

Update on critical evidence for use of carnitine analogs in clinical practice in CNS disorders

Giovanna Traina

Department of Economics and Food Sciences, University of Perugia, Perugia, Italy

Abstract: L-carnitine (LC) is part of the carnitine shuttle system at the mitochondrial inner membrane (MIM) and transports long chain fatty acids over the MIM route. Acetyl-L-carnitine (ALC), the acetyl ester of LC, plays an essential role in intermediary metabolism. To ALC are ascribed neurotrophic actions, antioxidant and antiapoptotic activity, positive effects on mitochondrial metabolism, and stabilization of intracellular membranes. Acylcarnitine and LC supplementation have shown beneficial effects in the treatment of aging, chronic degenerative pathologies and the slowing of the progression of mental deterioration in neurodegenerative diseases, and painful neuropathies. ALC is reported to affect brain energy and phospholipid metabolism and to interact with cell membranes, proteins, and enzymes. It also shows a neuromodulatory effect on synaptic morphology and neurotransmitter synaptic transmission, including that of acetylcholine and dopamine. All these data suggest that ALC can affect several targets in the central nervous system. The roles and effects of LC and ALC have led researchers to investigate carnitine's involvement in a variety of neuropathological states and treatments, including autism, Parkinson's disease, Alzheimer's disease, Down's syndrome, Huntington's disease, cerebellar ataxia, age-associated mental decline, hepatic encephalopathy, and ammonia neurotoxicity. This review summarizes evidence that carnitine analogs play many roles in serious neurological pathologies.

Keywords: L-carnitine, acetyl-L-carnitine, brain, neural disorders

Introduction Carnitine metabolism

Carnitine plays a critical role in energy balance across cell membranes and in energy metabolism of tissues, in particular in carnitine free fatty acid metabolism.

L-Carnitine (LC) is the biologically active carnitine stereoisomer, a compound widely distributed in the food from animal sources. In humans, about 75% of carnitine is obtained from the diet.¹ The endogenous part is synthesized in kidney, liver, and brain from the essential amino acids lysine and methionine.² Ascorbic acid, ferrous iron, pyroxidine, and niacin are necessary cofactors.³ In mammals the pathway is unique, using protein-bound lysine methylated to form trimethyllysine (TML) as post-translational modification of protein synthesis.^{1,4} The normal plasma concentration of LC in healthy adults with a mixed diet is 40 to 50 μM . Omnivorous humans ingest 2 to 12 $\mu\text{mol/kg}$ of body weight/day.⁵ The absorption of LC decreases as the intake of LC increases, maintaining a constant LC concentration. Unabsorbed LC is degraded by microorganisms in the large intestine.⁵ Carnitine is not metabolized and is excreted, mostly as free carnitine in the urine, with daily urinary carnitine excretion equal to the sum of dietary absorption and

Correspondence: Giovanna Traina
Via San Costanzo, 06126, Perugia, Italy
Tel +39 75 5857977
Fax +39 75 5857904
Email traina@unipg.it

endogenous synthesis.⁶ Exogenous LC is almost completely excreted during the first 12 hours after administration whereas dietary LC is reabsorbed.

The main biological role of LC is to facilitate the transport of fatty acids to mitochondria. In fact, LC performs several biochemical and physiological roles, including: i) transporting fatty acids acetyl-CoA across the mitochondrial inner membrane (MIM) for β -oxidation; ii) providing acyl deposit that keeps appropriate levels of free CoA; iii) facilitating the oxidation of pyruvate and branched-chain ketoacids and, by preventing their accumulation, contributing to the protection of cells from potentially membrane-destabilizing acyl-CoAs.⁷ LC has an amphiphilic structure, making it very mobile throughout the cell. The free hydroxyl group has the potential for many different molecules to attach, creating a wide array of possible acylcarnitines. The ability to esterify and transport metabolites throughout the body distinguishes LC as a unique metabolite and suggests that the acylcarnitine profile might be a useful indicator of metabolic changes, particularly related to disease states. In addition, this wide array of possibilities also leads to a broad range of structures that are very different both chemically and metabolically.

Plasma concentration of free carnitine is in dynamic balance with acylcarnitine. Acylcarnitine esters are formed intracellularly during regular metabolic activity. LC undergoes acetylation in rodents and human intestine thus forming esterified compounds such as acetyl-L-carnitine (ALC). ALC is the principal acylcarnitine ester, an endogenous intermediate synthesized in many tissues including brain, liver, heart, kidney, and muscle.⁸ It is involved in trans-mitochondrial membrane traffic of acetyl units, participating in both anabolic and catabolic pathways in cellular metabolism.^{8,9} When injected into the brain, the acetyl groups were mostly incorporated into saturated fatty acids. ALC plays an essential role in energy production as 'shuttles' of long-chain fatty acids between the cytosol and the mitochondria for subsequent β -oxidation.¹⁰⁻¹² Together with LC, ALC is involved in the control of mitochondrial acetyl-CoA ratio, and peroxisomal oxidation of fatty acids.⁷

Carnitine and acylcarnitines cross the blood-brain barrier primarily via the high affinity through Na⁺-dependent organic cation/carnitine transporter (OCTN2), and secondarily via ATB^{0,+}, a Na⁺, Cl⁻-dependent amino acid transporter expressed in the hippocampus.¹³⁻¹⁶ ALC spreads across membranes much better than LC and its efflux in the systemic circulation was 4 times greater than that of LC.

Cao et al¹⁷ have shown that LC has a greater maximum plasma concentration than ALC and palmitoyl-L-carnitine

(PLC), and LC has a longer half-life than ALC and PLC. Yet a long-chain acylcarnitine, such as PLC, needs a transporter to cross the plasma membrane and, therefore, may be more restricted in its actions. As a result, changes in individual acylcarnitines may imply changes in specific metabolic pathways.

The roles of long-chain acylcarnitines, specifically PLC, in the brain have been investigated. Owing to the amphiphilic nature of PLC, it can react on the membrane surface and influence membrane fluidity and the activity of membrane enzymes and transporters.^{18,19} PLC is involved in phospholipid and fatty acid turnover in rat fetal neurons.²⁰

Carnitine in the brain

The importance of carnitine in the brain is emphasized by carnitine deficiency symptoms, many of which involve major deleterious effects.²¹

Carnitine deficiency

Two types of carnitine deficiency states exist. Primary carnitine deficiency is a genetic disorder consisting in a recessive mutation of the cellular carnitine-transporter system (OCTN2) that usually manifests itself by 5 years of age with symptoms of cardiomyopathy, skeletal-muscle weakness, and hypoglycemia. Secondary carnitine deficiency is an acquired carnitine depletion that may occur due to certain disorders (chronic renal failure) or under particular conditions, such as the use of antibiotics, which can reduce carnitine absorption or increase its excretion.^{6,7}

Since the brain is dependent on oxidative metabolism, impairment of fatty acid metabolism and energy production due to lack of carnitine leads to metabolic encephalopathy.²² The majority of the brain is composed of fatty acids, which are needed for incorporation into structural lipids.²³ Glucose is the primary energy source for the adult brain under normal conditions, but fatty acids become pivotal energy substrates for the brain under metabolically compromised conditions such as fasting or starvation. For this reason, carnitine and acylcarnitines functions in fatty acid metabolism, ketosis, and buffering of the concentration ratio of acyl-CoA to free CoA, are significant in brain metabolism, particularly metabolic disturbances present in neurological disease.

The enzymes required for the synthesis of carnitine are present in brain tissue.²⁴ LC is stored in neurons of the cerebral cortex and forms acylcarnitines. Isolated neurons of the adult brain contain approximately 80% free carnitine, 10% to 15% ALC, and less than 10% long-chain acylcarnitines. Since carnitine and its acylcarnitines have chemical structures comparable to choline and acetylcholine (ACh), it has been suggested that they are involved in neurotransmission. In

particular, many studies showed a modulation of synaptic transmission by LC and ALC, through an increase in ACh synthesis and release, an enhancement of dopamine release, and an increase in γ -aminobutyric acid (GABA).^{21,25–27} The transporters OCTN1, OCTN2, and OCTN3 are expressed in the central nervous system of the mouse in regions that suggest they play a role in modulating cerebral bioenergetics and in synthesis of ACh.²⁸

Acetyl-L-carnitine in the brain

ALC is present at relatively high levels in the brain.²⁹ It is highest in the hypothalamus,³⁰ where the level of the ALC-synthesizing enzyme, carnitine acetyltransferase (CAT), is high. Since ALC can readily cross the blood–brain barrier,³¹ its supplementation could possibly affect brain metabolism. Injection of ALC in rats leads to reduced oxidation of glucose and increased glycogen synthesis in brain.³²

ALC has been proposed to have beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders (Table 1). The main mechanism of action of ALC is the improvement of mitochondrial respiration which allows the neuron to produce ATP necessary to maintain the normal membrane potential.³³

The main effects of ALC on the nervous system can be summarized as follows:

1. ALC has been shown to be neuroprotective through a variety of effects (see below) such as the increase in protein kinase C (PKC)³⁴ activity and gene expression.^{21,35,36} In particular, an increase in PKC in the rat brain cortex is correlated with an improvement of the performance in a spatial learning task, reversing the age-related decline.^{33,34}
2. ALC counteracts the loss of N-Methyl D-Aspartate (NMDA) receptors in neuronal membrane.³³

Table 1 Role of acetyl-L-carnitine in nervous system diseases

Neural disorders	Cited references
Alzheimer's disease	31,37,53,54,80,100–103,105,106
Parkinson's disease	84,87,90
Autism spectrum disorders	93,94
Neuronal ceroid lipofuscinosis	99,116–121
Cerebellar ataxia	72
Huntington's disease	81,90
Down's syndrome	79,108
Aging	27,31,33,56,57,58,60
Neurodegeneration	50,51
Intractable epilepsy	21,85,115
Chronic fatigue	21,109–111
Antioxidant and antiapoptosis	56,61–71

3. ALC increases the production of neurotrophins.³³ Many studies have focused on the neurotrophic effects of ALC in the nervous system. ALC modulates the activity of nerve growth factor (NGF) and a number of hormones.³⁷ In particular, ALC increases NGF production and NGF binding in vivo.^{38,39} NGF affects neuronal development and maintenance of neurons in the peripheral and central nervous system. ALC has influence on neuronal repair and nerve fiber regeneration. In diabetic Worcester rats prolonged treatments with ALC promote nerve fiber regeneration, correct both the Na⁺/K⁺ ATPase and nerve conduction defect, and prevent structural changes associated with diabetes pathology.⁴⁰ ALC prevents the age-dependent structural changes in rat peripheral nerves and, in lesioned animals, ALC treatment promotes regeneration of nerves by significantly increasing both the density of regenerating myelinated fibers and axon diameter.⁴¹ ALC has also exhibited both neuroprotective and neurotrophic activity in primary motoneurons exposed to excitotoxic agents or deprived of brain-derived neurotrophic factor (BDNF).⁴² ALC exerts cytoprotective, antioxidant, and antiapoptotic activity, and there is some experimental evidence that ALC might also have antiaging effects and cardioprotective activity.^{33,43,44} Feeding ALC to older Fisher rats (22–28 months of age) increases the cellular consumption of oxygen, and reverses the declines in mitochondrial membrane potential and cardiolipin content.⁴⁵ ALC improves different aspects of the neuronal metabolism,^{33,46–48} and has wide neuromodulatory effects.^{7,26,43,46} It plays a neuromodulatory role by increasing the synthesis of phospholipids for membrane formation and integrity.⁷ When ALC was added to α -lipoic acid (LA) to rats, reversals in the age-associated decline of mitochondrial membrane potential and the levels of ascorbate and malondialdehyde were observed.^{49,50} The density of neuronal mitochondria associated with lipofuscin and vacuoles has been reduced by feeding ALC and LA to aged rats. In addition, an increase of the number of intact mitochondria has been observed.⁵¹ Aged rats showed significant improvements on cognitive tasks, including the Morris water maze test.⁵²

Anti-aging and neurodegeneration

ALC has been shown to have beneficial effects treating symptoms of cerebral dysfunction caused by aging and in some disorders of aging associated with cholinergic deficiency, such as Alzheimer's disease (AD).^{53,54}

Listed below are the most important examples of ALC effects on aging.

- a. Sershen et al²⁷ studied the effect of ALC on dopamine release and age-related changes in dopamine receptors. These receptors declined with age, and treatment with ALC for 3 months diminished the reduction in receptor binding.
- b. Both sphingomyelin and cholesterol tend to accumulate in the brain of older rats. Such increments are reduced by long-term ALC supplementation.⁵⁵
- c. ALC can reverse alterations in membrane lipid content and function, and it can improve age-related changes in metabolism, either directly through supplying high-energy acyl groups or indirectly through restoring membranes.
- d. In rats, chronic ALC treatment increases life-span, improves cognitive behavior in aged animals, and guarantees long-term memory performance.³³
- e. Aging produces both a decline in mitochondrial energetics and an augment in oxidative stress. ALC prevents age-related changes in mitochondrial respiration and decreases oxidative stress biomarkers through the upregulation of heme oxygenase-1 (HO-1), Hsp70, and superoxide dismutase-2 in senescent rats, and a high expression of the redox-sensitive transcription factor Nrf2.⁵⁶⁻⁵⁸ ALC is involved in cognitive functions in rats.²⁶ A recent study suggests that supplementation with ALC improves attention, learning, and spatial working memory deficits, reduces oxidative stress, and inhibits apoptotic cascade induced by hypoxia.⁵⁹
- f. ALC restores the age-associated decline of learning and memory in aging animals.⁶⁰

Antioxidant and antiapoptotic functions

ALC can be protective against oxidative stress by: 1) a reduction in tissue lactic acidosis, which brings about the formation of reactive oxygen species (ROS); 2) shifts in both the mitochondrial and cytosolic redox state;⁶¹ and/or 3) the induction of antioxidant genes.^{56,61} Such events could lead to an increase of reducing power necessary for detoxification through the glutathione system.⁶¹ Protection against mitochondrial alterations and cell death from cytokines along with an increased expression of HO-1 has been observed in primary rat cortical astrocytes treated with ALC.⁵⁶ Traina et al³⁵ reported an upregulation of hsp72 gene expression in rat brain after chronic treatment with ALC. Hsp72 gene plays a protective role against brain oxidative stress, and works as a relevant cellular protection molecule against protein aggregation.⁶²⁻⁶⁴ These changes restored

the ratio of reduced to oxidized glutathione and reversed the inhibition of complex IV. Further studies have evidenced that ALC decreases both 4-hydroxy-2-nonenal (HNE) formation and protein carbonyls, indicators of oxidative stress.^{58,65} Pretreatment of cortical neurons with ALC and LA decreased HNE-mediated neurotoxicity, protein and lipid oxidation, and apoptosis in a dose-dependent manner as well as increased cellular reduced glutathione and heat-shock proteins (hsps).⁶⁶ These results showed that ALC induces upregulation of HO-1, hsp60, and hsp72, and that this effect may involve the transcription factor Nrf2, implying the possibility that ALC, by promoting acetylation of DNA-binding proteins, can induce post-translational modifications of critical target proteins. Such a new role of ALC as a molecule able to enhance the cellular stress response pathways appears to be promising as an alternative therapeutic approach for those pathophysiological conditions where stimulation of the HO pathway is guaranteed.⁵⁶

Oxidative stress underlies the neuropathology of AD and other disorders. ALC treatment leads to the activation of phosphoinositol-3 kinase, protein kinase, and extracellular signal-regulated kinase pathways that are important in neuronal cell survival and differentiation.⁶⁶ Both ALC and LC treatments have also been shown to reduce apoptosis through the mitochondrial pathway.⁶⁷⁻⁶⁹ Oxidative stress from insults such as hypoxia and deprivation of trophic factors can cause apoptosis both *in vitro* and *in vivo*. ALC treatment reduced apoptosis in serum-deprived mouse fibroblasts, an effect that was confirmed by an assessment of cytochrome c release and immunoreactivity to caspase 3. ALC as well as LC promoted neuronal survival and mitochondrial activity and have antiapoptotic effects in serum-deprived primary culture neurons.⁶⁹ ALC is involved in the energy response and maintenance and appears to stimulate cell proliferation.

Traina et al⁷⁰ reported that ALC upregulates the voltage-dependent anion channel. This channel exerts an important role in cellular homeostasis, in apoptosis, and in synaptic plasticity.

ALC downregulates ferritin-H gene expression.³⁶ Several studies suggest that multiple independent pathways exist which converge in the increase of ferritin synthesis in response to various forms of oxidative insult. Ferritin, with its ability to oxidize and sequester intracellular iron in an internal mineral core, limits the levels of catalytically available iron, owing to the generation of free radicals, as a critical cytoprotective protein that constitutes an integral part of the

antioxidant response. A recent study reported that ALC exerts antioxidant effect and reverses iron-induced oxidative stress in human fibroblast.⁷¹ It is possible that ALC might reduce available iron, by reducing ferritin expression.³⁶

Finally, despite the different genetic defects underlying degenerative cerebellar ataxia, it has been suggested that mitochondrial energy production and antioxidative metabolism dysfunction may be common biochemical alterations related to this disease. Treatments with ALC in patients with degenerative ataxia produced an improvement of some symptoms and reduced the progression of the disease.⁷²

Energy metabolism

According to its role in whole energy metabolism, ALC can repair neurological injury through metabolic pathways.²¹ In rats, ALC supplementation enhances the level of phosphocreatine and decreases the concentrations of lactate and inorganic phosphate in aging and post-ischemic brain models, providing protection during metabolic stress, such as ischemia, hypoxia, reperfusion of the brain, alcohol, and brain injury.^{73,74} In particular, studies by ³¹P and ¹H-NMR spectroscopy suggested a therapeutic role for ALC in the treatment of cerebral ischemia through a faster recovery.^{21,74} Dogs treated with ALC exhibited a reduction in neurological deficit scores after cerebral ischemia and reperfusion, suggesting that ALC improves neurological effect, as a result of potentiating brain energy metabolism.^{21,75} ALC increases fluidity in rat brain microsomes and liposomes.^{76,77} ALC, as well as LC, may affect membrane fluidity due to its amphiphilic structure.⁷⁸ Alterations in neural phospholipid composition and further effects on signal transduction pathways have been found to be characteristic of many neurological disorders such as AD, Down syndrome, and Huntington's disease.^{79–81}

Evidence suggests that ALC exerts a function in the elongation–desaturation of the *n*–3 polyunsaturated fatty acids to form 22:6*n*–3, docosahexaenoic acid (DHA), in mitochondria.⁸² It is believed that ALC represents an intramitochondrial source of acetyl groups and supplies them in the elongation pathway. Changes in DHA content of membranes can affect synaptic plasticity, enzymatic activities, immunity, inflammatory response, gene expression, ion channels, membrane-bound proteins, and neurotransmission.⁸³

Neuroprotection mechanisms from excitotoxicity

It is known that either the formation of ROS or mitochondrial dysfunction lead to metabolic and oxidative stress and are

the basic processes in many neurotoxic and neurodegenerative disorders, such as AD and Parkinson's disease (PD).⁸⁴ The oxidative stress affects the activities of the respiratory chain complexes I–V, changes that are crucial in many neurological disorders. ALC treatment is neuroprotective. ALC upregulates cytochrome b oxidase, and complex bc 1 gene expression.³⁶ ALC increases the energy status of the cell by increasing the activities of cytochrome b oxidase, thus maintaining energy levels of the cells and stabilizing mitochondrial activity. Cassano et al⁸⁵ have found an increase in transcripts related to mitochondrial biogenesis with ALC supplementation in a rat model for hind-limb muscle atrophy. ALC could also help to generate more mitochondria under certain conditions, preserving or improving overall metabolic function.⁸⁵

Pretreatment of young rats with ALC exerted effect neuroprotection at the mitochondrial level against 3,4-methylenedioxymethamphetamine (ecstasy).⁸⁶ Neurotoxicity was induced in vitro by rotenone, an inhibitor of mitochondrial complex I, in rat cortical neurons. Coincubation of the cells with ALC increases survival and partial protection from cell death. An inhibitor of complex I, 1-methyl-4-phenylpyridinium (MPP+), results in symptoms similar to those in patients with PD. Virmani et al⁸⁷ reported that the inhibitory action of MPP+ was partly reversed by incubation of cells with ALC. In neuroblastoma cells, treatment with ALC, but not LC, prevented MPP+(+) toxicity and partially restored intracellular ATP concentrations. However, ALC did not reverse the MPP+(+)-induced loss of mitochondrial oxygen consumption suggesting that protective effects are independent of oxidative phosphorylation. ALC may exert protection through maximizing cellular glucose efficiency under both normal and MPP+-treated conditions.⁸⁸ Studies suggest that the mechanism of neuroprotection may be through restoration of mitochondrial function and/or improved energy use since at least part of the toxicity of MPP+ is due to mitochondrial inhibition.^{87,88}

It has been observed that neurons are protected by LC after inducing neurotoxicity through 3-nitropropionic acid, a molecule that induces neurotoxicity through irreversible binding to succinate dehydrogenase (complex II of the mitochondrial respiratory chain), suggesting that LC's protective effects against neurotoxicity mainly seem to be due to its antioxidant ability.⁸⁹ Defects in the function of complexes II and III have been observed in Huntington's disease patients.⁹⁰ Due to the reduction of both mortality and neuronal degeneration, LC appeared to be protective against neurotoxicity. More-

over, LC inhibited the increase in oxidized glutathione and mitochondrial dysfunction in the hippocampus and prevented neuronal hypoglycemia-induced damage.^{91,92}

Several studies have described an association between autism spectrum disorders and mitochondrial dysfunction. Also carnitine deficiency is commonly found in autistic patients.⁹³ Carnitine deficiency results in impaired β -oxidation. In autistic patients, as a consequence of impaired β -oxidation polyunsaturated long-chain fatty acids and/or saturated very long chain fatty acids were elevated. None of the autistic patients had a well-recognized cause of a primary deficiency. None had the carnitine deficiency or the clinical picture seen with the classical OCTN2 transport defect.⁹⁴

Traina et al³⁶ reported that ALC treatment upregulates the expression of brain-specific Na^+ -dependent inorganic phosphate transporter gene. This evidence is consistent with studies that have ascribed to vesicular glutamate transporter 1 a role in the protection against excitotoxic injury. In addition, ALC upregulates prostaglandin D2 synthase (PGD2S) gene expression.³⁶ Liang et al⁹⁵ showed that in the brain the activation of DP1, a receptor of PGD2, can prevent neuronal injury in paradigms of acute excitotoxicity, and Lin et al⁹⁶ indicated that a product of PGD2 exhibits anti-inflammatory properties, supporting an emerging and neuronal protective role for prostaglandins that may present novel therapeutic targets in neurological diseases.

Both a decline in mitochondrial energetics and an increase in oxidative stress are some of the effects of aging. ALC can decrease brain lipid peroxidation in old rats whereas LC was ineffective. However, treatment with both carnitine and acylcarnitines has shown to significantly reduce the levels of circulating tumor necrosis factor- α (TNF- α) and interleukins, which could then protect against oxidative stress.^{97,98}

Alzheimer's disease

Preclinical studies suggested that ALC treatment could be beneficial for the treatment of age-related diseases. ALC exerts advantageous effects on AD, the most common disorder in the geriatric population. The clinical efficacy of ALC was previously reported, and several molecular mechanisms were evoked to support it.³⁷ Many studies suggest that the mode of action of ALC in AD may involve, as well as synaptic function, an increase of cholinergic activity, restoration, protection against toxins, acetylation of proteins, and neurotrophic effects stimulating NGF. Patients affected by AD treated with ALC at doses ranging from 1 to 2 g/day for 6 to 12 months have shown an improved performance on several cognitive tests.

Recent studies reported that chronic ALC treatment induces modulation of expression of genes involved in neural

disorders.^{99,100} In particular, ALC upregulates kinesin light chain 1 (KLC1) gene expression.¹⁰⁰ Kinesin-1 is needed to move different types of cargoes in neuronal axons. A receptor that attaches KLC1 to vesicular cargoes is the amyloid precursor protein (APP). It is known that the deposition of APP degradative product in the brain is a major pathological finding in AD. Axonal transport of APP is mediated by direct binding of the KLC1, and leads to the suggestion that abnormal interaction of APP and KLC1 could play a role in the pathogenesis of AD.¹⁰¹ Reduction in KLC1 increases $\text{A}\beta$ levels in the brain, and accelerates and enhances amyloid deposition. Reductions in microtubule-dependent transport may stimulate proteolytic processing of APP, resulting in the development of senile plaques and AD.¹⁰¹

ALC may therefore, in some way, modify key pathogenic elements in AD, such as amyloid processing. Another hypothesis is that it can alter membrane fluidity or composition and that this modification can influence disintegrin and metalloprotease domain 10 (ADAM10) activity. This is supported by findings that describe the activation of ADAM10 as the major target of the cholesterol effects on APP metabolism because of increased membrane fluidity.¹⁰² Supplementation of ALC has been shown to normalize the levels of high-energy phosphate in the brain of AD patients as measured by ³¹P magnetic resonance spectroscopy.¹⁰³

ALC has well-established antioxidant properties and studies in humans and animals suggest ALC has a favorable role in restoring cerebral energy metabolism, ie, ALC increases the activity of both cytochrome oxidase and α -ketoglutarate dehydrogenase in intrasynaptic but not in nonsynaptic mitochondria from rat cerebral cortex.^{32,104} It was hypothesized that the antioxidant properties of ALC on compromised mitochondrial function could be involved in the effect of this compound on α -secretase activity and APP metabolism. Accumulation of the $\text{A}\beta$ peptide has been implicated as the cause of the cognitive decline seen with AD. The $\text{A}\beta$ peptide can suppress levels of acetyl-CoA and the activity of choline acetyltransferase in cell culture.¹⁰⁵ In this study ALC reversed these effects, but it did not change the mortality of the undifferentiated cells. In other cases of neurotoxicity from $\text{A}\beta$ fragments, ALC was able to attenuate the oxidative stress, ATP depletion, and cell death.⁷ ALC may be acting through buffering of oxidative stress and maintaining energy levels.

However, results are variable on the extent to which ALC improves clinical symptoms of AD: some studies have observed significant improvements in biochemical assays and psychometric tests, whereas others have not observed such large differences on a large scale.^{106,107}

Finally, since there is an increased prevalence of AD in people with Down's syndrome, it was suggested that ALC administration might affect central nervous function positively and decrease mental deterioration in older people with Down's syndrome.¹⁰⁸

Chronic fatigue and neurotransmitter modulation

Chronic fatigue patients show reduced biosynthesis of neurotransmitters.^{21,109} ALC treatment reduces physical and mental fatigue in the elderly, and improve cognitive status, suggesting effects on endogenous ACh levels.¹¹⁰ ALC supplementation induces an increase in choline uptake, ACh synthesis, and ACh release in synaptosomes, striatum, and hippocampus of rats.¹¹ Together with choline, carnitine was found to stimulate ACh synthesis in a synergistic way in rat cortex cells.¹¹¹ Pretreatment with ALC determines a progressive and dose-dependent recovery of field potential amplitude, which is an index of functional activity of striatal neurons. The addition of a choline transporter inhibitor blocks this protective effect of ALC. It is believed that choline transporters support presynaptic ACh synthesis and release. Neuroprotection by ALC is prevented by the addition of a nonselective muscarinic antagonist and by an M2-like receptor antagonist. By changing the ACh production in the brain, ALC increases cholinergic neurotransmission.^{11,21,38} Studies suggest that ALC may improve transmitter function of cholinergic neurons by enhancing the acetyl-CoA concentration in the cytosol.¹⁰⁵

Intractable epilepsy

The ketogenic therapy (KT) is a therapeutic, alternative diet for pharmaco-resistant epilepsies. It mimics the state of starvation through a low-carbohydrate, high-fat regimen. Since dietary sources of glucose are dramatically reduced, during KT treatment the body synthesizes ketones as an energy supplement to the brain.

A study on rat hippocampus identified modulation of expression of genes after KT treatment, including genes involved in energy metabolism, signal transduction, oxidative phosphorylation, accompanied with mitochondrial biogenesis.¹¹² ALC upregulates mitochondrial transcripts in soleus muscle and improves mitochondrial morphology and function reinforcing the effects of KT.^{85,113}

Both KT and ALC have comparable effects: increased β -oxidation, mitochondrial biogenesis, and enhanced energy reserves by reducing "the levels of circulating proinflammatory cytokines (TNF- α and IL-1 β). Many of

the neuroprotective functions of ALC could have beneficial effects for epileptic patients, such as neurotransmitter modulation, upregulation of hsp's, and protection against excitotoxicity. Since free radical concentrations and apoptosis may be elevated in states of enhanced fat metabolism, a free radical quencher with antiapoptotic effects such as ALC may be useful".^{21,114,115} Future studies will be needed to improve our knowledge on ALC's role in KT.

Neuronal ceroid lipofuscinosis

In order to study the role of ALC in molecular mechanisms Traina et al^{35,36,70,99,100} have identified the differentially expressed genes in the rat brain in response to ALC treatment and gene expression was compared at the mRNA level using suppression subtractive hybridization (SSH). These authors comprehensively analyzed all the genes that are up- or downregulated after long-lasting ALC treatment. SSH combines normalization and subtraction of cDNAs in a single procedure and allows enrichment of differentially expressed sequences, generating an equalized representation of differentially expressed genes irrespective of their relative abundance. It is an excellent technology to search for differentially expressed genes in the tissues, and, in particular, for rare transcripts and unknown genes. Two different mRNA populations are compared so as to obtain clones of genes that are differentially expressed. The studies proved that chronic ALC treatments modulate different gene expression in the rat brain, and that the majority of detected clones are involved in the neuroprotection and/or in neuromodulation.

In particular, Traina et al⁹⁹ observed the effects of ALC on important molecular elements involved, to different degrees, in neuronal ceroid lipofuscinosis (NCL). ALC treatment: 1) upregulates the expression of the lysosomal H⁺/ATPase, V1 subunit D gene; 2) downregulates the expression of the myelin basic protein (MBP) gene; 3) downregulates the expression of the ATP synthase lipid-binding protein, subunit c gene. The neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessively inherited monogenetic storage disorders. Since there are no effective therapies available, all forms of NCL invariably prove to be fatal after a prolonged period of disability. Indeed, for the forms of NCL that are the result of mutations in transmembrane proteins, the therapeutic outlook remains uniformly bleak. NCLs are considered as lysosomal storage diseases (LSDs). In NCLs the ceroid lipopigments are accumulated in the lysosomes, such as subunit c of mitochondrial ATP synthase. In particular, a loss of H⁺/ATPases determines a strong accumulation

of the subunit c of mitochondrial ATP synthase and increased amounts of lysosomal enzymes.¹¹⁶ Since the low pH of lysosomes is necessary to maintain the activity of acid hydrolases in the lysosomal lumen, a deficit in proton pump leads to severe neurodegeneration. The upgrading of the lysosomal protonic pump by ALC treatment might be a compensatory mechanism of the abnormal higher lysosomal pH.⁹⁹ Holopainen et al¹¹⁷ have measured intracellular and lysosomal pH in fibroblast cell lines of patients with 6 different types of NCLs. The highest alkalinization was found in lysosomes of the most severe form of NCL. The elevated lysosomal pH might interfere with the catalytic activity of the lysosome by inactivating hydrolases.¹¹⁷ There are studies suggesting that the regulation of lysosomal pH may be the underlying cause of Batten disease. In the CLN3 form, the lysosomal pH has been shown to be elevated.¹¹⁷

It has been observed that ALC exerts a downregulation of the subunit c of ATP synthase gene expression.^{35,99} This protein is the major protein accumulated in the storage bodies of animals or humans affected by NCL. The fact that this protein, initially located in the mitochondria, is accumulated in lysosomes of NCL cells strongly suggests that the intracellular trafficking of specific molecules to lysosome is severely altered. In a canine model for the juvenile form of the human disease, a major constituent of the storage bodies is the subunit c protein of mitochondrial ATP synthase that contains an ϵ -N-TML residue.¹¹⁸ TML is a precursor in carnitine biosynthesis. The changes in plasma carnitine and TML levels support the possibility that the disease involves a defect in the carnitine biosynthetic pathway.¹¹⁸ Both TML and carnitine levels were significantly depressed in the affected individuals. In addition, dietary supplementation with carnitine delayed the progression of cognitive decline in NCL dogs.¹¹⁹ Prolonged treatment with LC in isolated fibroblast cells from NCL patients fully restored the mitochondrial enzyme activities.¹²⁰ ALC and LA supplementation diet not only eliminated the mitochondrial damage, but also prevented the formation of lipofuscin and/or myelin-like structures in neurones.⁵¹

These results are the first studies in which, by applying a molecular biological approach, it has been possible to identify a direct effect of ALC on gene expression related to neuronal activity. The results are of relevant importance for possible therapeutic intervention, contributing to the increasingly recognized importance of the role of ALC in neuroprotection and suggesting a pathway for the treatment of NCL.⁹⁹

Wei et al¹²¹ have shown that endoplasmic reticulum (ER) and oxidative stresses (ER stress) are common manifestations in cells

from both neurodegenerative and non-neurodegenerative LSD. These ER stresses might cause apoptosis. Wei et al¹²¹ claim that lysosomal dysfunction, through the alteration of pH, produces ER and oxidative stress, supporting the idea that all the abnormalities originate by altering the pH of organelles. According to these findings, neuroprotective therapeutic strategies, involving substances that reduce the risk of neurodegeneration, are emerging. These substances are chemical and pharmacological chaperones that stabilize the conformation of proteins, increase the protein-folding capacity of the ER, and facilitate the trafficking of mutant proteins, including one of the main intracellular redox systems involved in neuroprotection, the vitagene system, as a potential target for novel cytoprotective intervention. Vitagene encode for cytoprotective hsp70 and HO-1 as well as thioredoxin reductase and sirtuins proteins.⁶³ In addition, dietary antioxidants, including curcumin, ALC, and carnosine, have neuroprotective roles. In conclusion, these chemical chaperones might protect LSD cells.

Disclosure

The author reports no conflicts of interest in this work.

References

- Steiber A, Kerner J, Hoppel C-L. Carnitine: a nutritional, biosynthetic, and functional perspective. *Mol Aspects Med.* 2004;25:455–473.
- Cave MC, Hurt RT, Frazier TH, et al. Obesity, inflammation, and the potential application of pharmacconutrition. *Nutr Clin Pract.* 2008;23:16–34.
- Kendler BS. Carnitine: an overview of its role in preventive medicine. *Prev Med.* 1986;15:373–390.
- Rebouche CJ. Carnitine function and requirements during the life cycle. *Faseb J.* 1992;6:3379–3386.
- Vaz FM, Wanders RJ. Carnitine biosynthesis in mammals. *Biochem J.* 2002;361:417–429.
- Stanley CA. Carnitine deficiency disorders in children. *Ann NY Acad Sci.* 2004;1033:42–51.
- Virmani A, Binienda Z. Role of carnitine esters in brain neuropathology. *Mol Aspects Med.* 2004;25:533–549.
- Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann NY Acad Sci.* 2004;1033: 30–41.
- Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. *Annu Rev Nutr.* 1998;18:39–61.
- Bieber LL. Carnitine. *Annu Rev Biochem.* 1988;57:261–283.
- Nalecz KA, Miecz D, Berezowski V, Cecchelli R. Carnitine: transport and physiological functions in the brain. *Mol Aspects Med.* 2004;25: 551–567.
- Shea TB. Effects of dietary supplementation with N-acetyl cysteine, acetyl-L-carnitine and S-adenosyl methionine on cognitive performance and aggression in normal mice and mice expressing human ApoE4. *Neuromolecular Med.* 2007;9:264–269.
- Kido Y, Tamai I, Ohnari A, et al. Functional relevance of carnitine transporter OCTN2 to brain distribution of L-carnitine and acetyl-L-carnitine across the blood-brain barrier. *J Neurochem.* 2001;79: 959–969.
- Nakanishi T, Hatanaka T, Huang T, et al. Na⁺- and Cl⁻-coupled active transport of carnitine by the amino acid transporter ATB[0, +] from mouse colon expressed in HRPE cells and Xenopus oocytes. *J Physiol.* 2001;531:297–304.

15. Sloan JL, Mager S. Cloning and functional expression of a human Na⁺ and Cl⁻-dependent neutral and cationic amino acid transporter B₀,⁺. *J Biol Chem*. 1999;274:23740–23745.
16. Miecz D, Januszewicz E, Czeredys M, et al. Localization of organic cation/carnitine transporter [OCTN2] in cells forming the blood–brain barrier. *J Neurochem*. 2008;104:113–123.
17. Cao Y, Wang YX, Liu CJ, Wang LX, Han ZW, Wang CB. Comparison of pharmacokinetics of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine after single oral administration of L-carnitine in healthy volunteers. *Clin Invest Med*. 2009;32:E13–E19.
18. Haruna T, Horie M, Takano M, et al. Alteration of the membrane lipid environment by L-palmitoylcarnitine modulates K[ATP] channels in guinea-pig ventricular myocytes. *Pflugers Arch*. 2000;441: 200–207.
19. Liu QJ, Rosenberg RL. Activation and inhibition of reconstituted cardiac L-type calcium channels by palmitoyl-L-carnitine. *Biochem Biophys Res Commun*. 1996;228:252–258.
20. Arduini A, Denisova N, Virmani A, Avrova N, Federici G, Arrigoni-Martelli E. Evidence for the involvement of carnitine-dependent long-chain acyltransferases in neuronal triglyceride and phospholipid fatty acid turnover. *J Neurochem*. 1994;62:1530–1538.
21. Jones LL, McDonald DA, Borum PR. Acylcarnitine: role in brain. *Prog Lipid Res*. 2010;49:61–75.
22. Kimura S, Amemiya F. Brain and liver pathology in a patient with carnitine deficiency. *Brain Dev*. 1990;12:436–439.
23. Nalecz KA, Nalecz MJ. Carnitine – a known compound, a novel function in neural cells. *Acta Neurobiol Exp [Warsz]*. 1996;56:597–609.
24. Rebouche CJ, Engel AJ. Tissue distribution of carnitine biosynthetic enzymes in man. *Biochim Biophys Acta*. 1980;630:22–29.
25. Falchetto S, Kato G, Provini L. The action of carnitines on cortical neurons. *Can J Physiol Pharmacol*. 1971;49:1–7.
26. Ando S, Tadenuma T, Tanaka Y, et al. Enhancement of learning capacity and cholinergic synaptic function by carnitine in aging rats. *Neurosci Res*. 2001;66:266–271.
27. Sershen H, Harsing LG Jr, Banay-Schwartz M, Hashim A, Ramacci MT, Lajtha A. Effect of acetyl-L-carnitine on the dopaminergic system in aging brain. *J Neurosci Res*. 1991;30:555–559.
28. Lamhonwah AM, Hawkins CE, Tam C, Wong J, Mai L, Tein I. Expression patterns of the organic cation/carnitine transporter family in adult murine brain. *Brain Dev*. 2008;30:31–42.
29. Shug AL, Schmidt MJ, Golden GT, Fariello RG. The distribution and role of carnitine in the mammalian brain. *Life Sci*. 1982;31:2869–2874.
30. Bresolin N, Freddo L, Vergani L, Angelini C. Carnitine, carnitine acyltransferases, and rat brain function. *Exp Neurol*. 1982;78:285–292.
31. Parnetti L, Gaiti A, Mecocci P, Cadini D, Senin U. Pharmacokinetics of IV and oral acetyl-L-carnitine in a multiple dose regimen in patients with senile dementia of Alzheimer type. *Eur J Clin Pharmacol*. 1992;42: 89–93.
32. Aureli T, Di Cocco ME, Pucetti C, et al. Acetyl-L-carnitine modulates glucose metabolism and stimulates glycogen synthesis in rat brain. *Brain Res*. 1998;796:75–81.
33. McDaniel MA, Maier SF, Einstein GO. “Brain-specific” nutrients: a memory cure? *Nutrition*. 2003;19:957–975.
34. Pascale A, Milano S, Corsico N, et al. Protein kinase C activation and anti-amnesic effect of acetyl-L-carnitine: in vitro and in vivo studies. *Eur J Pharmacol*. 1994;265:1–7.
35. Traina G, Valleggi S, Bernardi R, et al. Identification of differentially expressed genes induced in the rat brain by acetyl-L-carnitine as evidenced by suppression subtractive hybridisation. *Mol Brain Res*. 2004;132:57–63.
36. Traina G, Federighi G, Brunelli M, Scuri R. Cytoprotective effect of acetyl-L-carnitine evidenced by analysis of gene expression in the rat brain. *Mol Neurobiol*. 2009;39:101–106.
37. Pettegrew JW, Levine J, McClure RJ. 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;5:616–632.
38. Furlong JH. Acetyl-L-carnitine: metabolism and applications in clinical practice. *Altern Med Rev*. 1996;1:85–93.
39. Foreman PJ, Perez-Polo JR, Angelucci L, Ramacci MT, Tagliatela G. Effects of acetyl-L-carnitine treatment and stress exposure on the nerve growth factor receptor [p75 NGFR] mRNA level in the central nervous system of aged rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 1995;19:117–133.
40. Sima AAF, Ristic H, Merry A, et al. Primary preventive and secondary interventional effects of acetyl-L-carnitine on diabetic neuropathy in the bio-breeding Worcester rat. *J Clin Invest*. 1996;97: 1900–1907.
41. De Angelis C, Scarfò C, Falcinelli M, et al. Acetyl-L-carnitine prevents age-dependent structural alterations in rat peripheral nerves and promotes regeneration following sciatic nerve injury in young and senescent rats. *Exp Neurol*. 1994;128:103–114.
42. Bigini P, Larini S, Pasquali C, Muzio V, Mennini T. Acetyl-L-carnitine shows neuroprotective and neurotrophic activity in primary culture of rat embryo motoneurons. *Neurosci Lett*. 2002;329:334–338.
43. Calò LA, Pagnin E, Davis PA, et al. Antioxidant effect of L-carnitine and its short chain esters. Relevance for the protection from oxidative stress related cardiovascular damage. *Int J Cardiol*. 2006;107:54–60.
44. Manfredi A, Forloni GL, Arrigoni-Martelli E, Mancina M. Culture of dorsal root ganglion neurons from aged rats: effects of acetyl-L-carnitine and NGF. *Int J Dev Neurosci*. 1992;10:321–329.
45. Hagen TM, Ingersoll RT, Wehr CM, et al. Acetyl-L-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity. *Proc Natl Acad Sci U S A*. 1998;95:9562–9566.
46. Calvani M, Arrigoni-Martelli E. Attenuation by acetyl-L-carnitine of neurological damage and biochemical derangement following brain ischemia and reperfusion. *Int J Tissue React*. 1999;21:1–6.
47. Ori C, Freo U, Pizzolato G, Dam M. Effects of acetyl-L-carnitine on regional cerebral glucose metabolism in awake rats. *Brain Res*. 2002;951:330–335.
48. Tanaka M, Nakamura F, Mizokawa S, Matsumura A, Matsumura K, Yasuyoshi W. Role of acetyl-L-carnitine in the brain: revealed by bioradiography. *Biochem Biophys Res Commun*. 2003;306: 1064–1069.
49. Hagen TM, Liu JK, Lykkesfeldt J, et al. Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc Natl Acad Sci U S A*. 2002;99: 1870–1875.
50. Long JG, Gao F, Tong LQ, Cotman CW, Ames BN, Liu JK. Mitochondrial decay in the brains of old rats: ameliorating effect of alpha-lipoic acid and acetyl-L-carnitine. *Neurochem Res*. 2009;34:755–763.
51. Aliev G, Liu J, Shenk JC, et al. Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats. *J Cell Mol Med*. 2009;13:320–333.
52. Beal MF. Bioenergetic approaches for neuroprotection in Parkinson’s disease. *Ann Neurol*. 2003;53:S39–S47.
53. Calvani M, Lannuccelli M, Colasimone D, Orfalian Z. Chronicle of the development of an endogenous substance for dementia: acetyl-L-carnitine. In: Bes A, editor. *Senile Dementias: Early Detection*. John Libbey Eurotext; 1986:394–400.
54. Bonavita E. Study of the efficacy and tolerability of L-acetylcarnitine therapy in the senile brain. *Int J Clin Pharmacol*. 1986;24:511–516.
55. Aureli T, Di Cocco ME, Capuani G, et al. Effect of long-term feeding with acetyl-L-carnitine on the age-related changes in rat brain lipid composition: a study by P-31 NMR spectroscopy. *Neurochem Res*. 2000;25:395–399.
56. Calabrese V, Ravagna A, Colombrita C, et al. Acetylcarnitine induces heme oxygenase in rat astrocytes and protects against oxidative stress: involvement of the transcription factor Nrf2. *J Neurosci Res*. 2005; 79:509–521.
57. Calabrese V, Colombrita C, Sultana R, et al. Redox modulation of heat shock protein expression by acetylcarnitine in aging brain: relationship to antioxidant status and mitochondrial function. *Antioxid Redox Signal*. 2006;8:404–416.
58. Calabrese V, Stella AMG, Calvani M, Butterfield DA. Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *J Nutr Biochem*. 2006;17:73–88.

59. Barhwal K, Singh SB, Hota SK, Jayalakshmi K, Llavazhagan G. Acetyl-L-carnitine ameliorates hypobaric hypoxic impairment and spatial memory deficits in rats. *Eur J Pharmacol.* 2007;570:97–107.
60. Seidman MD, Khan MJ, Bai U, Shirwany N, Quirk WS. Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. *Am J Otol.* 2000;21:161–167.
61. Zanelli SA, Solenski NJ, Rosenthal RE, Fiskum G. Mechanisms of ischemic neuroprotection by acetyl-L-carnitine. *Ann NY Acad Sci.* 2005; 1053:153–161.
62. Kravets A, Hu Z, Miralem T, Torno MD, Maines MD. Biliverdin reductase, a novel regulator for induction of activating transcription factor-2 and heme oxygenase-1. *J Biol Chem.* 2004;279:19916–19923.
63. Calabrese V, Cornelius C, Mancuso C, et al. Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. *Neurochem Res.* 2008;33:2444–2471.
64. Glover JR, Lindquist S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell.* 1998;94: 73–82.
65. Poon HF, Calabrese V, Calvani M, Butterfield DA. Proteomics analyses of specific protein oxidation and protein expression in aged rat brain and its modulation by L-acetylcarnitine: insights into the mechanisms of action of this proposed therapeutic agent for CNS disorders associated with oxidative stress. *Antioxid Redox Signal.* 2006;8:381–394.
66. Abdul HM, Butterfield DA. 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.* 2007;42:371–384.
67. Furuno T, Kanno T, Arita K, et al. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. *Biochem Pharmacol.* 2001;62:1037–1046.
68. Pillich RT, Scarsella G, Risuleo G. Reduction of apoptosis through the mitochondrial pathway by the administration of acetyl-L-carnitine to mouse fibroblasts in culture. *Exp Cell Res.* 2005;306:1–8.
69. Ishii T, Shimpo Y, Kinoshita K, Kinoshita K. Anti-apoptotic effect of acetyl-L-carnitine and L-carnitine in primary cultured neurons. *Jpn J Pharmacol.* 2000;83:119–124.
70. Traina G, Bernardi R, Rizzo M, Calvani M, Durante M, Brunelli M. Acetyl-L-carnitine up-regulates expression of voltage-dependent anion channel in the rat brain. *Neurochem Int.* 2006;48:673–678.
71. Lal A, Atamna W, Killilea DW, Suh JH, Ames BN. Lipoic acid and acetyl-carnitine reverse iron-induced oxidative stress in human fibroblasts. *Redox Rep.* 2008;13:2–10.
72. Sorbi S, Forleo P, Fani C, Piacentini S. Double-blind, crossover, placebo-controlled clinical trial with L-acetylcarnitine in patients with degenerative cerebellar ataxia. *Clin Neuropharmacol.* 2000;23: 114–118.
73. Aureli T, Miccheli A, Ricciolini R, et al. Aging brain: effect of acetyl-L-carnitine treatment on rat brain energy and phospholipid metabolism. A study by ³¹P and ¹H NMR spectroscopy. *Brain Res.* 1990;526:108–112.
74. Aureli T, Miccheli A, di Cocco ME, et al. Effect of acetyl-L-carnitine on recovery of brain phosphorus metabolites and lactic acid level during reperfusion after cerebral ischemia in the rat. Study by ³¹P- and ¹H-NMR spectroscopy. *Brain Res.* 1994;643:92–99.
75. Rosenthal RE, Williams R, Bogaert YE, Getson PR, Fiskum G. Prevention of postischemic canine neurological injury through potentiation of brain energy metabolism by acetyl-L-carnitine. *Stroke.* 1992;23:1312–1318.
76. Arduini A, Mancinelli G, Radatti GL, Dottori S, Molajoni F, Ramsay RR. Role of carnitine and carnitine palmitoyltransferase as integral components of the pathway for membrane phospholipid fatty acid turnover in intact human erythrocytes. *J Biol Chem.* 1992;267:12673–12681.
77. Arienti G, Ramacci MT, Maccari F, Casu A, Corazzi L. Acetyl-L-carnitine influences the fluidity of brain microsomes and of liposomes made of rat brain microsomal lipid extracts. *Neurochem Res.* 1992;17: 671–675.
78. Virmani MA, Caso V, Spadoni A, Rossi S, Russo F, Gaetani F. The action of acetyl-L-carnitine on the neurotoxicity evoked by amyloid fragments and peroxide on primary rat cortical neurones. *Neuroprotect Agents.* 2001;939:162–178.
79. Murphy EJ, Schapiro MB, Rapoport SI, Shetty HU. Phospholipid composition and levels are altered in down syndrome brain. *Brain Res.* 2000;867:9–18.
80. Farooqui AA, Rapoport SI, Horrocks LA. Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. *Neurochem Res.* 1997;22:523–527.
81. Puri BK. Impaired phospholipid-related signal transduction in advanced Huntington's disease. *Exp Physiol.* 2001;86:683–685.
82. Infante JP, Huszagh VA. Secondary carnitine deficiency and impaired docosahexaenoic [22:6n-3] acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and beta-oxidation. *FEBS Lett.* 2000;468:1–5.
83. Horrocks LA, Farooqui AA. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostag Leukotr Essent Fatty Acids.* 2004;70: 361–372.
84. Hinerfeld D, Traini MD, Weinberger RP, et al. Endogenous mitochondrial oxidative stress: neurodegeneration, proteomic analysis, specific respiratory chain defects, and efficacious antioxidant therapy in superoxide dismutase 2 null mice. *J Neurochem.* 2004;88: 657–667.
85. Cassano P, Sciancalepore AG, Pesce V, et al. Acetyl-L-carnitine feeding to unloaded rats triggers in soleus muscle the coordinated expression of genes involved in mitochondrial biogenesis. *Biochim Biophys Acta Bioenerg.* 2006;1757:1421–1428.
86. Alves E, Binienda Z, Carvalho F, et al. Acetyl-L-carnitine provides effective in vivo neuroprotection over 3,4-methylenedioxymethamphetamine-induced mitochondrial neurotoxicity in the adolescent rat brain. *Neuroscience.* 2009;158:514–523.
87. Virmani A, Gaetani F, Binienda Z, Xu A, Duhart H, Ali SF. Role of mitochondrial dysfunction in neurotoxicity of MPP+ – Partial protection of PC12 cells by acetyl-L-carnitine. *Ann NY Acad Sci* 2004;1025:267–273.
88. Mazzeo E, Yoon KJ, Soliman KFA. Acetyl-L-carnitine cytoprotection against 1-methyl-4-phenylpyridinium toxicity in neuroblastoma cells. *Biochem Pharmacol.* 2003;66:297–306.
89. Silva-Adaya D, Perez-De La Cruz V, Herrera-Mundo MN, et al. Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. *J Neurochem.* 2008;105:677–689.
90. Schapira AHV. Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic paraplegia and Friedreich's ataxia. *Biochim Biophys Acta Bioenerg.* 1999;1410:159–170.
91. Binienda Z, Virmani A, Przybyla-Zawislak B, Schmued L. Neuroprotective effect of L-carnitine in the 3-nitropropionic acid [3-NPA]-evoked neurotoxicity in rats. *Neurosci Lett.* 2004;367:264–267.
92. Hino K, Nishikawa M, Sato E, Inoue M. L-carnitine inhibits hypoglycemia-induced brain damage in the rat. *Brain Res.* 2005;1053:77–87.
93. Palmieri L, Persico AM. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? *Biochim Biophys Acta.* 2010;1797: 1130–1137.
94. Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. *J Autism Develop Disord.* 2004;34: 615–623.
95. Liang X, Liejun W, Tracey H, Katrin A. Prostaglandin D2 mediates neuronal protection via the DP1 receptor. *J Neurochem.* 2005;92: 477–486.
96. Lin TN, Cheung WM, Wu JS, et al. 15d-prostaglandin J2 protects brain from ischemia-reperfusion injury. *Arterioscler Thromb Vasc Biol.* 2006;26:481–487.
97. Liu J, Head E, Kuratsune H, Cotman CW, Ames BN. 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 NY Acad Sci.* 2004;1033:117–131.

98. Winter BK, Fiskum G, Gallo LL. Effects of L-carnitine on serum triglyceride and cytokine levels in rat models of cachexia and septic shock. *Br J Cancer*. 1995;72:1173–1179.
99. Traina G, Bernardi R, Cataldo E, Macchi M, Durante M, Brunelli M. In the rat brain acetyl-L-carnitine treatment modulates the expression of genes involved in neuronal ceroid lipofuscinosis. *Mol Neurobiol*. 2008;38:146–152.
100. Traina G, Federighi G, Brunelli M. Up-regulation of kinesin light-chain I gene expression by acetyl-L-carnitine: therapeutic possibility in Alzheimer's disease. *Neurochem Int*. 2008;53:244–247.
101. Stokin GB, Lillo C, Falzone TM, et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science*. 2005;307:1282–1288.
102. Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10. *Proc Natl Acad Sci U S A*. 2001;98:5815–5820.
103. Pettegrew JW, Klunk WE, Panchalingam K, Kanfer JN, McClure RJ. Clinical and neurochemical effects of acetyl-L-carnitine in Alzheimer's disease. *Neurobiol Aging*. 1995;16:1–4.
104. Gorini A, D'Angelo A, Villa RF. Action of L-acetylcarnitine on different cerebral mitochondrial populations from cerebral cortex. *Neurochem Res*. 1998;23:1485–1491.
105. Szutowicz A, Bielarczyk H, Gul S, Zielinski P, Pawelczyk T, Tomaszewicz M. Nerve growth factor and acetyl-L-carnitine evoked shifts in acetyl-CoA and cholinergic SN56 cell vulnerability to neurotoxic inputs. *J Neurosci Res*. 2005;79:185–192.
106. Montgomery SA, Thal LJ, 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.
107. Thal LJ, Calvani M, Amato A, Carta A. A 1-year controlled trial of acetyl-L-carnitine in early-onset AD. *Neurology*. 2000;55:805–810.
108. Pueschel SM. The effect of acetyl-L-carnitine administration on persons with Down syndrome. *Res Dev Disabil*. 2006;27:599–604.
109. Kuratsune H, Yamaguti K, Lindh G, et al. Brain regions involved in fatigue sensation: reduced acetylcarnitine uptake into the brain. *Neuroimage*. 2002;17:1256–1265.
110. Malaguarnera M, Gargante MP, Cristaldi E, et al. Acetyl-L-carnitine [ALC] treatment in elderly patients with fatigue. *Arch Gerontol Geriatr*. 2008;46:181–190.
111. Wawrzenczyk A, Nalecz KA, Nalecz MJ. Effect of externally added carnitine on the synthesis of acetylcholine in rat cerebral cortex cells. *Neurochem Int*. 1995;26:635–641.
112. Bough KJ, Wetherington J, Hassel B, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol*. 2006;60:223–235.
113. Liu JK, Atamna H, Kuratsune H, Ames BN. Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites. Increasing healthy life span: conventional measures and slowing the innate aging process. *Ann NY Acad Sci*. 2002;959:133–166.
114. Ma W, Berg J, Yellen G. Ketogenic diet metabolites reduce firing in central neurons by opening K[ATP] channels. *J Neurosci*. 2007;27:3618–3625.
115. Li SY, Liu Y, Sigmon VK, McCort A, Ren J. High-fat diet enhances visceral advanced glycation end products, nuclear O-Glc-Nac modification, p38 mitogen-activated protein kinase activation and apoptosis. *Diabetes Obes Metab*. 2005;7:448–454.
116. Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K. Loss of the chloride channel CIC-7 leads to lysosomal storage disease and neurodegeneration. *EMBO J*. 2005;24:1079–1091.
117. Holopainen JM, Saarikoski J, Kinnunen PKJ, Jarvela I. Elevated lysosomal pH in neuronal ceroid lipofuscinosis (NCLs). *Eur J Biochem*. 2001;268:5851–5856.
118. Katz ML, Siakotos AN. Canine hereditary ceroid-lipofuscinosis: evidence for a defect in the carnitine biosynthetic pathway. *Am J Med Genet*. 1995;57(2):266–271.
119. Siakotos AN, Hutchins GD, Farlow MR, Katz ML. Assessment of dietary therapies in a canine model of Batten disease. *Eur J Paediatr Neurol*. 2001;5:151–156.
120. Dawson G, Kilkus J, Siakotos AN, Singh I. Mitochondrial abnormalities in CLN2 and CLN3 forms of Batten disease. *Mol Chem Neuro-pathol*. 1996;29(2–3):227–235.
121. Wei H, Kim S-J, Zhang Z, Tsai P-C, Wisniewski KE, Mukherjee AB. ER and oxidative stresses are common mediators in both neurodegenerative and non-neurodegenerative lysosomal storage disorders and are alleviated by chemical chaperones. *Hum Mol Gen*. 2008;17:469–477.

Nutrition and Dietary Supplements

Publish your work in this journal

Nutrition and Dietary Supplements is an international, peer-reviewed, open access journal focusing on research into nutritional requirements in health and disease, impact on metabolism and the identification and optimal use of dietary strategies and supplements necessary for normal growth and development. The journal welcomes papers covering

Submit your manuscript here: <http://www.dovepress.com/nutrition-and-dietary-supplements-journal>

Dovepress

original research, basic science, clinical & epidemiological studies, reviews and evaluations, guidelines, expert opinion and commentary, case reports and extended reports. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use.