



## Review

## Acylcarnitines: Role in brain

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## ABSTRACT

L-carnitine is present in mammalian cells as free carnitine and acylcarnitines. The acylcarnitine profile has been shown to be useful in identifying inborn errors of metabolism and to be altered under different metabolic conditions. While carnitine's most widely known function is its involvement in  $\beta$ -oxidation of fatty acids, it may also have other roles in metabolism. The importance of acylcarnitines in tissues with high rates of  $\beta$ -oxidation such as heart and muscle is intuitive. However, acylcarnitine and carnitine supplementation have resulted in beneficial effects in the treatment of various neurological diseases, even though fat is not the major fuel for brain. Recent data indicate new, multifactorial roles for acylcarnitines in neuroprotection. Brain acylcarnitines can function in synthesizing lipids, altering and stabilizing membrane composition, modulating genes and proteins, improving mitochondrial function, increasing antioxidant activity, and enhancing cholinergic neurotransmission. Currently a relatively small subset of acylcarnitines is usually investigated. More research is needed on the use of acylcarnitines in the treatment of neurological diseases using a list of acylcarnitines encompassing a wide range of these molecules. In summary, carnitine is not merely a cofactor in  $\beta$ -oxidation, but rather it has many known and yet to be discovered functions in physiology.

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**Abbreviations:** LC, L-carnitine; ALC, acetyl-L-carnitine; CPT 1, carnitine palmitoyltransferase 1; acyl-CoA, acyl-Coenzyme A; CAT, carnitine acetyltransferase; VLCAD, long-chain acyl-CoA dehydrogenase deficiency; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; PLC, palmitoyl-L-carnitine; AD, Alzheimer's disease; DHA, docosahexaenoic acid; ROS, reactive oxygen species; PD, Parkinson's disease; LDH, lactate dehydrogenase; FCCP, p-(trifluoromethoxy)phenylhydrazone; MPP+, 1-methyl-4-phenylpyridinium; 3-NPA, 3-nitropropionic acid; SOD, superoxide dismutase; HO-1, heme oxygenase-1; Hsp, heat shock protein; HNE, 4-hydroxy-2-nonenal; LA,  $\alpha$ -lipoic acid; PI3K, phosphoinositol-3 kinase; MDA, malonyldialdehyde; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; NGF, nerve growth factor; CNS, central nervous system; BDNF, brain-derived neurotrophic factor; NT, neurotransmitter; ACh, acetylcholine; GABA,  $\gamma$ -aminobutyric acid; SSH, subtractive hybridization; VDAC, voltage-dependent anion channel; MPT, mitochondrial permeability transition; mGlu2, metabotropic glutamate receptor 2; DRG, dorsal root ganglia; NF- $\kappa$ B, nuclear factor- $\kappa$ B; A $\beta$ , amyloid  $\beta$ ; KT, Ketogenic Therapy.

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## 1. Introduction

L-Carnitine (trimethylamino- $\beta$ -hydroxybutyrate) (LC) is present in cells and tissues as both free carnitine and acylcarnitines, including acetyl-L-carnitine (ALC). LC is a naturally occurring, endogenous compound in all mammalian species and its most widely known function is as an important transporter of long-chain fatty acids into mitochondria for  $\beta$ -oxidation. Humans obtain carnitine from their diet, predominately from meat and dairy, and through endogenous biosynthesis. LC is synthesized *in vivo* from L-lysine and L-methionine, mostly in liver and kidney [1,2].

Under normal conditions, carnitine palmitoyltransferase 1 (CPT 1) catalyzes the transfer of acyl groups from acyl-Coenzyme A (acyl-CoA) to carnitine to produce acylcarnitines and free coenzyme A (CoA). This reaction is one of the first highly regulated steps that is common to both pathways of fatty acid oxidation and ketogenesis [3,4] (Fig. 1). Therefore, the critical substrates carnitine and acylcarnitines are important in understanding the metabolic pathway associated with ketosis. While carnitine is an important metabolite involved in  $\beta$ -oxidation of fatty acids, it may also be important in transporting other metabolites due to its ability to form esters with many carboxylic acids. The free hydroxyl group, shown in Fig. 2, can be enzymatically esterified to activated acetate groups or to another activated carboxylic acid including fatty acids of all chain lengths, to form acylcarnitines (examples of which are shown in Fig. 3). Carnitine acetyltransferase (CAT) catalyzes the synthesis of short-chain acylcarnitines, specifically ALC, and is located on the inner mitochondrial membrane as well as in microsomes and peroxisomes [5,6]. ALC and other acylcarnitines can be transported across the inner mitochondrial membrane by carnitine–acylcarnitine translocase and transported out of the mitochondria into the cytosol. It is evident, then, that acylcarnitines are activated molecules that are

transportable throughout the body, delivering various acyl groups for a wide range of functions.

### 1.1. Acylcarnitine profile and clinical applications

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 the acylcarnitine profile may 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. For instance, ALC is a small, water-soluble molecule that is easily transportable and may be used to deliver acetyl groups to a variety of locations. Yet a long-chain acylcarnitine, such as palmitoyl-L-carnitine (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. Monitoring specific acylcarnitines should lead to a better understanding of mechanisms of disease and allow

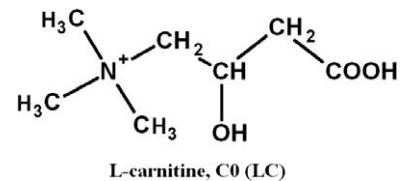


Fig. 2. L-carnitine structure.

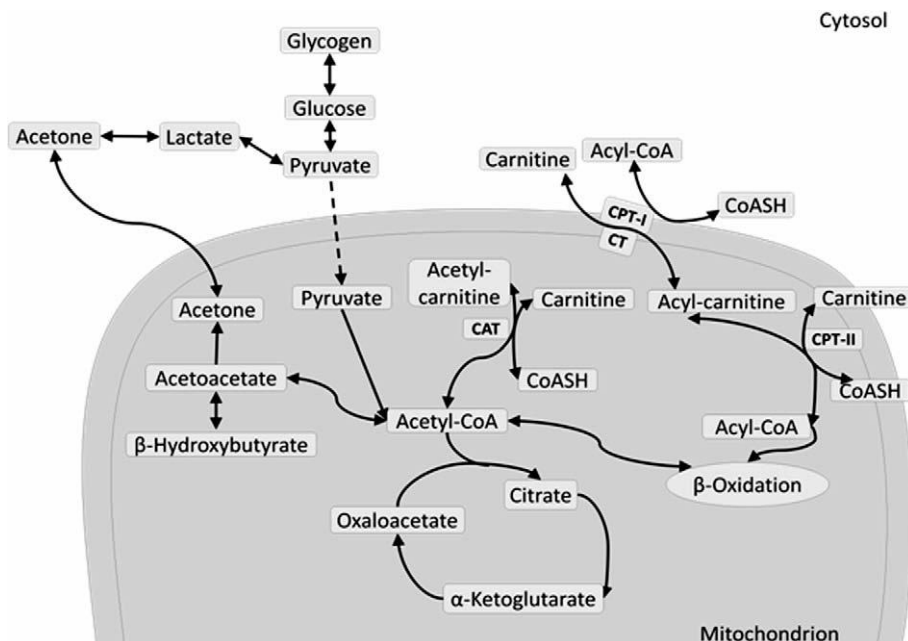


Fig. 1. Carnitine in overall energy metabolism.

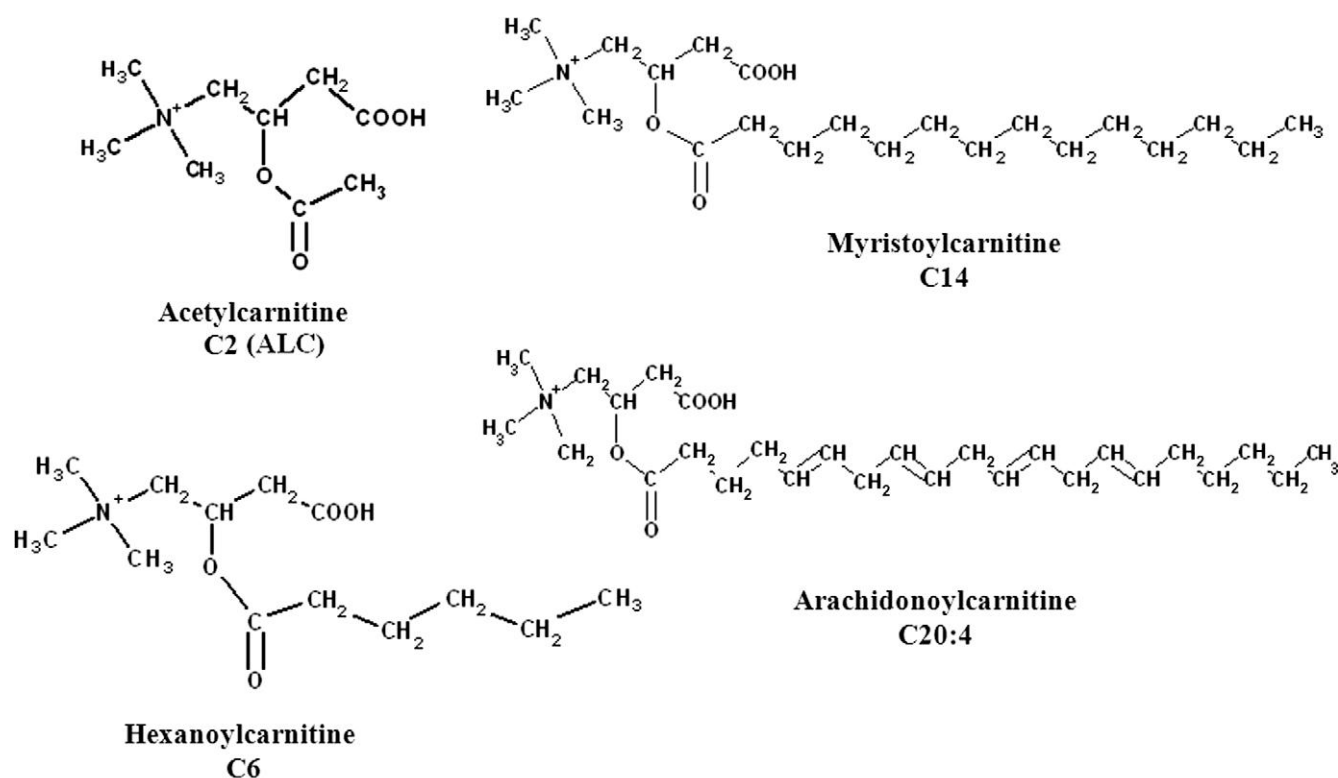


Fig. 3. Examples of acylcarnitine structures.

for better design of treatment regimens. The acylcarnitine profile has been shown to be useful in identifying inborn errors of metabolism in neonatal screening using tandem mass spectrometry (MS/MS) based analysis. Important examples are fatty acid oxidation defects, such as long-chain acyl-CoA dehydrogenase deficiency (VLCAD) and disorders of organic acid metabolism, such as propionyl-CoA carboxylase deficiency [7–9]. Recently metabolomics methodology has been used to identify patients with methylmalonic and propionic acidemia [10].

Inborn errors of metabolism can lead to a build-up of toxic metabolites and can be fatal or result in serious health problems early in life. For this reason, early comprehensive neonatal screening is used to detect abnormalities to avoid major physical and neurological effects [11]. Mass spectrometry-based analysis is used to diagnose inborn errors of metabolism in newborns by identifying and quantifying specific metabolites [12,13]. Newborn screening by MS/MS has identified compounds and provided early treatment to infants with disorders of mitochondrial  $\beta$ -oxidation, organic acidemias, disorders of the urea cycle, and rare disorders of metabolism before the onset of symptoms. For example, after the application of tandem mass spectrometry was introduced clinically, infants with short-chain acyl-CoA dehydrogenase deficiency (SCAD) and isobutyryl-CoA dehydrogenase deficiency have been identified based on elevated butyryl-carnitine/isobutyryl-carnitine concentrations in newborn blood spots [14], even though two out of three infants remained asymptomatic at the time of diagnosis. Age-related variations in acylcarnitine and free carnitine concentrations have been observed and should be taken into consideration when diagnosing and managing inborn errors of metabolism [12,15]. Inborn errors of metabolism detected by acylcarnitine profile analysis adapted from Rinaldo and coworkers [16] and the corresponding acylcarnitine changes are listed in Table 1.

In addition to identifying inborn errors of metabolism, the acylcarnitine profile may also be useful in identifying other meta-

bolic perturbations. In healthy neonates, cord blood concentrations of total acylcarnitines strongly correlated to birth weight, and lower umbilical artery pH caused accumulation of long-chain acylcarnitines [12]. The acylcarnitine profile may additionally be a useful parameter for identifying perinatal asphyxia and other metabolic disturbances *in utero*.

Outside of newborn screening, other disease states and alterations in metabolism may be detected through monitoring of the acylcarnitine profile. Ulcerative colitis is a disorder involving chronic inflammation of the colonic mucosa for which the etiology and pathogenesis are unknown. Alterations in short-chain fatty acid metabolism have been identified in patients with this disease [17]. Furthermore, celiac disease is an autoimmune disorder derived from gluten intolerance. Bene and coworkers found a significant difference in the acylcarnitine profile in plasma of adult patients with ulcerative colitis and patients with celiac disease compared to controls with no change to free carnitine levels [18,19]. Comparison of the plasma acylcarnitine profile of diabetic patients to non-diabetic controls found a 300% increase of acylcarnitines with a chain length of 10–12 carbons indicating incomplete long-chain fatty acid  $\beta$ -oxidation [20]. In these patients ALC increased and propionyl-L-carnitine decreased as glycosylated hemoglobin increased.

The metabolome and the targeted carnitinome or acylcarnitine profile are becoming increasingly popular tools. Non-targeted metabolomics is being used to create a more comprehensive metabolic profile of the plasma that allows the investigator to assay thousands of metabolites and identify significantly different metabolic features [10]. Many labs have evaluated the utility of acylcarnitine profiles in the diagnosis of inborn errors of metabolism, some of which were reviewed by Pasquali and coworkers in 2006 [21]. Supplementation of specific acylcarnitines, furthermore, may lead to benefits for particular disease states. For example, propionyl-L-carnitine supplementation may improve general fatigue [3] and may

**Table 1**

Inborn errors of metabolism detected by acylcarnitine profile analysis: disorders of fatty acid oxidation and organic acid metabolism.

Fatty acid oxidation disorders	Acylcarnitine changes
Carnitine uptake defect	↓ LC
Carnitine palmitoyltransferase I (CPTI) deficiency	↑ LC and ↓ C16 and C18
Carnitine-acylcarnitine translocase (CACT) deficiency	↑ C16 with C18:2, C18:1, C18
Carnitine palmitoyltransferase II (CPTII) deficiency	↑ C16 with C18:2, C18:1, C18
Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	↑ C14:1 with C14, C14:2
Long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	↑ C16-OH with C16:1-OH, C18:1-OH, C18-OH
Trifunctional protein (TFP) deficiency	↑ C16-OH with C16:1-OH, C18:1-OH, C18-OH
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	↑ C8 with C6, C10, C10:1
Medium/short-chain L-3-hydroxyacyl-CoA (M/SCHAD) dehydrogenase deficiency	↑ C4-OH and Inc C10-OH
Medium-chain 3-ketoacyl-CoA thiolase (MCKAT) deficiency	↑ C10-OH
Short-chain acyl-CoA dehydrogenase (SCAD) deficiency	↑ C4
Multiple-CoA dehydrogenase (MAD) deficiency ( $\alpha$ -ETF, $\beta$ -ETF, ETF-QO deficiency) (glutaric acidemia type II)	↑ C4 (with C5, and other longer chain species)
Dienoyl-CoA reductase deficiency	
Organic acid metabolism disorders	Acylcarnitine changes
Propionyl-CoA carboxylase deficiency (propionic acidemia)	↑ C3
Multiple carboxylase deficiency (holocarboxylase synthetase deficiency and biotinidase deficiency)	↑ C5-OH
Methylmalonyl-CoA mutase deficiency (methylmalonic acidemia)	↑ C3
Disorders of cobalamin metabolism (Cbl A/B/C/D/F deficiencies)	
Succinyl-CoA synthetase deficiency (SUCLA2)	↑ C3 and Inc C4DC
Isobutyryl-CoA dehydrogenase deficiency	↑ C4
Ethylmalonic encephalopathy	↑ C4 with C5
$\beta$ -Ketothiolase (2-methylacetoacetyl-CoA thiolase, or 3-oxothiolase) deficiency	↑ C5-OH with C5:1
Isovaleryl-CoA dehydrogenase deficiency (isovaleric acidemia)	↑ C5
2-Methylbutyryl-CoA dehydrogenase deficiency (short/branched chain acyl-CoA dehydrogenase (SBCAD) deficiency)	↑ C5
2-Methyl 3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency	↑ C5-OH
3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency	↑ C5-OH
3-Hydroxy-3-methylglutaryl-CoA lyase (HMG-CoA lyase) deficiency	↑ C5-OH with C6DC
3-Methylglutaconyl-CoA hydratase deficiency	↑ C5-OH
Malonyl-CoA carboxylase deficiency	↑ C3DC
Glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I)	↑ C5DC
Glutamate formiminotransferase deficiency (formiminoglutamic aciduria)	↑ C4 (with more prominent peak at <i>m/z</i> 287)

Adapted from Rinaldo et al. [16].

LC = free carnitine; C3 = propionyl-carnitine; C4 = butyryl-carnitine, isobutyryl-carnitine; C5 = isovaleryl-carnitine, methylbutyryl-carnitine; C4-OH = 3-hydroxybutyryl-carnitine; C5-OH = 3-hydroxyisovaleryl-carnitine, 3-hydroxy-2-methylbutyryl-carnitine; C8 = octanoyl-carnitine; C3DC = malonyl-carnitine; C4DC = succinyl-carnitine/methylmalonyl-carnitine; C5DC = glutaryl-carnitine; C10-OH = 3-hydroxy-decanoyl-carnitine; C14:1 = tetradecenoyl-carnitine; C16 = palmitoyl-carnitine; C16-OH = 3-hydroxypalmitoyl-carnitine.

**Table 2**

Changes in carnitine and acylcarnitine concentrations under dietary manipulations and alterations in metabolism.

Model	Diet	Free L-carnitine (LC)	Acylcarnitine/ALC	Ref.
Rat	Starvation/fasting	↓ (same in brain)	↑	[24,28,179,181]
	High-fat	↓	↑	[24,28]
	High CHO	↑	↓	[24]
Human	Starvation/fasting	↓	↑	[25,27,29]
	High-fat/fat load	↓	↑	[29,182]
	Diabetic ketosis	↓	↑	[26,27]

protect heart health [22], while PLC supplementation may regulate lipid esterification [23].

### 1.2. Changes in carnitine and acylcarnitine concentrations

Concentrations of carnitine and acylcarnitines change under altered dietary conditions. During starvation and after eating a high-fat diet, the proportion of carnitine that is acetylated in liver and kidney significantly increases and, oppositely, a high carbohydrate diet causes very low levels of ALC in liver [24]. In humans, there appears to be a delayed decrease in plasma LC and a rapid increase in both long- and short-chain acylcarnitines during fasting or diabetic ketosis [25–27]. While plasma levels do not directly correlate with cerebral levels, in neonatal rats starvation led to a significant increase in mean brain acylcarnitine concentration compared to control rats, with almost all of the increase attributed to short-chain acylcarnitines [28]. The authors concluded that carnitine and its relative esters may be redistributed to the brain during fasting and the brain may use them for energy production or, possibly, for the delivery of acetyl groups.

Plasma acylcarnitine levels were analyzed in 1–7 year old children after fasting and ingestion of sunflower oil, which is made up largely of linoleic acid (66%), a polyunsaturated fatty acid (PUFA), and oleic acid (21%), a monounsaturated fatty acid (MUFA). Under both conditions there was an increase in all plasma straight-chain acylcarnitines and ALC was the largest contributor to the increase in total esterified carnitine with an almost 4-fold increase after fasting [29]. These and other changes to carnitine concentrations are summarized in Table 2.

## 2. Carnitine and acylcarnitines in brain

The importance of carnitine in brain is emphasized by carnitine deficiency symptoms, many of which involve major deleterious effects in brain. Because the brain is highly reliant on oxidative metabolism, impairment of fatty acid metabolism and energy production due to lack of carnitine leads to metabolic encephalopathy. Structurally, the astrocyte swells and mitochondria are expanded in nerve cells under carnitine deprivation [30]. In addition, LC is

taken up by neuronal cells through a Na<sup>+</sup>- and energy-dependent mechanism. Therefore conditions of metabolic disturbances, as seen in many neurological disorders, may exacerbate a carnitine deficiency.

While glucose is thought to be the primary energy source for the adult brain under normal conditions, recently it was shown that fatty acids can be used by the brain as well. Almost 20% of the total oxidative energy produced in brain was from the oxidation of <sup>13</sup>C-octanoate in adult male Sprague–Dawley rats [31]. In addition, the majority of the brain is composed of fatty acids, which are needed for incorporation into structural lipids [32]. Similarly, fatty acids become key energy substrates for brain under metabolically compromised conditions such as fasting or starvation. Therefore functions of carnitine and acylcarnitines 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.

Carnitine is found to accumulate to a lower extent in brain as compared to peripheral tissues [33]. The enzymes needed for the synthesis of carnitine are present in brain tissue [34] as well as the acyltransferases necessary for the synthesis of acylcarnitines [35]. Carnitine can also be transported into the brain. Although more work is needed, thus far it appears carnitine can be transported through the blood–brain barrier via two transporters: OCTN2, a Na<sup>+</sup>-dependent transporter shown through reverse transcriptase polymerase chain reaction to be present in brain endothelial cells [36], and; ATB<sup>0,+</sup>, a Na<sup>+</sup>-, Cl<sup>-</sup>-dependent amino acid transporter expressed in the hippocampus [37,38]. Transport of carnitine in brain has been previously reviewed [39]. More recently, a detailed experiment indicates localization of OCTN2 in cells forming the blood–brain barrier and this suggests that carnitine can also be transported out of the brain [40]. Therefore, carnitine and acylcarnitines may have a role in brain to remove acyl esters in addition to delivering specific acyl moieties.

LC accumulates in neurons of the cerebral cortex and forms acylcarnitines. Isolated neurons of the adult brain contain approximately 80% free carnitine, 10–15% ALC, and less than 10% long-chain acylcarnitines. The ALC concentration is higher and free carnitine is lower in suckling rats compared to adult rats [41]. Because carnitine and its acyl derivatives have chemical structures comparable to choline and acetylcholine, it has been proposed that they play a role in neurotransmission [42]. 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 acetylcholine for neurotransmission [43]. Acylcarnitine and LC supplementation have shown beneficial effects in the treatment of aging, chronic degenerative diseases and slowing the progression of mental deterioration in Alzheimer's disease (AD) [44,45]. Therefore, these important roles of acylcarnitines and LC in brain and their potential therapeutic mechanisms need to be further elucidated.

### 3. Palmitoyl-L-carnitine (PLC): role in brain

#### 3.1. Energy metabolism and membranes

The roles of long-chain acylcarnitines, specifically PLC, in brain have been studied and appear to involve interaction with membranes, acylation of lipids, as well as protein interaction. Mainly due to the amphiphilic nature of PLC, it can react on the surface of membranes and influence membrane fluidity and the activity of membrane enzymes and transporters [46–49]. PLC has been shown to be involved in phospholipid and fatty acid turnover in rat fetal neurons [50,51]. PLC administration *in vitro* led to incorporation of palmitate into lipids such as spingomyelin, phosphatidylserine and

phosphatidylcholine, important phospholipids associated with neural membranes [52]. Because the activity of brain-specific carnitine palmitoyltransferase (CPT1c) is low compared to other tissues [35] and the energy produced from octanoate oxidation in brain primarily occurs in astrocytes [31], roles outside of energy production must exist for PLC. The CPT1c isoform appears to be involved in metabolism of specific brain lipids and in maintaining energy homeostasis [53,54]. Originally it was suggested CPT1c had no catalytic activity with palmitoyl-CoA as a substrate [53]. Eventually it was found that CPT1c knockout mice have lower body weight and food intake so, it does appear to be needed for energy regulation [54]. More recently CPT1c showed carnitine palmitoyltransferase activity and was found in neurons but not in astrocytes, specifically localized in the endoplasmic reticulum of cells, not in mitochondria [55]. In addition palmitoyl-CoA was the only product increased in neural cells over expressing CPT1c. PLC is most likely involved in the synthesis of complex lipids which influence neural membranes, brain plasticity and downstream signal transduction.

#### 3.2. Protein modulation

In addition to influencing membrane lipids, protein palmitoylation has been shown to alter the activity of various proteins and influence signaling pathways. The level of PLC was shown to increase during apoptosis, stimulating the activity of caspase enzymes, whereas free carnitine did not [56]. Specific proteins shown to be affected by palmitoylation are the trimeric G proteins, important in a variety of signaling pathways [57]. In addition, PLC appears to interact with and inhibit protein kinase C, [58,59] an enzyme involved in signal transduction pathways leading to cell growth and differentiation. Another protein involved in signaling pathways that is palmitoylated by PLC is GAP-43 (B-50, neuromodulin, F1), a protein involved in neuronal development, neuroplasticity, and neurotransmission [60]. PLC could be directly providing the activated palmitoyl group for these post-translational modifications.

### 4. Acetyl-carnitine (ALC): role in brain

ALC is present in relatively high levels in the brain [61] and it is highest in the hypothalamus, [33] where the level of the ALC synthesizing enzyme, CAT, is also high. ALC can readily cross the blood–brain barrier [62], so supplementing with this compound could feasibly affect brain metabolism. Injection of ALC in rats led to reduced oxidation of glucose and increased glycogen synthesis in brain [63]. Changes in the activities of specific enzymes involved in the tricarboxylic acid (TCA) cycle, electron transport chain and amino acid metabolism have also been observed after treatment with ALC [64]. Specifically, the activities of citrate synthase and glutamate dehydrogenase were decreased while the activities of  $\alpha$ -ketoglutarate dehydrogenase and cytochrome oxidase were increased.

Some of ALC's proposed neuroprotective benefits involve improved mitochondrial energetics and function, antioxidant activity, stabilization of membranes, protein and gene expression modulation, and enhancement of cholinergic neurotransmission [39,65–68]. This section will highlight these mechanisms of action for ALC in the brain and how these may be applicable in various clinical situations.

#### 4.1. Energy metabolism and membranes

Carnitine's primary function in the cell is in lipid metabolism. Animal studies have traced the fate of the acetyl group in ALC using radiolabeled [1-<sup>14</sup>C]ALC. Injection of this compound into mice

showed a rapid incorporation of the acetyl groups into lipid biosynthesis pathways in the liver [69]. When injected into the brain, the acetyl groups were mostly incorporated into saturated fatty acids (about 60% of the radioactivity present in the tissues), but they were also found in MUFAs and PUFAs [70]. Interestingly, in this experiment labeled [ $U$ - $^{14}C$ ]glucose was not incorporated into PUFAs, unlike [ $1$ - $^{14}C$ ]ALC.

Due to ALC's role in overall energy metabolism, many researchers have investigated how ALC can repair neurological damage through metabolic pathways. Supplementation of ALC has been shown to normalize the levels of high-energy phosphate in brain of AD patients as measured by  $^{31}P$  magnetic resonance spectroscopy [71]. In rats, ALC treatment increased the concentration of phosphocreatine and decreased the concentrations of lactate and inorganic phosphate in aging [72] and post-ischemic brain models [73]. ALC also seems to provide protection during metabolic stress, such as ischemia, hypoxia, aging, alcohol, and brain injury [67]. For example, dogs treated with ALC showed significantly lower neurological deficit scores and more normal cerebral cortex lactate/pyruvate concentration ratios than control animals after cerebral ischemia and reperfusion [74]. These results indicate that ALC improves neurological outcome, potentially as a result of normalizing brain energy metabolites.

Aging is associated with mitochondrial dysfunction and altered metabolic states [75]. Many groups have researched carnitine's utility in reversing the metabolic side effects of aging. Feeding ALC to older rats (22–28 months of age) increases the cellular consumption of oxygen, a parameter which decreases with increasing age [76]. In this same study, ALC reversed the declines in mitochondrial membrane potential and cardiolipin (a phospholipid) concentrations that are associated with aging. Ames and Liu reviewed support for these results as well as a role for ALC in stabilizing the inner mitochondrial membrane [77]. Sphingomyelin and cholesterol tend to accumulate in the cerebra of older rats, and both of these increases are attenuated by long-term ALC supplementation [78]. It is apparent that 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.

Many studies have shown other beneficial effects of ALC in either protecting mitochondria against biochemical insult or reversing damage to mitochondria. ALC appears to have neuromodulatory action through increasing the synthesis of phospholipids that are required for membrane formation and integrity [67]. When ALC was supplemented with  $\alpha$ -lipoic acid (LA) to rats, reversals in the age-associated decline of mitochondrial membrane potential and the levels of ascorbate and malondialdehyde (an indicator of lipid peroxidation) were observed [79,80]. Feeding ALC and LA to 21-month-old rats, reduced the density of mitochondria associated with vacuoles and lipofuscin and increased the number of intact mitochondria [81]. Another potential role for ALC was elucidated by Cassano et al., who found an increase in transcripts related to mitochondrial biogenesis with ALC supplementation in a rat model for hindlimb muscle atrophy [82]. ALC could, then, not only help preserve mitochondrial membrane integrity, but it could also help to generate more mitochondria under certain conditions, helping to preserve or improve overall metabolic function. ALC supplementation prior to cyanide injection in rats had beneficial protective effects on preventing behavioral changes, but did not affect phosphoinositide metabolism [83]. The authors propose carnitine's beneficial effect may not be through supplying energy to the brain, but rather through providing activated acyl groups for the reacylation of membrane phospholipids. This role for carnitine was previously found in the deacylation–reacylation process in the membrane phospholipids

of human red blood cells [50] and has since been supported by other studies [51,84].

Researchers have reported that ALC increases fluidity in rat brain microsomes and liposomes [50]. ALC, as well as LC, may affect membrane fluidity due to its amphiphilic structure that may directly interact with the surface charges on cell membranes [85]. The carboxylic group can interact with charges on membrane phospholipids, glycolipids, and proteins. Neural membranes contain a large amount of phospholipids which can be degraded through the action of phospholipase A2 to important lipid mediators such as docosahexaenoic acid (DHA) and arachidonic acid (AA), which further form docosanoids and eicosanoids found to be important in inflammatory and oxidative stress responses [86]. In addition, alterations to the composition of these phospholipids in membranes can alter membrane fluidity, permeability, and functioning of membrane proteins that act as important receptors and signals for multiple downstream reactions. Alterations in neural phospholipid composition and further effects on signal transduction pathways have been found to be characteristic of many neurological disorders [87–90]. Recent evidence suggests that ALC also plays a role in the elongation–desaturation of the  $n$ -3 polyunsaturated fatty acids to form 22:6 $n$ -3, DHA, in mitochondria [91–93]. ALC is thought to provide an intra-mitochondrial source of acetyl groups and donate them in the elongation pathway. As stated earlier, labeled ALC is found to be incorporated into PUFAs [70]. Changes in DHA content of membranes can markedly affect synaptic plasticity, inflammatory response, gene expression, ion channels, membrane-bound proteins and neurotransmission [94].

#### 4.2. Protection from excitotoxicity

The formation of reactive oxygen species (ROS) and mitochondrial dysfunction lead to metabolic and oxidative stress and are the underlying processes in many neurotoxic and neurodegenerative diseases, such as AD and Parkinson's disease (PD) [95]. The oxidative stress affects the activities of the respiratory chain complexes I–V, changes which are key in many neurological disorders. Application of sodium azide (an inhibitor of mitochondrial complex IV) to rat brain slices induced a pathological synaptic potentiation. ALC treatment was neuroprotective, but the neuroprotection was lost if the mitochondria were uncoupled [96]. Pre-treatment of rats with ALC exerted neuroprotection at the mitochondrial level against 3,4-methylenedioxymethamphetamine (ecstasy, a worldwide abused stimulant) [97]. Neurotoxicity was induced *in vitro* by an inhibitor of complex I, rotenone, in rat cortical neurons which led to mitochondrial dysfunction and cell death. Co-incubation of the cells with 1 mM ALC resulted in increased survival and partial protection from cell death. Toxicity and cell damage, as assessed by lactate dehydrogenase (LDH) release from the mitochondrial uncoupler, p-[trifluoromethoxy]phenylhydrazone (FCCP), was significantly reduced in cultured rat cortical neurons by both LC and ALC [84]. Another inhibitor of complex I, 1-methyl-4-phenylpyridinium (MPP+), results in symptoms similar to patients with PD. In a study in 2004, Virmani et al. found that the inhibitory action of MPP+ was partly reversed by co-incubation of cells with ALC [98]. Similar protection has been shown *in vivo* in primates [99]. In neuroblastoma cells, ALC, but not LC, prevented the toxicity and cell death from MPP+ and partially restored intracellular ATP concentrations with no effect on oxygen consumption. ALC was also effective in decreasing the utilization of glucose and increasing lactate production slightly in control and treated cells. Administration of insulin and malonyl-CoA in order to down-regulate fatty acid entry into mitochondria did not block the protective effect of ALC [100]; therefore, ALC may be protective through supporting anaerobic glucose metabolism and not through its

traditional role in oxidation of fatty acids. Researchers suggest the mechanism of neuroprotection by ALC may be through restoration of mitochondrial function and/or improved energy usage since at least part of the toxicity of MPP<sup>+</sup> is due to mitochondrial inhibition [98,100].

Part of ALC's function may derive from the carnitine portion of the molecule since LC exhibits many beneficial functions by itself. Neurons have also been shown to be protected by LC after inducing neurotoxicity through 3-nitropropionic acid (3-NPA), a compound which induces neurotoxicity through irreversible binding to succinate dehydrogenase (complex II of the mitochondrial respiratory chain) [101–103]. Defects in the function of complexes II and III have been observed in Huntington's disease patients [104]. LC pre-treatment before administration of 3-NPA prevented the increase in activities of endogenous free radical scavengers, catalase and superoxide dismutase (SOD), caused by 3-NPA alone, suggesting LC protected rats from oxidative stress associated with increased activity of free radical scavenging enzymes [101]. More recently, a neurohistological method was used to examine the effect of LC pre-treatment against 3-NPA-induced toxicity on 20 male Sprague–Dawley rats (10 receiving LC and 10 not). Using a fluorescent marker, Fluoro-Jade B, researchers were able to localize and identify degenerating neurons. Three animals in the control group survived the 3-NPA administration, 2 of which had damage and neuronal degeneration. Six out of 7 surviving animals that were treated with LC exhibited no lesions. Due to the reduced mortality and significantly reduced neuronal degeneration, LC again appeared to be protective against neurotoxicity [105]. In a 2005 study, LC inhibited the increase in oxidized glutathione and mitochondrial dysfunction in the hippocampus and prevented neuronal injury caused by a hypoglycemic insult [106]. Also, LC inhibited the decrease in mitochondrial membrane potential and generation of ROS in hippocampal neuronal cells cultured in glucose-deprived medium [106]. Recent research suggests LC's protective effects against neurotoxicity mainly seem to be due to its antioxidant ability [103].

Mitochondrial disorders and diseases affecting oxidative phosphorylation,  $\beta$ -oxidation and energy metabolism preferentially affect brain [107–109]. In addition, mitochondria have a high concentration of 22:6n–3-containing phospholipids [110,111] and, therefore, these phospholipids may be important for assembly of the respiratory chain complexes. This finding relates back to ALC preventing damage associated with inhibiting these complexes. Again, ALC may be contributing to the reacylation of membranes and, ultimately, membrane composition, which in turn affects downstream signaling pathways and oxidative phosphorylation.

#### 4.3. Antioxidant and anti-apoptotic functions

ALC may be protective against oxidative stress through a reduction in tissue lactic acidosis, which leads to formation of ROS, through shifts in both the mitochondrial and cytosolic redox state, and/or through the induction of antioxidant genes [66,112]. This could lead to an increase in reducing power available for detoxification through the glutathione system. Protection against mitochondrial alterations and cell death from cytokines along with an increased expression of heme oxygenase-1 (HO-1) was found in primary rat cortical astrocytes treated with ALC [112]. This increased antioxidative potential from treatment with ALC also led to the up-regulation of heat shock protein (Hsp) 60, Hsp72, SOD, and a high expression of the redox-sensitive transcription factor Nrf2 [112,113]. These changes restored the ratio of reduced to oxidized glutathione and reversed the inhibition of complex IV. Further studies have also shown ALC decreases both HNE (4-hydroxy-2-nonenal) formation and protein carbonyls, other indicators of oxidative damage [65,114].

Oxidative stress underlies the neuropathology of AD and other disorders. HNE is a highly reactive product of lipid peroxidation of unsaturated lipids that can be used to induce oxidative damage. Pre-treatment of cortical neurons with ALC and LA significantly reduced the HNE-associated cytotoxicity, protein and lipid oxidation, and apoptosis in a dose-dependent manner as well as increased cellular reduced glutathione and Hsps as compared to controls [115,116]. The induction of the heat shock response and HO-1 assists in the maintenance and repair systems important for survival of brain cells and may contribute to protection in the brain [112–114]. ALC treatment also leads to the activation of phosphoinositol-3 kinase (PI3K), PKG, and ERK1/2 pathways that are important in neuronal cell survival and differentiation [116].

ALC has been found to be protective against lipid peroxidation and membrane breakdown, indicating it may have antioxidant capacity in the mitochondria [84,85]. In streptozotocin-induced diabetic rats, treatment with ALC improved nerve conduction velocities (the speed of signal through the nerves), and this was associated with a reduction in elevated malonyldialdehyde (MDA) content, an indicator of lipid peroxidation [117]. Both a decline in mitochondrial energetics and an increase in oxidative stress are some of the effects of aging. Hagen et al. in 2002 found ALC increased hepatocellular oxygen consumption and partially reversed the decrease in mitochondrial membrane potential associated with aging [79]. They found a reduction in MDA levels in rats fed ALC in combination with LA. A restoration of hepatocellular ascorbate levels was also shown, suggesting that together ALC and LA lowered the oxidative stress response in aging rats [79]. Comparatively, ALC can decrease brain lipid peroxidation in old rats whereas LC was ineffective [118]. ALC is also more efficient to cross the blood brain barrier than LC [36]. However, treatment with both carnitine and acylcarnitines has been shown to significantly reduce the levels of circulating tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukins [119], which could then protect against oxidative stress.

In addition to affecting lipid peroxidation, ALC and LC have been shown to reduce apoptosis through the mitochondrial pathway [120–122]. Oxidative stress from insults such as hypoxia and deprivation of trophic factors can cause apoptosis *in vitro* and *in vivo*. To test whether ALC can improve mitochondrial function and protect against apoptosis, mouse fibroblasts in culture were administered ALC at different concentrations and after serum deprivation [121]. ALC treatment led to less apoptosis in serum-deprived cells which was confirmed by an assessment of cytochrome c release and immunoreactivity to caspase 3. ALC and LC promoted neuronal survival and mitochondrial activity in addition to having anti-apoptotic effects in serum-deprived primary culture neurons [122]. In another tissue culture study, ALC treatment of cells suppressed oxidative stress in mitochondria which prevented the mitochondrial signaling pathway leading to apoptosis [123]. ALC seems to be involved in the energy response and maintenance and repair systems in neurons and appears to stimulate cell proliferation.

#### 4.4. Neuromodulatory

##### 4.4.1. NGF and nerve regeneration

ALC has been shown to increase nerve growth factor (NGF) production and enhance NGF binding *in vivo* [124,125]. NGF affects neuronal development and maintenance of neurons in the peripheral and central nervous systems (CNS), and NGF binding is decreased in rodent CNS after stress exposure. This reduction was prevented by treatment with ALC in the CNS of aged rats [125]. ALC has effects on neuronal repair and nerve fiber regeneration. Treatment with ALC for 8 months in diabetic Worcester rats promoted nerve fiber regeneration by twofold compared to untreated

control rats [126]. Similarly, following sciatic nerve injury, ALC prevented the age-dependent structural changes in rat peripheral nerves and, in lesioned animals, ALC treatment promoted regeneration of nerves by significantly increasing the density of regenerating myelinated fibers and axon diameter [127]. ALC also has shown both neuroprotective and neurotrophic activity in primary motoneurons exposed to excitotoxic agents or deprived of brain-derived neurotrophic factor (BDNF) [128].

In addition to regeneration, ALC appears to correct altered peripheral nerve function. Rats with streptozotocin-induced diabetes were treated with ALC resulting in normal nerve conduction velocity [117]. Four month treatment with ALC in the diabetic Worcester rat corrected the Na<sup>+</sup>/K<sup>+</sup> ATPase defect, nerve conduction defect, and prevented structural changes associated with diabetes pathology [126]. Recently a review of clinical trials involving a total of 1679 subjects showed improvement in electrophysiological factors in one study and nerve generation in a different study [129]. Another study has suggested effects of ALC on Na<sup>+</sup>/K<sup>+</sup> ATPase activity in the leech T sensory neurons [130]. This resulted in a larger after hyperpolarization, leading to a decrease in the number of action potentials that reach synaptic terminals. Therefore, ALC's neuromodulatory actions may be through both alterations in structural and electrical properties in the nervous system.

#### 4.4.2. Neurotransmitter modulation

Other neuromodulatory actions of ALC involve neurotransmitter (NT) synthesis and function. Chronic fatigue patients have been found to have reduced biosynthesis of neurotransmitters from ALC [131] and ALC treatment has been reported to reduce physical and mental fatigue in the elderly with improved cognitive status [132]. Some researchers suggest that some of ALC's neuroprotective actions in neurons may be due to effects on endogenous acetylcholine (ACh) levels. Most likely due to its potential to donate acetyl groups [133], ALC affects many aspects of ACh metabolism. ALC administration leads to an increase in choline uptake, ACh synthesis and ACh release in synaptosomes, striatum and hippocampus of rats, [134,135] which was previously reviewed by [39]. Carnitine and choline treatments stimulated ACh synthesis in cerebral cortex cells from adult rats [41]. ALC-mediated protection against brain ischemia was analyzed *in vitro* from a rat corticostriatal slice preparation [136]. Pre-treatment with ALC resulted in a progressive and dose-dependent recovery of field potential amplitude, a measure of functional activity of striatal neurons by using electrophysiological recordings, following oxygen and glucose deprivation. The addition of a choline transporter inhibitor blocked this protective effect of ALC. Choline transporters are thought to sustain pre-synaptic ACh synthesis and release. In addition, neuroprotection by ALC was prevented by the addition of a non-selective muscarinic antagonist and by an M2-like receptor antagonist. Protection was not prevented after addition of M1-like receptor antagonist [136]. Results indicate the mechanism behind some of ALC's protective effects against ischemia requires the activity of choline uptake and M2 muscarinic receptors. By modifying the ACh production in the brain, ALC also enhances cholinergic neurotransmission [39,124,137]. One study suggests ALC may improve transmitter function of cholinergic neurons by increasing the acetyl-CoA concentration in the cytoplasmic compartment [138].

ALC has also been shown to have beneficial effects treating symptoms of cerebral dysfunction caused by aging [139] and in some disorders of aging associated with cholinergic deficiency, such as AD [139,140]. A second NT system involved in the aging brain in addition to ACh is the dopaminergic system. Sershen et al. studied the effect of ALC on dopamine release and age-related changes in dopamine receptors on brain amino acids [141]. In brain tissue from adult mice incubated with labeled dopamine, ALC increased dopamine release followed by electrical stimulation. Dopamine

receptors declined with age and treatment with ALC for 3 months diminished the reduction in receptor binding [141]. These findings suggest multiple actions of ALC in brain and on age-related changes in the dopaminergic system.

Another NT that appears to be affected by ALC is  $\gamma$ -aminobutyric acid (GABA), a major inhibitory NT in the mammalian brain. A significant dose-dependent increase in GABA in the substantia nigra region of the brain following intraperitoneal ALC administration for 5 days was observed in young adult mice [142]. A further neuromodulatory action was observed in mouse retinal ganglion cells in which the GABA-activated chloride currents are blocked by ALC [143]. ACh and ALC reduced GABAergic inhibitory postsynaptic currents and responses to exogenous GABA and this block was voltage-independent. This effect continued after co-administration with muscarinic ACh receptor antagonists, indicating a potential independent mechanism of ALC other than the production of ACh [143]. A study using autoradiography found ALC used in the production of glutamate, a precursor to GABA, as opposed to entering a metabolic pathway [144].

#### 4.5. Protein modulation

ALC may prevent the age-associated changes to proteins. Oxidative modification to proteins appears to increase with age, as evidenced by increased levels of protein carbonyls, 3-nitrotyrosine and 4-hydroxynonenal [114]. Supplementing ALC to the older rats in this study decreased many of these parameters in most of the brain regions measured. Additionally, proteins are altered during and after translation, most notably through phosphorylation, ubiquitination, glycosylation, methylation, acetylation, and palmitoylation. There is evidence, as stated earlier, that the presence of PLC increases the palmitoylation of proteins [23]. ALC may modulate protein function and concentrations by directed acetylation or by merely increasing the pool of available activated acetyl groups. One area of the cell for which acetylation plays a major role is histones [145], proteins involved in the activation and silencing of DNA through chromatin remodeling. If ALC can affect the acetylation of histones, it can effectively augment gene transcription.

#### 4.6. Gene expression modulation

Based on studies of ALC treatment producing effects on processes in the brain such as learning and memory, researchers suggested the involvement of gene expression modulation by ALC. ALC treatment has been found to produce several changes in gene expression [82,146–149]. Poly(A)<sup>+</sup> RNAs were isolated from control and ALC treated rat brain and, using suppression subtractive hybridization (SSH) for gene expression profiling, the expression of two genes was modified by ALC, the isoform  $\gamma$  14-3-3 protein was up-regulated and the precursor mitochondrial P3 of ATP synthase lipid-binding protein was down-regulated [146]. The 14-3-3 proteins are implicated in mediation of signal transduction pathways and the activation of Raf-1, which plays an important role in cell differentiation and growth [150,151]. Levels in brain tissue are increased in patients with AD and decreased in Down's syndrome [152], and have been suggested to modulate AD risk [153]. P3 is a protein that forms one of the chains of the non-enzymatic membrane component of mitochondrial ATPase which functions in transmembrane proton conduction catalyzing ATP synthesis and ATP hydrolysis with transmembrane proton transport [154]. In addition, the gene coding for Hsp72 was up-regulated by treatment with ALC, which appears to help establish a cytoprotective state in inflammation, neurodegenerative disorders, and aging [146,155].

Another gene identified that is positively modulated by ALC treatment in the brain is the mitochondrial voltage-dependent



**Table 3**  
Summary of ALC neuroprotective mechanisms.

	ALC neuroprotective effects
Energy metabolism	Incorporation into acetyl-CoA and lipids [69,70] Normalize high-energy phosphates [71] Normalize lactate and pyruvate [72–74] Increase cellular consumption of oxygen [76] Reduced glucose oxidation and increased glycogen synthesis [63] Changes in enzymes in TCA cycle, ETC, and amino acid metabolism [64]
Membrane	Protection of mitochondrial membrane [77,78,84,85] Restoration of cardiolipin [76] Synthesis of <i>n</i> -3 PUFA in membrane Deacylation/reacylation of membrane [50,51,67,84] Increase membrane fluidity [50]
Protection from excitotoxicity Anti-oxidant	Protection from respiratory chain complex inhibitors [67,84,99,100] Increase in expression of HO-1 [112] Up-regulation of Hsps, SOD, Nrf2 [65,112,113] Restored GSH/GSSG [65,112,113] Decrease formation of HNE, protein carbonyls, and MDA [65,84,85,76,113,114,117] Reduced levels of pro-inflammatory cytokines [119]
Anti-apoptotic	Decreased cytochrome <i>c</i> release and caspase 3 [120–122] Changes in mitochondrial membrane potential [76]
NGF and nerve regeneration	Increase NGF production and NGF binding [124,125] Effects on Na <sup>+</sup> /K <sup>+</sup> ATPase activity [126,130]
Protein modulation Gene modulation	Acetylation of transcription factors [159] Up regulate VDAC, isoform $\gamma$ 14-3-3 protein, Hsp72 [146,147,155] Down regulate mitochondrial P3 of ATP synthase lipid-binding protein [146] Increase in transcripts related to mitochondrial biogenesis in skeletal muscle [82]
NT modulation	Increase in ACh synthesis and release [134,135] Influences cholinergic neurotransmission [39,124,137] Increase dopamine release and receptor binding [141] Increase in GABA [142] Reduce GABAergic inhibitory postsynaptic currents [143]

anion channel (VDAC) protein [156]. VDAC, also known as porin, is a small pore-forming protein of the mitochondrial outer membrane. It is the primary pathway for diffusion of ions and metabolites across the outer membrane and plays a key role in apoptosis by forming the outer pore component of the mitochondrial permeability transition (MPT) complex. Through involvement in the transport of acyl-CoAs, such as palmitoyl-CoA, across the outer membrane, it also contributes to ADP/ATP uptake into mitochondria [67,157]. Outside of the brain, ALC treatment caused an up-regulation in the expression of mitochondrial transcripts (i.e. COX-I, ATP6, NDP6, 16S rRNA) and mitochondrial proteins involved in mitochondrial biogenesis in a rat model for hindlimb muscle disuse atrophy, as stated above [82]. ALC's ability to modulate gene expression and to cause up-regulation of transcripts involved in energy and mitochondrial metabolism may be important for a large number of disease states related to energy deficits.

Additionally, ALC has been used in the clinical setting for the treatment of neuropathic pain. In rodents the mechanism of action has been suggested by up-regulating the expression of metabotropic glutamate receptor 2 (mGlu2) in dorsal root ganglia (DRG) of the spinal cord [158,159]. A recent study further investigating the mechanism found this up-regulation involves transcriptional activation mediated by nuclear factor- $\kappa$ B (NF- $\kappa$ B) through the acetylation of p65/RelA by ALC [159]. Further studies should examine ALC's mechanism of action and if it involves the acetylation of histones and/or other proteins involved in gene transcription.

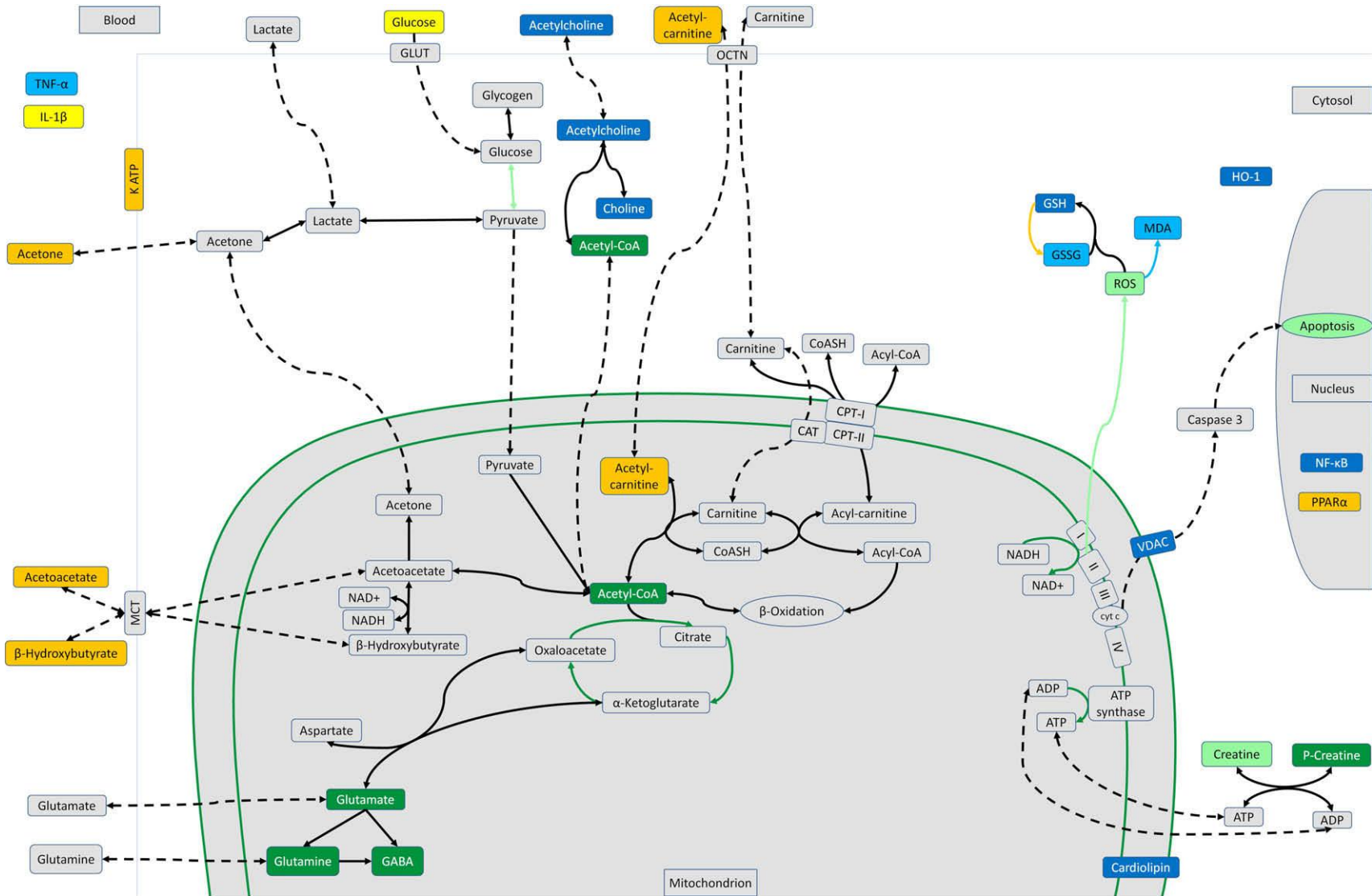
#### 4.7. Clinical applications

All of these roles and effects of ALC have led researchers to investigate carnitine's involvement in a variety of neurological disease states and treatments, including autism [160], PD [161], Down syndrome [162], hypoxia [163], Huntington's disease [164], cerebellar ataxia [165,166], age-associated mental decline [167], hepatic encephalopathy [168] and ammonia neurotoxicity [169,170]. A recent pharmacokinetic study of 12 healthy adults documented the

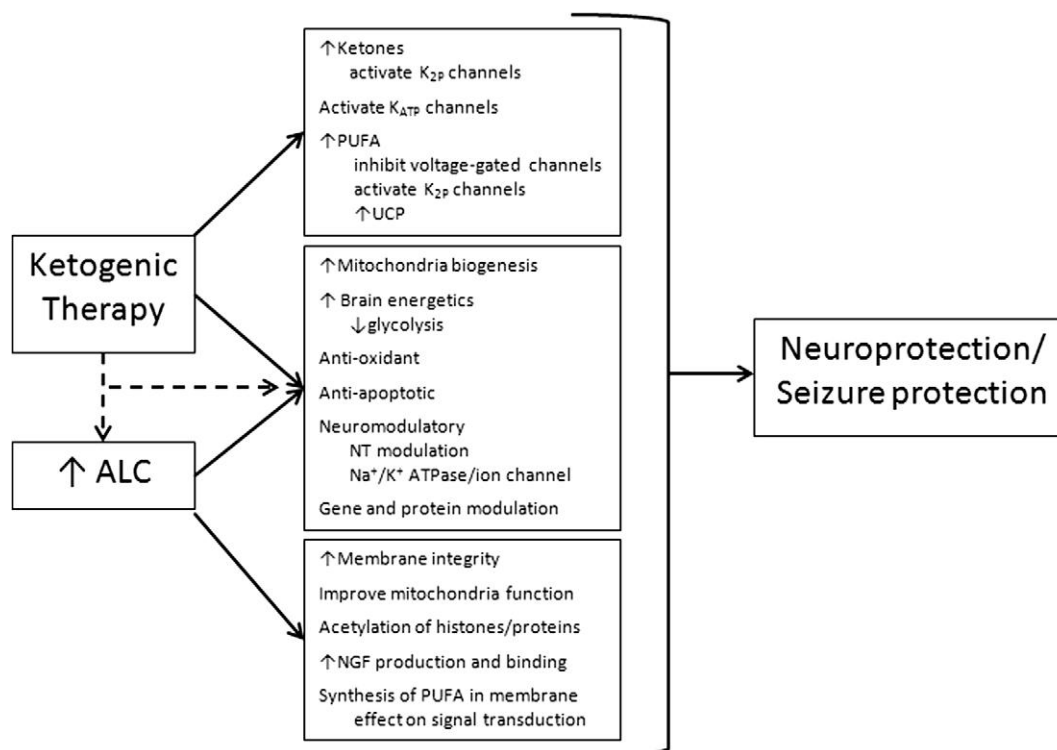
time course of the increase in plasma LC, ALC, and propionyl-L-carnitine [171]. Endogenous plasma concentrations of a number of acylcarnitines in 60 healthy subjects using the analytical techniques of radioenzymatic assay, HPLC and MS have been published [172]. A summary of ALC's neuroprotective mechanisms is listed in Table 3.

While neurodegenerative diseases such as AD involve mitochondrial dysfunction and oxidative stress, they also involve reduced levels of synaptic transmission [173]. Pre-synaptic terminals have a high number of mitochondria [174] and neurons specifically rely heavily on mitochondria because of their high-energy needs. Mitochondria generate both ATP and ROS and may, in turn, be vital in regulating neurotransmission [173]. Because of ALC and LC's role in increasing mitochondrial energetics and function, these molecules may also be playing a role in the prevention of neurodegeneration and the regulation of neurotransmission. Accumulation of the amyloid  $\beta$  (A $\beta$ ) peptide has been implicated as the cause of the cognitive decline seen with AD. Metabolically speaking, the A $\beta$  peptide can suppress levels of acetyl-CoA and the activity of choline acetyltransferase in cell culture [138]. ALC in this study reversed these effects, but it did not change the mortality of the undifferentiated cells. In other cases of neurotoxicity from A $\beta$  fragments, ALC was able to attenuate the oxidative stress, ATP depletion, and cell death [67,175]. ALC may be acting through buffering of oxidative stress and maintaining energy levels. As far as the extent to which ALC improves clinical symptoms of AD, results are variable: some studies have observed significant improvements in biochemical assays and psychometric tests [176]; others have not observed such large differences on a large scale [177]. Many of the possible roles of ALC in treating AD have been reviewed by Pettegrew [68], so those specific details are omitted here.

Ketogenic Therapy (KT) is a diet-based treatment for intractable epilepsy. It mimics the state of starvation through a high-fat, low-carbohydrate diet. Since the brain is usually reliant upon glucose for the majority of its energy, alternative sources of energy are



**Fig. 4.** Overview of ALC and KT metabolism and neuroprotective actions. Yellow = KT downregulation/decrease; orange = KT up-regulation/increase; bright blue = ALC downregulation/decrease; dark blue = ALC up-regulation/increase; light green = KT and ALC downregulation/decrease; dark green = KT and ALC up-regulation/increase.



**Fig. 5.** Summary of KT and ALC synergistic and/or additive effects on neuroprotection. Many of the KT mechanisms summarized here are not discussed in this review and have been reviewed previously.

needed for the brain under ketogenic conditions. In states of diabetes, fasting and high-fat diets, the body can enter a state of ketosis where the liver produces alternative energy substrates (most notably ketone bodies) to provide energy to the rest of the body, including the brain. Ketones such as acetoacetate and  $\beta$ -hydroxybutyrate are transported through monocarboxylic transporters (MCT1-4), along with pyruvate and lactate [178]. An increase in the glucagon:insulin concentration ratio (from starvation and high-fat diets) has been found to increase the total carnitine concentration of the liver and this change has been correlated to the concomitant increase in ketone production [179]. However, infusion of exogenous carnitine does not further enhance ketone production. Under these conditions, carnitine may increase fatty acid flux through carnitine acyltransferase to produce ketones [180], but it may also accumulate in the liver to accept activated acyl groups from CoA, thereby producing free CoA and acylcarnitines such as ALC (see Fig. 1).

In states of starvation, the concentration of ALC increases in liver [179,181]. Fasting increases total carnitine concentrations not only in the cytosol, but also in mitochondria, due, at least in part, to an increase in the  $V_{max}$  of carnitine transport [180]. When children on KT were monitored by subcutaneous microdialysis for acylcarnitines, the concentration of ALC was found to be increased [182]. Since the concentrations of ALC are increased under these conditions, it is likely the liver is releasing ALC, potentially to provide high-energy substrates to other parts of the body including the brain. If this is true, the liver would have competing systems for producing mobile energy substrates: ketones and ALC. Whereas ketones require ATP hydrolysis to regenerate acetyl-CoA, ALC does not. A study on 11-year old boys found a normal increase in blood acetoacetate after 12- and 17-h fasts [183]. When these boys were injected with DL-carnitine after 12 h of fasting, the increase in blood acetoacetate was attenuated. These results may indicate the presence of additional exogenous carnitine would enable the liver to produce more ALC, which would prevent the increase in blood ketones since the acetyl groups of acetyl-CoA could be direc-

ted towards producing ALC instead of ketones. In the brain of starved monkeys, the uptake of carnitine was relatively slow but the uptake of ALC was faster with rapid metabolism of the acetyl group [184], implying ALC can act as an alternative energy source for the brain.

A microarray study on the hippocampus from rats treated with KT found significant changes in specific sets of transcripts [185]. Metabolic genes were up-regulated and synaptic transmission genes were down-regulated. The number of mitochondria in the hippocampus was also found to be increased on KT. Overall brain energy reserves were increased on KT, synaptic transmission was resistant to low glucose concentrations and the seizure threshold of the rats was elevated. The authors believe mitochondrial biogenesis to be a major factor in the efficacy of KT. As previously mentioned, ALC has been found to up-regulate mitochondrial transcripts in soleus muscle [82] as well as to also improve mitochondrial morphology and function [186], so it could potentially bolster the effects of KT on mitochondria.

KT can be effective in reducing seizures in many different types of epilepsy [187], which implies this therapy may activate an endogenous mechanism for reducing seizures. The use of ketones and acylcarnitines to meet the brain's energy needs could be the basis for such a mechanism. The KT and the presence of ALC have similar and often overlapping results: increased  $\beta$ -oxidation, mitochondrial biogenesis and increased energy reserves (Figs. 4 and 5). Both can reduce the levels of circulating pro-inflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ). Many of the previously detailed neuroprotective functions of ALC could also have beneficial effects for epileptic patients, namely modulation of neurotransmitters, up-regulation of Hsps, and protection against excitotoxicity. Since free radical concentrations and apoptosis may be elevated in states of increased fat metabolism [188], a free radical quencher with anti-apoptotic effects such as ALC may be particularly useful. More research is needed, however, to determine ALC's role in KT and to assess the benefits of ALC supplementation.

## 5. Conclusions and future directions

Much of the research on carnitine and its esterified derivatives, such as ALC and PLC, has centered on the role of these molecules in metabolism. There is a great deal of evidence in the literature, however, to support new and multifactorial roles for these compounds in neuroprotection. ALC can provide high-energy acetyl groups to metabolic pathways to improve the overall energy status of the brain and to alter the biosynthesis patterns of some neurotransmitters. The acyl groups of acylcarnitines also have the potential to be involved in modulating proteins and gene expression. Other work has found acylcarnitines to improve mitochondrial function through improvements in membrane lipid content and enzyme activities. Pre-treatment of cells or animals with ALC before an excitotoxic insult can protect neuronal cells and treatment after such an insult can improve the condition of neurons. Some of these beneficial effects may also stem from the carnitine portion of ALC, which has antioxidant and anti-apoptotic functions by itself.

All of these functions of ALC make it a potentially beneficial molecule in the treatment of various neurological diseases, most notably AD and epileptic patients on KT. The aforementioned roles of ALC, metabolic and otherwise, could be part of the currently unknown mechanism behind KT. More research on use of ALC in the treatment of these diseases, including a supplementation regimen, is necessary to determine if these effects would translate to positive changes in a clinical setting. Work with acylcarnitines should also be expanded to encompass a wider range of these molecules instead of merely focusing on a relatively small subset. What is known, though, is that carnitine is not merely a cofactor in  $\beta$ -oxidation, but rather it has many known and yet to be discovered functions in physiology.

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