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Analyzing Mitochondrial Dysfunction, Oxidative Stress, and Apoptosis: Potential Role of L-carnitine

Mona Modanloo,[1] Mohammad Shokrzadeh[2]

[1]Pharmaceutical Sciences Research Centre, Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

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Review

Mitochondrial dysfunction, apoptosis and oxidative stress, are the interrelated events underlining the pathology of numerous diseases including cardiovascular, neurologic, and metabolic disorders. Due to playing a critical role in glucose and fatty acids' metabolism, L-carnitine probably has the potential to adjust these unfavorable events. The present review has evolved based on existing literature that investigated the mechanisms of L-carnitine and its derivatives based mitochondrial dysfunction, oxidative stress, and apoptosis related modulation. The released studies have been searched with the databases including Google Scholar, Scopus, and PubMed out of which overall 76 full-length articles have been chosen and recruited in this review. L-carnitine exerts protective effects against these cellular events in several manners including the maintenance of mitochondrial functions and decreasing the production of reactive oxygen species at different points. In clinical setting, these effects could be applied to treat a variety of associated diseases.

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ABBREVIATED EXPRESSIONS

3-NPA, 3-nitropropionic acid; Acetyl-CoA, acetyl-coenzyme A; ACOX, Peroxisomal acyl-coenzyme A oxidase 1; ATP, Adenosine triphosphate; Apaf-1, Apoptotic protease activating factor-1; AMPK, Adenosine monophosphate-activated protein kinase; ARE, Antioxidant Response Element; Bcl-2, B-cell lymphoma 2; Bcl-X_L, B-cell lymphoma-extra-large; Bax, Bcl-2-associated X protein; CAT, catalase; CPT, carnitine palmitoyltransferase; DNA, Deoxyribonucleic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ETC, electron transport chain; ERK, extracellular-related kinase; $FADH_2$, Flavin adenine dinucleotide; GSH, glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; HNE, 4-hydrox-nonenal; HSP, heat shock protein; iNOS, inducible Nitric oxide synthase; IAP, inhibitors of apoptosis proteins; LKB1, liver kinase B1; mtDNA, mitochondrial DNA; MPT, Mitochondrial permeability transition; Nrf2, nuclear factor erythroid 2-related factor 2; NADH, Nicotinamide adenine dinucleotide; NOX, Nicotinamide adenine dinucleotide phosphate oxidase; NQO1, NAD (P) H Quinone dehydrogenase 1; NF-κB, nuclear factor-kappa B; PDH, pyruvate dehydrogenase; PTP, permeability transition pore; PPAR-α, Peroxisome proliferator-activated receptor alpha; PKG, Protein Kinase G; PGC-1α, peroxisome proliferator-activated receptor coactivator-1 α; PGI_2, Prostaglandin I_2; PI3K, Phosphoinositide 3-kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, tricarboxylic acid; Trx, thioredoxin; TFAM, mitochondrial transcription factor A; XO, xanthine oxidase; XIAP, X-linked inhibitor of apoptosis protein;

INTRODUCTION

Perhaps, mitochondria can be considered as the most fascinating cell organelles, drawing researchers attention since being discovered for over a century. The first biological function of a mitochondria is related to cellular respiration

and energy production.[1] However, cell signaling, growth, adaptation, and death as other functions have been revealed by the studies done within the past few decades. The mitochondria's complex role in cells has brought about an intriguing subject in various diseases' pathology. Here a basic review is presented on the critical functions of mitochondria influenced by exogenous compounds that result in its better performance, targeting a benefit for the mitochondrial-based disorders.

Mitochondria and Energy Production

The energy produced as ATP basically depends on aerobic respiration. Aerobic respiration is made up of a chain of 4-steps reaction. First, glucose is converted into pyruvate by glycolysis. After that, Pyruvate is decarboxylated by pyruvate dehydrogenase (PDH) complex that produces Acetyl-CoA molecules. Moreover, acetyl-CoAs are produced from fatty acids during β-oxidation process.[2] Acetyl-CoA enters tricarboxylic acid (TCA) cycle and high-energy molecules such as NADH and $FADH_2$ plus a few ATP molecules are yielded. The last step is oxidative phosphorylation which produces the majority of ATP by transferring electrons from NADH and $FADH_2$ along the electron transport chain (ETC) to O_2 as the final recipient. The ETC consists of complex I or NADH dehydrogenase, complex II also known as succinate dehydrogenase, Coenzyme Q, complex III or bc_1 complex, cytochrome c and complex IV or cytochrome c oxidase. The electrons passing along these complexes give them the chance to pump H^+ ions from the inner mitochondria matrix to the intermembrane spaces and hence, to generate a proton motive force empowering ATP synthase complex also known as complex V to create ATP as protons flux back into the matrix.[3] Two elements of the proton motivating forces are proton concentration gradient and transmembrane electric potential ($\Delta\Psi_m$).[4] It is necessary to sustain the transmembrane potential for the proper mitochondrial functioning and this fact stresses the key role played by an intact inner mitochondrial membrane. In fact, the inner mitochondrial membrane is a unique structure that prepares an appropriate media for ETC's occurrence. A required component of this media is perhaps a phospholipid known as cardiolipin, almost exclusive to the inner mitochondrial membrane.[5,6] Nearly 25% of total phospholipids are made up of this membrane.[7] The unique cardiolipin structure optimizes ETC components' function; however, on the downside, it makes this membrane highly susceptible to oxidative insults.[8]

Mitochondria and Oxidative Stress

Some of ETC byproducts are different reactive oxygen species (ROS) such as superoxide radical $O_2^{\bullet-}$, hydrogen peroxide H_2O_2 and hydroxyl radical $OH^{\bullet}$. Complex I and III are behind the production of these ROS;[9] however, they can also be generated by non-mitochondrial sources including NADPH oxidase (NOX) and xanthine oxidase (XO) activities, and by ionizing radiations such as gamma radiation. These reactive radicals impose oxidative damage to cell components including lipids, proteins and DNA. Thus, it is required to take some defensive antioxidant measures in order to maintain cell homeostasis, including small molecule antioxidants such as glutathione (GSH) and vitamins E and C, with the macromolecules and enzymes such as thioredoxin (Trx) catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). Any sort of imbalance between the cell ROS generation and antioxidant capabilities leads to an oxidative stress, which brings about the cell dysfunction and death by exposing the energy production and cellular function at risk.[10]

Mitochondria and Apoptosis

As a scheduled cell death, apoptosis happens in multicellular organisms. It is considered as a defensive measure to remove the unnecessary or dysfunctional cells in a controlled manner to block the surrounding harmful cell components' dissemination. "Death signals" activation triggers apoptosis, mainly as a result of excessive DNA damage by the insults including radiation, ROS, and noxious compounds. Apoptosis activation results in morphological cell changes such as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and fragmentation of the nucleic acid content, all of which are diagnostic apoptotic cell markers. Two main pathways are engaged in the apoptosis progression, namely, the extrinsic and the intrinsic pathways, also known as the mitochondrial pathways. Using complex and closely regulated signaling pathways, mitochondria decide the cell's

fate, as to whether the excessive injuries should happen to cell components. The following section is a brief summary of these pathways: Death signals promote the pro-apoptotic protein Bax binding with permeability transition pores (PTP), which creates an opening for cytochrome c and apoptosis-inducing factors to enter cytoplasm. This opening, known as the mitochondrial permeability transition (MPT), is boosted by Ca^{2+} ions accumulation in the mitochondria, in addition to $\Delta\Psi$ reduction, and ROS insult. In the cytosol, cytochrome c and apoptosis-inducing factors with the cytosolic apoptotic protease activating factor-1 (Apaf-1), bind to and transform the protease pro-caspase-9 to caspase-9, leading to the cascade activation of executioner caspases, such as caspase-3 which digest the cellular components, finally, resulting in cell death. The proteins such as the inhibitors of apoptosis proteins (IAP) and Bcl-2 protein family including Bcl-2 and Bcl-X_L can antagonize the process of apoptosis.[11]

Dysfunction of Mitochondria as an Underling Cause of Diseases

The mitochondrial based decisive functions grant a critical role to this organelle in order to be protected against various disorders. In fact, mitochondrial dysfunction could yield alarming outcomes observed in the conditions like cancer,[12] diabetes,[13] nonalcoholic steatohepatitis,[14] cardiovascular diseases,[15] fatigue,[16] aging, and numerous neurological disorders such as Huntington, Parkinson, and Alzheimer's disease, and anxiety-related disorders.[17-19]

Recently, using nutraceuticals has attracted many researchers and clinicians to modify the dysfunctions of mitochondria, as a fundamental approach to treat the relevant disorders.[20] Unlike the common symptomatic therapies, this approach could modify the underlying disease pathology, probably occurring as an additional advantage of less adverse side effects. L-carnitine, as a natural compound closely related to mitochondrial functions is an example of such nutraceutical. In the present paper, current information on the L-carnitine mechanisms for mitochondrial disorders' treatment is collected hoping to pave the ground for developing further studies in this field.

Role of L-Carnitine

L-carnitine (LC) or Levocarnitine, as a naturally occurring amino acid plays its role in long-chain fatty acids' mitochondrial transfer and oxidation. It was discovered in 1905[21] and its chemical structure was established in 1927 (3-hydroxy-4-trimethylaminebutirate). Although, LC was initially labeled as vitamin $B_{T,}$[22] its animal related biosynthesis discovery made this term extinct. Human kidney, as the liver and brain, is a source of endogenous L-carnitine synthesis[21,23,24] from essential amino acids (lysine, and methionine) and the contributing ascorbic acid, niacin, pyridoxine, and iron.[21,25] However, dietary intake from food sources like meat and dairies supplies the majority of physiologic L-carnitine demand.[26] Under stress and genetic conditions, the required L-carnitine level may exceed its available amount, which brings about primary or secondary L-carnitine deficiencies. Thus it is imperative to consider L-carnitine "conditionally essential".[27] Kidney can involve in both primary and secondary deficiencies. The primary carnitine deficiency indicates a defective metabolism, including those of its synthesis, transport to tissues, or LC or its esters excretion by kidney. The secondary carnitine deficiency has several physiological and pathological etiologies like hepatic and/or renal impairment, physiological situations such as pregnancy, diverse drugs' administration, the deficiency of its precursors (lysine and/or methionine) or the necessary cofactors (iron, vitamins C, B3 or B6[27, 28]).

The carnitine system, made up of LC along with its acylated derivatives including acetyl-L-carnitine (Ac-LC), is engaged in the metabolic mitochondrial functions concerning glucose and lipid metabolism.[29] The basic L-carnitine function involves mitochondria, in which a series of reactions, labeled as the "carnitine shuttle", get to happen. Carnitine shuttle paves the ground for long-chain fatty acids' transfer across mitochondrial membranes, otherwise impermeable to these compounds.[26] Acyl-CoA synthase is the first enzyme involved, activating long-chain fatty acids by converting them into fatty acyl-CoAs. Following this stage, the enzyme carnitine palmitoyltransferase I (CPT I), labeled as carnitine acyltransferase I in the outer mitochondrial membrane, employs carnitine to convert fatty acyl-CoAs into acylcarnitines [30].Subsequently, in exchange for free L-carnitine, acylcarnitines

are transferred across the inner mitochondrial membrane by carnitine/acyl-carnitine translocase. Next, the enzyme carnitine palmitoyltransferase II (CPT II) based in the inner mitochondrial membrane turns acyl-carnitine back into acyl-CoAs and free L-carnitine.[30] What yields is acyl-CoAs undergoing β-oxidation that produces ATP, while the free L-carnitine can either serve as a substrate for CPT I to form more acyl- carnitines or for carnitine acetyltransferase (LCAT) to make the short- and medium- chain acyl-CoAs conjugates as acyl-carnitines. This reaction boosts the mitochondrial matrix excess acyl-CoAs extraction because of their accumulation's toxicity to mitochondrial functions.[30,31]

METHODOLOGY

Surfing the scientific databases like Google Scholar, Pubmed, and Scopus was done with the search terms "L-carnitine" "Mitochondrial Dysfunction" or "Oxidative Stress" or "Apoptosis" up to July 2017 as PRISMA guideline prescribed. At first, 250 related results were scanned and the latest papers were prioritized (1990). After that, articles considered appropriate by thoroughly scanning their abstracts, on the condition of dealing with L-carnitine's or any of its derivatives' effects on mitochondrial function, oxidative stress or apoptosis. As the criteria indicate, overall 45 full-length papers were chosen. The other associated papers were discovered either via reviewing the primary articles' references or searching the discussed topics like "Mitochondrial Apoptosis" or "Oxidative Stress". Another 35 articles were resulted and after 5 duplicates' deletion, 76 articles remained and recruited in this review.

MECHANISMS OF L-CARNITINE PRODUCTION

Given the protection of cellular homeostasis and function, L-carnitine exerts pleiotropic effects; obviously the majority of them originate from its function in mitochondria. In order to come up with a better understanding of molecular mechanisms of L-carnitine protective effects these are divided into several categories. It is noteworthy to mention that the mechanisms and the corresponding pathways are inherently interrelated, and may overlap at

Table 1. LC Treatment Effects in Different Study Settings

Agent	Study type	Subject	Type of insult	Results	Notes
Ac-LC / LC[62]	*In vitro*	DT40 cell line	TRX2 knockout	↑survival; ↓Caspase-3 & -9; ↓ROS; ↑GSH; ↓ANT oxidation; ↓Cyt-c & SOD1 release	Only Ac-LC was effective
Ac-LC[29]	*In vivo*	Rats	Hypoxia	↓Apoptosis; ↑ mitochondria biogenesis; ↑Nrf-2 expression; ↓cytoplasmic Ca^{2+};	ERK1/2 activation by Ac-LC; ↑Ca^{2+} uniporter expression; failure to restore complex I activity
Ac-LC / LC[58]	*In vitro*	Primary cultured neurons	Serum deprivation	↑survival; ↓apoptosis	-
Ac-LC[56]	*In vitro*	Primary cultured neurons	Amyloid $\beta_{(1-42)}$ peptide	↓mitochondrial dysfunction; ↓oxidation of proteins and lipids; ↓apoptosis; ↑GSH; ↑HO1; ↑Hsp72; ↓iNOS	Ac-LC reduced iNOS levels
Ac-LC[64]	*In vitro*	Primary cultured neurons	Hypoxia	↑survival; ↓mitochondrial dysfunction; ↓ROS; ↑GSH; ↓apoptosis; ↓caspase-3; ↓cyt-c release	Ac-LC restored NGF and TrkA expression with subsequent ERK1/2 activation
LC[61]	*In vitro*	HK2 cell line	H_2O_2	↓ROS; ↑Gpx, CAT & SOD; ↑mitochondrial dysfunction; ↓apoptosis; ↓caspase-3, Bax & cyt-c release; ↑bcl-2	-
LC[59]	*In vivo*	Rats	Cisplatin	↓mitochondrial dysfunction; ↓apoptosis; ↓oxidative damage	The tumoricidal activity of cisplatin was unaffected
LC[31]	*In vivo*	Mice	Non-alcoholic steatohepatitis	↑ expression of genes involved in long-chain fatty acid transport, β-oxidation, antioxidant enzymes; ↓oxidative stress, inflammatory cytokines, tumorigenesis	Diverse genomic-level activities of LC

Table 1. Continued

Agent	Study type	Subject	Type of insult	Results	Notes
LC[45]	*In vitro*	Neuro-2a cell line	Nickel chloride	↑cell viability; ↓LDH release; ↓oxidative stress; ↓mitochondrial dysfunction; ↑mtDNA	-
LC[60]	*In vitro*	Primary cultured neurons	1-Methyl-4-phenylpyridinium (MPP^+)	↓apoptosis; ↑Bcl-X_L, ↓Bax	-
LC[53]	*In vitro*	Primary cultured cardio-myocytes	doxorubicin	↓apoptosis; ↓caspase-3, cyt-c release; ↑Bcl-X_L; ↓ROS; ↓NOX; ↑PGI_2; ↑PPAR-α	PGI_2 mediated PPAR- α activation is possibly involved in reducing apoptosis
LC[70]	*In vitro*	Isolated rat liver mitochondria	Oleic acid	↓apoptosis; ↓mitochondrial dysfunction; ↓cyt-c release; ↑β-oxidation	Degradation of LCFAs is possibly involved in reducing apoptosis
LC[73]	*In vitro*	Isolated rat liver mitochondria	3-nitropropionic acid	↓mitochondrial dysfunction; ↓cyt-c release; ↓MPT; ↑Ca^{2+} capacity of mitochondria; ↑β-oxidation	LC only partially recovered 3-NP deactivation of complex II
LC[72]	*In vitro*	Isolated rat cardiac mitochondria	The palmitoyl-CoA	↓mitochondrial dysfunction; ↓cyt-c release; ↑β-oxidation	Degradation of LCFAs is possibly involved in reducing apoptosis
Ac-LC/ LC[63]	*In vivo*	Rats	Sciatic nerve ligation	↓apoptosis; ↓caspase-3, cyt-c release; ↑XIAP	Only Ac-LC was effective
Ac-LC(doi:10.1016/j.yexcr.2005.01.019)	*In vitro*	Murine fibroblast 3T6 cell line	Serum deprivation	↓apoptosis; ↓caspase-3, cyt-c release; ↑PCNA; ↑cell proliferation	-
Ac-LC[32]	*In vivo*	Rats	Aging	↑MMP; ↑cardiolipin; ↑O_2 consumption; ↓GSH; ↓ascorbate	Ac-LC could increase ROS generation
Ac-LC[38]	*In vivo*	Rats	Spinal cord injury	↑ mitochondrial respiration; ↑ tissue sparing & functional recovery	-
LC doi:10.1016/j.cbi.2003.10.010	*In vivo*	Rats	Aging	↑ activity of complexes I, II, III & IV	-
LC[37]	*In vitro*	Isolated human skeletal muscle mitochondria	-	↑ activity of PDHC; ↑ pyruvate oxidation	-
Ac-LC[34]	*In vivo*	Rats	Aging	↑ activity of complex IV; ↑ activity of ANT	-
AC-LC[33]	*In vivo*	Rats	Aging	↑MMP; ↑cardiolipin; ↑mitochondrial respiration	Ac-LC could increase ROS generation
Ac-LC[39]	*In vivo*	Rats	Aging	↑GST; ↓oxidative stress	Gpx levels were unaffected by Ac-LC
Ac-LC[40]	*In vivo*	Mice	Ethanol	↓ oxidative and nitrosative stress; ↓iNOS; ↓NOX	
LC[52]	*In vitro*	Human hepatocyte HL7702 cell line	H_2O_2	↓ ROS; ↑SOD; ↑CAT; ↑CPT1; ↑ACOX (acyl-CoA oxidase); ↑PPAR-α;	PPAR-α probably mediates the effects of LC on SOD & CAT
LC[41]	*In vitro;* *In vivo*	Rats	Quinolinic acid; 3-nitropropionic acid; Ferrous sulfate	↓ ROS; ↓ oxidative stress; ↓ mitochondrial dysfunction;	-
LC[42]	*In vivo*	Mice	Acetaminophen	↓ oxidative stress; ↑GSH	-
Ac-LC[43]	*In vivo*	Dogs	Ischemia-reperfusion	↓ oxidative stress	-
LC(doi:10.1002/ijc.20636)	*In vivo*	Rats	Hepatocarcinogenesis	↓ mitochondrial dysfunction; ↓ROS; ↓oxidative damage; ↓apoptosis; ↓liver tumors	-
LC(doi:10.1016/j.brainres.2005.06.062)	*In vivo*	Rats	Hypoglycemia	↓ oxidized GSH; ↑mitochondrial respiration	-
LC[71]	*In vitro;* *In vivo*	HepG2 cell line; Rats	Palmitic acid	↓apoptosis; ↑mtDNA; ↑β-oxidation; ↓ ROS; ↑CPT1; ↑PPAR-γ;	-
Ac-LC (doi:10.1016/j.febslet.2006.11.016)	*In vitro*	CEM cell line; U937 cell line	Zidovudine, stavudine and didanosine	↓ROS	Ac-LC was unable to preserve MMP
Ac-LC[46]	*In vivo*	Rats	γ-irradiation	↑SOD; ↑GPx; ↑GSH; ↓oxidative damage; ↓ total nitrate/nitrite	-

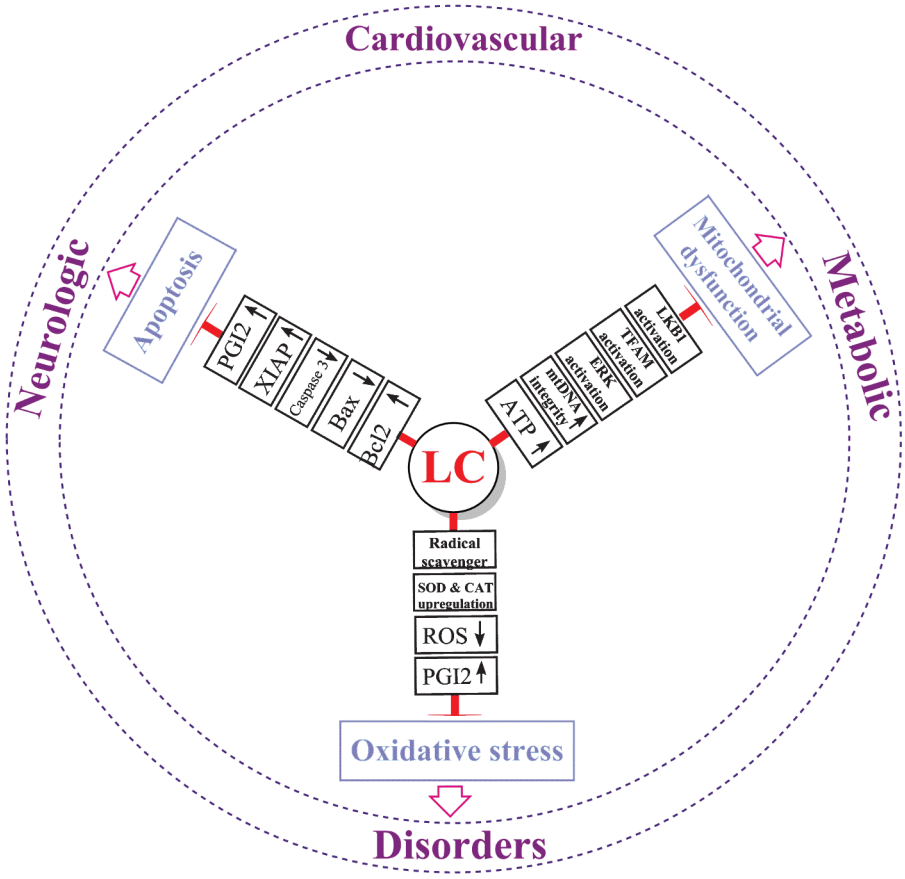

Figure 1. Mechanisms of L-carnitine Protection.

numerous points (see Table 1 and Figure 1).

Effects on Mitochondrial Biogenesis and Integrity

Studies in recent years have concentrated on the protective effects of L-carnitine treatment on the mitochondrial functions and the associated components. A research dealing with rotenone-induced Parkinson's disease in rats, Ac-LC (100mg/kg/day, oral) co-treatment enhanced mitochondrial DNA (mtDNA) integrity and quantity, and raised ATP production, indicating the mitochondria protection and their boosted function, and the final outcome, was survival of dopaminergic neurons and enhanced motor performance.[32] Through simulating hypobaric hypoxic conditions on rats, Barhwal et al.[33] studied the Ac-LC induced mitochondrial biogenesis mechanisms in neurons. The subjects responded to a 2-week treatment regimen of 75 mg/kg oral AC-LC revealed by better task learning. It was discovered that Ac-LC activated the transcription factor peroxisome proliferator-activated receptor coactivator-1a (PGC-1α), and subsequently, upregulated nuclear factor erythroid 2-related factor 2 (Nrf2) and NRF-1 downstream of PGC-1α. This signaling pathway activates the mitochondrial transcription factor A (TFAM), as an important contributing factor in mitochondrial DNA (mtDNA) transcription encoding several mitochondrial complex proteins. Moreover, it also activates liver kinase B1 (LKB1), a kinase engaged in AMP-activated protein kinase (AMPK) complex phosphorylative activation. It also activates PGC-1α, following mitochondrial biogenesis and ATP production. These pathways' activation both resulted from extracellular-related kinase (ERK 1/2) activation. Due to reduction of ATP/mtDNA ratio, these newly formed mitochondria were less functional than the pre-existing mitochondria, which indicate lower ATP production in these mitochondria. The Ac-LC inability to completely compensates complex I activity drop might be the reason behind it, which was prevented by hypoxia. Though, cytosolic Ca^{2+} buffering, following cell hypoxic injury, due to Ca^{2+} uniporter expression

increases and cytosolic Ca^{2+} reduction results in the boosting of active mitochondrial function and clarifying the net ATP production rise. In another study on rotenone-induced Parkinson's disease, a similar PGC-1α activation by 4-week pretreatment of Ac-LC neutralized ATP production drop by rotenone.[34] Ishikawa et al. performed a systematic genomics, proteomics and metabolomics study about LC exerted effects in non-alcoholic steatohepatitis (NASH) mice model[35] by lacing high fat diet mixed with 0.028% LC. After 4 weeks, the results suggested a significant rise in the expression of various genes including those of long chain fatty acids transport and β-oxidation, like medium-chain Acyl-CoA dehydrogenase (MCAD). L-carnitine treatment led to several TCA cycle related metabolites' rise.

In two studies, Hagen et al. compared old and young rats, revealed a drop of $\Delta\Psi_m$ and cardiolipin content of mitochondria during aging course.[36,37] One month dietary Ac-LC supplementation (~1% wt./vol in drinking water) for the aged rats restored both of these parameters to the juvenile levels and enhanced ambulatory activity of the rats (defined as total distance traveled per hour). The reason behind cardiolipin protection has been attributed to the cytochrome c oxidase and ADP translocase increased activity in the heart mitochondria of aged rats.[38] An age based drop in rats' LCAT activity has been revealed in another research, and the lipid peroxidation products, malondialdehyde, and 4-hydrox-nonenal (HNE has been identified) as the troublemakers of this decline in the *in vitro* analysis. This drop resulted from a 7-week 0.5% Ac-LC supplementation in drinking water [39]. Reviewing the potential mechanisms behind membranes and proteins' Ac-LC modulation is at hand.[40]

The additional engagement of LC in boosting the metabolic mitochondrial functions has been suggested in many studies. In a research which addressed human muscle cells, incubation with 1mM LC resulted in pyruvate oxidation increased by twice accompanied by PDH complex activity intensification in the same way compensated by LCAT inhibition. This mentions the LC critical role in removing the excess acetyl-CoA produced by high PDH activity and consequently, enhancing the function of this complex.[41] In the studies following the rats' spinal cord injury, as the result of the boosted PDH, NADH dehydrogenase and cytochrome c oxidase activity *in vivo*, Ac-LC treatment stimulated respiratory rate. *In vitro* investigations indicated that Ac-LC could replace this substrate in mitochondrial respiration in the absence of pyruvate, that finally a better functional recovery may achieve.[41,42]

Effects on Oxidative Stress

LC and its esters induced protective effects against oxidative stress as the result of diverse insults such as rotenone,[32] aging,[43] alcohol,[44] quinolinic and 3-nitropropionic acid (3-NPA),[45] acetaminophen toxicity,[46] the ischemia-reperfusion injury of brain,[47] and kidney,[48] as well as nickel toxicity,[49] and gamma radiation.[50] Post-renal transplantation ischemia-reperfusion injury typically trigers delayed graft function in the recipients and the reason behind probably results from the cellular homeostasis imbalance and collapsing energy balance. In addition, reperfusion of the ischemic tissue leads to some ischemia-reperfusion related complications. A research on the adult male rats by Mister et al. drew this conclusion that via prevention of lipid peroxidation, free radical generation, improvement of the tubular cells' structure and function and adjustment of tissue inflammation, propionyl-L-carnitine can probably limit ischemia-reperfusion injury and its post- renal transplant related complications.[48] However, as shown by Hagen et al., using Ac-LC treatment on senile liver parenchymal cells resulted in increased ROS production per consumed oxygen, and consequently, decreased cellular glutathione and ascorbate reserves.[36] The above-mentioned phenomenon can be explained by taking in to consideration that despite stimulating cell respiration, the Ac-LC works to raise the mitochondria number with non-optimal function, and consequently, increases the ROS production. Yet, LC and its derivatives potential power to neutralize the oxidative and nitrosative stress is distinguished well and occurs by a set of mechanisms including direct radicals scavenging, for example, $DPPH^{\cdot}$, superoxide and hydrogen peroxide,[51] chelation of metals catalyzing radical formation such as Fe^{2+},[51] inhibition of ROS-producing enzymes like XO and NOX, upregulation of antioxidant enzymes like CAT, SOD, GST, GR and GPx, heme oxygenase and endothelial nitric oxide synthase and other protective proteins including

HSPs.[52,53] A research by Zhang et al. conducted on the effects induced by 4-weeks Ac-LC alone compared with Ac-LC + lipoic acid on a rotenone-induced model of Parkinson's disease. The result of Ac-LC treatment was increased mitochondrial biogenesis and decreased ROS production, likely due to upregulating the PGC-1α. It has to be noted that as an upstream factor, PGC-1α activates NRF1/2 which in turn activates antioxidant response element (ARE). As a response element, ARE modulates vital cell antioxidants' expression, such as HO-1, NQO1, GST, CAT, SOD.[54] This study's fascinating finding was that even after stopping Ac-LC treatment, the cells exerted resistance for up to 4-weeks rotenone insult, suggesting that the protective effect of Ac-LC could be indirectly done via PGC-1α.[34] The upregulation of PGC-1α signaling by Ac-LC induced ERK1/2 activation and its implication in mitochondrial biogenesis has already been observed.[33]

Similarly, the antioxidant defense boosted through the restoration of expression and activity of GPx, SOD, and GR, and reduction of NOX expression via upregulating Nrf2 and PPAR-α, and the drop of nuclear factor-kappa B (NF-κB) spotted in the hypertensive rats' renal cortex, which further highlights the role of these transcription factors in mediating the LC induced effects.[55]

As recently mentioned in a study, L-carnitine exhibited to protect a human hepatocyte cell line, i.e., HL7702, against oxidative stress exerted by H_2O_2 and boosted the cell viability and survival. 12-hour pre-exposure to diverse LC concentrations accelerated the cells' antioxidant defense as the result of up-regulating SOD and CAT. The further investigation of the mechanisms came up with the PPAR-α involvement in mediating the LC-induced effects. PPAR-α as a transcription factor is necessary for lipid homeostasis and its downregulation, as seen by H_2O_2 in this case, will damage the cell metabolism. The expression of CPT1 and ACOX as the two components of β-oxidation controlled by PPAR-α, severely declined by H_2O_2, while compensated by LC treatment [56]. It is intriguing that PPAR-α activation could happen following LC induced rise in PGI_2 production . Moreover, a 24-hour cardio-myocytes pretreatment course by 1-30mM LC resulted in the reduced doxorubicin-induced ROS production and oxidative damage, basically via increasing PGI_2, the activation of PPAR-α, and the doxorubicin-induced NOX activity inhibition.[57] PGI_2 has been proposed as a ligand for PPAR-α that directly upregulates the gene expression of the antioxidants such as SOD, CAT, GPx, and HO,[58] while lowers the NOX expression.[59] This again supports the pleiotropic nature of LC protective effects.

Another research studied the Ac-LC's protective effect (concentrations range 10Mm-200mM) against amyloid-beta peptide $A\beta_{(1–42)}$ induced oxidative/nitrosative stress and apoptosis in neurons revealed that the results partly had been obtained by the rise in GSH level and Hsp32 (HO1) and Hsp72 upregulation and iNOS downregulation.[60] The group performed another study on HNE, as a reactive lipid peroxidation byproduct induced by $A\beta_{(1–42)}$, to induce oxidative stress and apoptosis in cortical neurons,which revealed the potential PI3K/Akt signaling pathway involvement in upregulation of Hsps and PKG-mediated ERK1/2 activation and consequently, increasing the antioxidant defense and survival.[61]

Modulation of Apoptosis

The final outcome of the accumulated mitochondrial dysfunction and oxidative damage is apoptotic cell death, to the point that it gets impossible to be repaired. Assuming that the LC's anti-apoptotic effect comes after its anti-oxidant and protective effects on mitochondria seems to be logical. Though, the LC-induced effects by signaling pathways are also required. The LC's and Ac-LC's anti-apoptotic effects in different cells have been revealed in various conditions including neurons serum deprivation, cisplatin injury and MPP toxicity [62-64]. Similarly, LC improved Bcl-2 expression in human proximal tubule epithelial cell line and deterred H_2O_2 apoptosis through maintaining Bcl-2 and counteracting Bax increase, which led to lower caspase-3 activity and apoptosis.[65] Likewise, Trx knockout cells that exhibit significant oxidative stress and apoptosis, were released by Ac-LC but not by LC, as the result of ROS drop and mitochondrial apoptosis prevention easily seen via declined caspase-3 and -9 and lowered cytochrome C and SOD1 getting released into the cytoplasm.[66] Apoptosis prevention by $A\beta_{(1–42)}$ in cortical neurons also occurs in a similar way.[60] The research by Mannelli et al. on LC and Ac-LC induced effects in a peripheral neuropathy model

reported that only Ac-LC could prevent neuronal apoptosis, highlighted the prevention of XIAP upregulation contributed in apoptosis by Ac-LC,[67] Ac-LC induced ERK1/2 signaling activation has ben discussed elsewhere. Though, under hypoxic conditions, this pathway also correlates with apoptosis drop, as the cytochrome c release declines, caspase-3 levels drop and Bcl-2 expression rises reported in hypoxic hippocampal neurons [68], later verified in animal models exposed to hypoxia.[33] In the latter test, there was the mitochondrial Ca^{2+} uniporter's increased expression accompanied by the cytoplasmic Ca^{2+} decline, signifying the Ca^{2+} uptake into mitochondria stimulated by Ac-LC. The role of Ca^{2+} in apoptosis performance or the cell survival promoted by Bcl-2 and NF-κB[69] is particularly fascinating. Thus, the rise in mitochondrial cytoplasmic Ca^{2+} uptake resulted in the cell survival as the Ac-LC's co-treatment with a Ca^{2+} uniporter inhibitor, which lowered caspase-3 decreased by Ac-LC. Besides, PKG activation in mediating the ERK1/2 signaling and Bcl-2 upregulation, with BAD phosphorylation increases by PI3K/AKT, likely plays role in decreasing HNE induced apoptosis by Ac-LC, and Ac-LC's pro-survival effects.[61] Apparently, in cardio-myocytes, LC-induced PGI_2 is required in order to shield against doxorubicin-induced apoptosis, and PPAR-α also has a remarkable role in mediating this effect.[57] As a matter of fact, the significance of PPAR-α in protection against the apoptotic cell death is widely recognized[70] and the other research approved the PGI_2 anti-apoptotic effects through its interaction with PPAR-α.[71] These pathways' utilization by LC leads to LC's improvement, which is used as an anti-apoptotic agent.

Ca^{2+} ion takes part in apoptosis' execution partly due to Ca^{2+} -dependent phospholipase A_2 activation by the free fatty acids released from membrane phospholipids.[72,73] Free fatty acids have the ability to boost MPT and depolarize mitochondria. LC can lower free fatty acid damage to mitochondria by increasing β-oxidation, thus acts against apoptosis.[74] As detected,by applying HepG2 human liver cell line, LC preserved the mitochondrial structure and prevented palmitate induced apoptosis due to increasing β-oxidation partly because of the increased CPT-1 and PPAR-γ expression.[75] Through applying the isolated heart mitochondria of the animals receiving palmitoyl-CoA with LC treatment,[76] similar results have been obtained. LC treatment resulted in inhibiting the palmitoyl-CoA depolarization and mitochondrial swelling and also prevented cytochrome C release into the cytoplasm as β-oxidation increased. 3-NPA has been reported as a potent complex II inhibitor, induces mitochondrial swelling, depolarization, and permeability transition, following with cytochrome C release by the mechanisms including of PLA_2 Ca^{2+} activation. When these effects were suppressed by PLA_2 inhibitors, LC treatment similarly protected against 3-NPA-induced MPT by oxidative degradation through PLA_2-generated fatty acids.[77]

DISCUSSION

The exogenous LC and its ester derivatives administration have some certain beneficial impacts on mitochondrial structure, metabolism, oxidative stress and apoptosis. LC acetylated form has some distinguished characteristics, leading to the improvement of its function in comparison with LC itself. One of these features is the higher efficacy of OCTN2, the Na^+-dependent organic cation transporter, in transporting the Ac-LC into the cells,[78,79] indicting that with reaching better accumulation in its target tissues and inside the cells, Ac-LC has more powerful action compared with LC. Another remarkable feature is Ac-LC being located in the acetyl moiety capable to be transferred to several cell components like membrane lipids, receptor proteins and histones, which consequently, changes the membranes' physicochemical properties, modulates their binding to ligands, modifies receptors' activity and changes gene expression. Further studies can be done on the Ac-LC's acetylation mechanism as a fascinating subject, regarding gene expression changes, the modulation of the receptors like tyrosine kinases and the enzymes such as cardiolipin synthase. Lately, the basal ROS levels' critical role in cell signaling has been clarified and the ROS's absolute annihilation adverse effects due to some potent antioxidants are gradually elucidated, as for example seen in the context of carcinogenesis. Though, based on what has been revealed by the above-mentioned studies, LC can't fully react to cellular ROS, it could promote ROS generation in mitochondria. This relative drop of ROS, as a type

of "ROS-buffering" could preserve the required functions of cells' ROS while blocking the oxidative stress. To sum up, carnitines' antioxidant effect may be superior to many other antioxidant molecules. What seems inevitable is the potential extrinsic pathway induced by modulating apoptosis, as the TNF-α's downregulation has been revealed by LC treatment.[35] Surprisingly, TNF-α, Fas, and caspase-8, as some of the significant components contributing to the extrinsic apoptosis pathway, are upregulated after LC treatment used for hepa1c1c7 mouse's cancer cells.[80] Our understating of the LC mechanisms behind the actions will certainly grow by the gained underlying reasons for this distinct response and the potential extrinsic apoptosis pathway's implications in other cells .

CONCLUSIONS

LC, equipped with its various interactions with different cell components and signaling pathways, guarantees the accomplishment of cell homeostasis and its proper functioning. LC possesses this potential to raise the mitochondrial biogenesis, increasing various mitochondrial components' gene expression and maintains their function via supplying their respective substrates and protecting them against insults including the toxic products' or reactive radicals' accumulation. This is the cell boosted bioenergetics that accompanies with improved viability and function. Higher production of ETC-induced ROS is the drawback of this process, which is optimistically neutralized when LC potently reacts to the oxidative stress by some direct mechanisms such as radical scavenging and Fe^{2+} ions' chelation and indirect actions due to the decrease of ROS and RNS producing enzymes like XO, NOX, and iNOS while raising the GSH, SOD, CAT, and GPx antioxidants' expression. LC accompanied with the above mechanisms also rigorously modulates the cells' apoptotic signaling seen by decreasing pro-apoptotic proteins, namely, BAX and BAD and boosts the anti-apoptotic components such as Bcl-2 proteins, XIAP and some HSPs. All together, LC-induced protection possesses the potential to counter diverse cell injuries, hence, using LC and its derivatives in clinical setting is motivated.

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Correspondence to:
Mohammad Shokrzadeh, MD
Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
E-mail: mslamuki@gmail.com

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