep knowledge of the mechanisms of action of this drug are needed before any conclusions about the therapeutic potential of ALCAR can be drawn.

Some authors have also used ALCAR in Parkinson's disease and Multiple Sclerosis, but the existing evidence on the therapeutic potential of ALCAR in these diseases is insufficient [64].

Morover the use of L acetyl carnitine is a therapy to be considered in order to slow bone loss in ovarian hormone deficiency. The findings from animal experiments suggest that carnitine supplementation slows bone loss and improves their microstructural properties by decreasing the bone turnover. (65). It has been shown that dietary carnitine signifi-

cantly decreased the mRNA level of tartrate-resistant acid phosphatase (TRAP), which is an indicator of bone resorption. Also carnitine reduces mRNA levels from alkaline phosphatase (ALP) and collagen type-1 (COL), measures of bone formation. (66).

In the wide range of metabolic pathways in which L-carnitine could be considered we can include its use in fertility treatment, where carnitine expresses its activity increasing concentration, motility and sperm viability. Therefore it plays a critical role in metabolism, morphology and maturation of sperm. [67,68]

CONCLUSIONS

The biological functions of L-carnitine go far beyond its role in the transport of fatty acids. It regulates apoptosis and protects from free radicals. In addition, a possible role as an acetyl donor in the acetylation of proteins has been proposed for acetylated Carnitine. Even though the existing evidence comes from *in vitro* experiments, the results are promising for the design of future clinical trials. In many of these cases, the precise mechanism by which the observed interactions occur has not yet been elucidated. Studies have been performed in a wide range of systems, from tissues to simple eukaryotes, like yeast. These microorganisms can potentially be used to identify the genes involved in these biological processes, which in turn can be correlated with the homologous genes in higher eukaryotes.

ABBREVIATIONS

Abeta	=	Beta Amyloid
ALCAR	=	Acetyl-L-Carnitine
APAF-1	=	Apoptosis protease-activating factor-1
BAX	=	Bcl-2-associated X protein
BCL-2	=	B-cell lymphoma 2
CPT1	=	Carnitine palmitoyltransferase 1
DCFH	=	2',7' – dichlorofluorescein - diacetate
FAS	=	Fatty Acid Synthase
GSH	=	Glutathione
GSH-Px	=	Glutathione Peroxidase
HIV	=	Human immunodeficiency virus
HSP	=	Heat Shock Proteins
MDA	=	Malondialdehyde
MPO	=	Myeloperoxidase
MTX	=	Metrotrexate
NF-κB	=	nuclear factor kappa-light-chain-enhancer of activated B cells
OAT	=	Organic Anion Transporters
OCT	=	Organic Cation Transporter
PPARα	=	Peroxisome proliferator-activated receptor-α
SOD	=	Superoxide Dismutase

TBARS = Thiobarbituric acid reactive substances

TNF-α = Tumor necrosis factor-alpha

REFERENCES

- [1] Stella C.A.; Burgos H.I.; Salellas M.; Cristaldo M.L.; Ramos E.H. and Krugier N. L-Carnitine Effect upon Iron Growth Inhibition on *Saccharomyces cerevisiae*. *Lett Drug Dis Develop.*, **2005**, 2, 184-193.
- [2] Ganapathy, M. E.; Huang, W.; Raja, D. P.; Carter, A. L.; Sugawara, M.; Iseki, K.; Leibach, F. H.; Ganapathy, V. Beta-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J Biol Chem.*, **2000**, 275, 1699-707.
- [3] Tamai, I.; Ohashi, R.; Nezu, J.; Sai, Y.; Kobayashi, D.; Oku, A.; Shimane, M.; Tsuji, A. Molecular and functional characterization of organic cation/carnitine transporter family in mice. *J Biol Chem.*, **2000**, 275, 40064-72.
- [4] Inano, A.; Sai, Y.; Kato, Y.; Tamai, I.; Ishiguro, M.; Tsuji, A. Functional regions of organic cation/carnitine transporter OCTN2 (SLC22A5): roles in carnitine recognition. *Drug Metab. Pharmacokin.*, **2004**, 19, 180-9.
- [5] Tamai, I.; Ohashi, R.; Nezu, J.; Yabuuchi, H.; Oku, A.; Shimane, M.; Sai, Y.; Tsuji, A. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem.*, **1998**, 273, 20378-82.
- [6] Grube, M.; Schwabedissen, H. E. U. M.; Präger D.; Haney, J.; Möritz, K.; Meissner, K.; Eckel, L.; Böhm, M.; Jedlitschky, G.; Kroemer, H. K. Uptake of cardiovascular drugs into the human heart: expression, regulation, and function of the carnitine transporter OCTN2 (SLC22A5). *Circulation*, **2006**, 113, 1114-22.
- [7] Maeda, T.; Wakasawa, T.; Funabashi, M.; Fukushima, A.; Fujita, M.; Motojima, K.; Tamai, I. Regulation of Octn2 transporter (SLC22A5) by peroxisome proliferator activated receptor alpha. *Biol. Pharm. Bull.*, **2008**, 31, 1230-6.
- [8] Wagner, C. A.; Lükewille, U.; Kaltenbach, S.; Moschen, I.; Bröer, A.; Risler, T.; Bröer, S.; Lang, F. Functional and pharmacological characterization of human Na(+)-carnitine cotransporter hOCTN2. *Am. J. Physiol. Renal Physiol.*, **2000**, 279, 584-91.
- [9] Wu, X.; Huang, W.; Prasad, P. D.; Seth, P.; Rajan, D. P.; Leibach F. H.; Chen, J.; Conway, S. J.; Ganapathy, V. Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J Pharmacol Exp Ther*, **1999**, 290, 1482-92.
- [10] Ohashi, R.; Tamai, I.; Yabuuchi, H.; Nezu, J.; Oku, A.; Sai, Y.; Shimane, M.; Tsuji, A. Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J Pharmacol Exp Ther.*, **1999**, 291, 778-84.
- [11] Ohashi, R.; Tamai, I.; Nezu, J.; Nikaido, H.; Hashimoto, N.; Oku, A.; Sai, Y.; Shimane, M.; Tsuji, A. Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. *Mol Pharmacology*, **2001**, 59, 358-66.
- [12] Kato, Y.; Sugiura, M.; Sugiura, T.; Wakayama, T.; Kubo, Y.; Kobayashi, D.; Sai, Y.; Tamai, I.; Iseji, S.; Tsuji, A. Organic cation/carnitine transporter OCTN2 (Slc22a5) is responsible for carnitine transport across apical membranes of small intestinal epithelial cells in mouse. *Mo Pharmacology*, **2006**, 70, 829-37.
- [13] Iwata, D.; Kato, Y.; Wakayama, T.; Sai, Y.; Kubo, Y.; Iseji, S.; Tsuji, A. Involvement of carnitine/organic cation transporter OCTN2 (SLC22A5) in distribution of its substrate carnitine to the heart. *Drug Metab. Pharmacokin.*, **2008**, 23, 207-15.
- [14] Berezowski, V.; Miecz, D.; Marszalek, M.; Bröer, A.; Bröer, S.; Cecchelli, R.; Nalecz, K. A. Involvement of OCTN2 and B0,+ in the transport of carnitine through an *in vitro* model of the blood-brain barrier. *J. Neurochem.*, **2004**, 91, 860-72.
- [15] Nalecz, K. A.; Miecz, D.; Berezowski, V.; Cecchelli, R. Carnitine: transport and physiological functions in the brain. *Mol. Aspects Med.*, **2004**, 25, 551-67.
- [16] Inano, A.; Sai, Y.; Nakaido, H.; Hashimoto, N.; Asamo, M.; Tsuji, A.; Tamai, I. Acetyl-L-carnitine permeability across the blood-brain barrier and involvement of carnitine transporter OCTN2. *Biopharm. Drug Dispos.*, **2003**, 24, 357-65.
- [17] Miecz, D.; Januszewicz, E.; Czeredys, M.; Hinton, B.T.; Berezowski, V.; Cecchelli, R.; Nalecz, K. A. Localization of organic

- cation/carnitine transporter (OCTN2) in cells forming the blood-brain barrier. *J. Neurochem.*, **2008**, *104*, 113-23.
- [18] Vaz, F. M.; Wanders, R. J. A. Carnitine biosynthesis in mammals. *Biochem. J.*, **2002**, *361*, 417-29.
- [19] Rebouche, C. J. Carnitine function and requirements during the life cycle. *FASEB J.*, **1992**, *6*, 3379-86.
- [20] Evans, A.M.; Fornasini, G. Pharmacokinetics of L-carnitine. *Clin. Pharmacokinet.*, **2003**, *42*, 941-67.
- [21] Rebouche, C.J.; Chenard, C. A. Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites. *J. Nutr.*, **1991**, *121*, 539-46.
- [22] Rebouche, C.J. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann. N. Y. Acad. Sci.*, **2004**, *1033*, 30-41.
- [23] Farrel, S.; Vogel, J.; Bieber, LL. Entry of acetyl-L-carnitine into biosynthetic pathways. *Biochim. Biophys. Acta*, **1986**, *876*, 175-7.
- [24] Pettegre, J. W.; Levine, J.; McClure, R. J. Acetyl-L-carnitine physical-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression. *Mol Psychiatry*, **2000**, *5*, 616-32.
- [25] Kiernan, R.; Brès, V.; NG, R. W. M.; Coudart, M.; El Messaoudi, S.; Sardet, C.; Jin, D.; Emiliani, S.; Benkirane, M. Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. *J Biol Chem.*, **2003**, *278*, 2758-66.
- [26] Chiechio, S.; Copani, A.; De Petris, L.; Morales, M. E. P.; Nicoletti, F.; Gereau IV, R. W. Transcriptional regulation of metabotropic glutamate receptor 2/3 expression by the NF-kappaB pathway in primary dorsal root ganglia neurons: a possible mechanism for the analgesic effect of L-acetylcarnitine. *Molecular Pain*, **2006**, *2*, 20.
- [27] Pomponi, M. G.; Neri, G. Butyrate and acetyl-carnitine inhibit the cytogenetic expression of the fragile X *in vitro*. *Am. J. Med. Genet.*, **1994**, *51*, 447-50.
- [28] Higa, H. H.; Butor, C.; Diaz, S.; Varki, A. O-acetylation and de-O-acetylation of sialic acids. O-acetylation of sialic acids in the rat liver Golgi apparatus involves an acetyl intermediate and essential histidine and lysine residues—a transmembrane reaction? *J Biol Chem.*, **1989**, *264*, 19427-34.
- [29] Mazzarelli, P.; Pucci, S.; Bonano, E.; Sesti, F.; Calvani, M.; Spagnoli, L. G. Carnitine palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation? *Cancer Biol Ther.*, **2007**, *6*, 1606-13.
- [30] Friedlander, R. M. Apoptosis and caspases in neurodegenerative diseases. *The N Engl J Med.*, **2003**, *348*, 1365-75.
- [31] Qi, S.; Zhang, Z.; Wang, Z.; Yoshida, A.; Ueda, T. L-carnitine inhibits apoptotic DNA fragmentation induced by a new spin-labeled derivative of podophyllotoxin via caspase-3 in Raji cells. *Onco Rep.*, **2006**, *15*, 119-22.
- [32] Vescovo, G.; Ravara, B.; Gobbo, V.; Sandri, Angelini, A.; Della Barbera, M.; Dona, M.; Peluso, G.; Calvani, M.; Moscón, L.; Dalla Libera, L. L-Carnitine: a potential treatment for blocking apoptosis and preventing skeletal muscle myopathy in heart failure. *Am. J. Physiol. Cell Physiol.*, **2002**, *283*, 802-10.
- [33] Tamilselvan, J.; Jayaraman, G.; Sivarajan, K.; Panneerselvan, C. Age-dependent upregulation of p53 and cytochrome c release and susceptibility to apoptosis in skeletal muscle fiber of aged rats: role of carnitine and lipoic acid. *Free Radic. Biol. Med.*, **2007**, *43*, 1656-69.
- [34] Ishii, T.; Shimpō, Y.; Matsuoka, Y.; Kinoshita, K. Anti-apoptotic effect of acetyl-L-carnitine and L-carnitine in primary cultured neurons. *J. Pharmacol.*, **2000**, *83*, 119-24.
- [35] Di Cesare Mannelli, L.; Ghelardini, C.; Calvani, M.; Nicolai, R.; Mosconi, L.; Vivoli, E.; Pacini, A.; Bartolini, A. Protective effect of acetyl-L-carnitine on the apoptotic pathway of peripheral neuropathy. *Eur. J. Neurosci.*, **2007**, *26*, 820-7.
- [36] Abdul, H. M.; Calabrese, V.; Calvani, M.; Butterfield, D. A. Acetyl-L-carnitine-induced up-regulation of heat shock proteins protects cortical neurons against amyloid-beta peptide 1-42-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. *J. Neurosci. Res.*, **2006**, *84*, 398-408.
- [37] Abdul, H. M.; Butterfield, D. A. Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Free Radic. Biol. Med.*, **2008**, *42*, 371-84.
- [38] Dhitavat, S.; Ortiz, D.; Shea, T. B.; Rivera, E. R. Acetyl-L-carnitine protects against amyloid-beta neurotoxicity: roles of oxidative buffering and ATP levels. *Neurochem. Res.*, **2002**, *27*, 501-5.
- [39] Liu, J.; Head, E.; Kuratsune, H.; Cotman, C. W.; Ames, B. N. Comparison of the effects of L-carnitine and acetyl-L-carnitine on carnitine levels, ambulatory activity, and oxidative stress biomarkers in the brain of old rats. *Ann. N. Y. Acad. Sci.*, **2004**, *1033*, 117-31.
- [40] Al-Majed, A. A.; Sayed-Ahmed M. M.; Al-Omar F. A.; Al-Yahya A. A.; Aleisa A. M.; Al-Shabanah, O. A. Carnitine esters prevent oxidative stress damage and energy depletion following transient forebrain ischaemia in the rat hippocampus. *Clin. Exp. Pharmacol. Physiol.*, **2006**, *33*, 725-33.
- [41] Sener, G.; Ekşioğlu-Demiralp, E.; Cetiner, M.; Ercan, F.; Sirvanci, S.; Gedik, N.; Yeğen, B. C. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. *Cell Biol. Toxicol.*, **2006**, *22*, 47-60.
- [42] Strauss, M.; Porras, N. Differential expression of HSP70 and ultra-structure of heart and liver tissues of rats treated with adriamycin: protective role of L-carnitine. *Invest. Clin.*, **2007**, *48*, 33-43.
- [43] Yapar, K.; Kart, A.; Karapehlivan, M.; Atakisi, O.; Tunca, R.; Erginsoy, S.; Cital, M. Hepatoprotective effect of L-carnitine against acute acetaminophen toxicity in mice. *Exp. Toxicol. Pathol.*, **2007**, *59*, 121-8.
- [44] Mansour H. H. Protective role of carnitine ester against radiation-induced oxidative stress in rats. *Pharmacol. Res.*, **2006**, *54*, 165-71.
- [45] Kocer, I.; Taysi, S.; Ertekin, M. V.; Karşlioglu, I.; Gepdiremen, A.; Sezen, O.; Serifoglu, K. The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model. *Graefes Arch. Clin. Exp. Ophthalmol.*, **2007**, *245*, 588-94.
- [46] Garcia, C. L.; Filippi, S.; Mosesso, P.; Calvani, M.; Nicolai, R.; Palitti, F. The protective effect of L-carnitine in peripheral blood human lymphocytes exposed to oxidative agents. *Mutagenesis*, **2006**, *21*, 21-7.
- [47] Calabrese, V.; Giuffrida Stella, A. M.; Calvani, M.; Butterfield, D.A. Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *J. Nutr. Biochem.*, **2006**, *17*, 73-88.
- [48] Derin, N.; Izgüt-Uysal, V. N.; Agac, A.; Aliciguzel, Y.; Demir, N. L-carnitine protects gastric mucosa by decreasing ischemia-reperfusion induced lipid peroxidation. *J Physiol Pharmacol.*, **2004**, *55*, 595-606.
- [49] Dokmeci, D.; Akpolat, M.; Aydogdu, N.; Doganay, L.; Turan, F. N. L-carnitine inhibits ethanol-induced gastric mucosal injury in rats. *Pharmacol. Report.*, **2005**, *57*, 481-8.
- [50] Chiechio, S.; Copani, A.; Gereau, R.W. 4th; Nicoletti, F. Acetyl-L-carnitine in neuropathic pain: experimental data. *C.N.S. Drugs*, **2007**, *21*, 31-8.
- [51] Malone, J. I.; Lowitt, S.; Salem, A. F.; Miranda, C.; Korthals, J. K.; Carver, J. The effects of acetyl-L-carnitine and sorbinil on peripheral nerve structure, chemistry, and function in experimental diabetes. *Metabolism*, **1996**, *45*, 902-7.
- [52] Hart, A. M.; Wiberg, M.; Youle, M.; Terenghi G. Systemic acetyl-L-carnitine eliminates sensory neuronal loss after peripheral nerve trauma. *Exp. Brain Res.*, **2002**, *145*, 182-9.
- [53] Chiechio, S.; Caricasole, A.; Barleta, E.; Storto, M.; Catania, M. V.; Copani, A.; Vertechy, M.; Nicolai, R.; Calvani, M.; Melchiorri, D.; Nicoletti, F. L-Acetyl-L-carnitine induces analgesia by selectively up-regulating mGlu2 metabotropic glutamate receptors. *Mol Pharmacol.*, **2002**, *61*, 989-96.
- [54] Ghelardini, C.; Galeotti, N.; Calvani, M.; Mosconi, L.; Nicolai, R.; Bartolini, A. Acetyl-L-carnitine induces muscarinic antinociception in mice and rats. *Neuropharmacology*, **2002**, *43*, 1180-7.
- [55] Chiechio, S.; Copani, A.; Nicoletti, F. Gereau, R.W. IV. L-acetylcarnitine: a proposed therapeutic agent for painful peripheral neuropathies. *Curr Neuropharmacol*, **2006**, *4*, 233-7.
- [56] De Grandis, D.; Minardi, C. Acetyl-L-carnitine (levacecarnine) in the treatment of diabetic neuropathy. A long-term, randomised, double-blind, placebo-controlled study. *Drugs R. D.*, **2002**, *3*, 223-31.

- [57] Sima, A. A. F.; Calvani, M.; Mehra, M.; Amato, A. Acetyl-L-carnitine improves pain, nerve regeneration, and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized placebo-controlled trials. *Diabetes Care*, **2005**, *28*, 89-94.
- [58] Montgomery, S. A.; Thal, L. H.; Amrein, R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease. *Int. Clin. Psychopharmacol.*, **2003**, *18*, 61-71.
- [59] Thal, L. J.; Calvani, M.; Amato, A.; Carta, A. A 1-year controlled trial of acetyl-L-carnitine in early-onset AD. *Neurology*, **2000**, *55*, 805-10.
- [60] Brooks III, J. O.; Yesavage, J. A.; Carta, A.; Bravi, D. Acetyl L-carnitine slows decline in younger patients with Alzheimer's disease: a reanalysis of a double-blind, placebo-controlled study using the trilinear approach. *Int. Psychogeriatr.*, **1998**, *10*, 193-203.
- [61] Thal, L. J.; Carta, A.; Clarke, W. R.; Ferris, S. H.; Friedland, R. P.; Petersen, R. C.; Pfeiffer, E.; Raskind, M. A.; Sano, M.; Tuszyński, M. H.; Woolson, R. F. A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer's disease. *Neurology*, **1996**, *47*, 705-11.
- [62] Bianchetti, A.; Rozzini, R.; Trabucchi, M. Effects of acetyl-L-carnitine in Alzheimer's disease patients unresponsive to acetylcholinesterase inhibitors. *Curr. Med. Res. Opin.*, **2003**, *19*, 350-3.
- [63] Mayeux, R.; Sano, M. Treatment of Alzheimer's disease. *N Engl J Med.*, **1999**, *341*, 1670-9.
- [64] Kidd P. M. Neurodegeneration from mitochondrial insufficiency: nutrients, stem cells, growth factors, and prospects for brain rebuilding using integrative management. *Altern Med Rev.*, **2005**, *10*, 268-93.
- [65] Hooshmand S., Balakrishnan A, Clark R.M., Owen K.Q., Koo S.I., Arjmandi B.H. Dietary l-carnitine supplementation improves bone mineral density by suppressing bone turnover in aged ovariectomized rats. *Phytomedicine*. **2008**, *15*, 595-601.
- [66] Colucci S., Mori G., Vaira S., Brunetti G., Greco G., Mancini L., Simone G.M., Sardelli F., Koverech A., Zallone A., Grano M. L-carnitine and isovaleryl L-carnitine fumarate positively affect human osteoblast proliferation and differentiation *in vitro*. *Calcif Tissue Int.* **2005**, *6*, 458-65.
- [67] Giorgio C.. Male idiopathic oligoasthenoteratozoospermia. *Asian J Androl.*, **2006**, *8*, 143-157
- [68] Agarwal A, Said T.M.. Carnitines and male infertility. *Reprod Biomed Online*. **2004**, *4*, 376-84.