ORIGINAL ARTICLE

Ultrastructural aspects of the effects of L-carnitine administration on epithelial cells in the aging rat tongue

Barlagiannis D[1], Dietrich EM[2], Papaliagkas V[3], Makri S[4], Toskas A[2], Papamitsou T[2]

[1]Catheterization Laboratory, Medical Clinic I, Lukas Hospital, Neuss, Germany
[2]Department of Histology and Embryology, Medical School, Aristotles University of Thessaloniki, Thessaloniki, Greece
[3]Department of Neurology, General Hospital Georgios Papanikolaou, Thessaloniki, Greece
[4]Department of Biomedical Sciences, Faculty of Health Sciences and Caring Professions, Technological Educational Institute, Thessaloniki, Greece

Abstract

Background: The investigators hypothesized that degenerative changes accumulate in epithelial cells in the aging rat tongue and that carnitine administration is effective at reversing these alterations.

Material and Methods: To gain insights into the effects of carnitine on epithelial cells of the tongue, the investigators used 15 Wistar rats [3 experimental groups: 5- (A), 12- (B) and 18- (C) month old rats] with 4 rats per group and 1 control group with 1 rat per age group). L-carnitine was administered intraperitoneally to animals of the experimental group for 35 days. Samples of the tongue were processed for electron microscopy.

Results: Degeneration of epithelial cells of the rat tongue was shown to begin early in life (5 months) and alterations were shown to accumulate while aging. L-carnitine administration eliminated degenerative changes when administered in the first age group A, while in the older rats the regeneration was only partial for the epithelium (groups B and C).

Conclusions: The results of this study suggest that profound ultrastructural alterations commence in the degenerating rat tongue and that L-carnitine administration results into partial regeneration of epithelial cells. Hippokratia 2014; 18 (1): 32-36.

Keywords: Carnitine, tongue, microscopy, electron

Corresponding author: Dietrich Eva-Maria, DDS, P.B. 1033, 57006 Vassilika, Thessaloniki, Greece, tel: +306993648667, fax: +302396023349, e-mail: aeffchen.dietrich@gmail.com

Introduction

Carnitine is an essential nutrient that is present in almost all animal species, microorganisms and plants[1]. It is involved in many different processes in the human body: energy production, membrane biosynthesis and repair, removal of toxic substrates, anti-apoptotic mechanisms, transport of fatty acids through the internal mitochondrial membrane with concomitant removal of toxic by-products of fatty acids metabolism[2,3].

Carnitine levels in the body are the result of exogenous uptake, endogenous synthesis and tubular reabsorption in the kidneys[4]. Endogenous synthesis is mainly done in the liver, the kidneys and brain from methionine and lysine[5]. Its concentration is increased in tissues that require high amounts of energy, like muscles and the myocardium[6]. This justifies the decreased carnitine plasma level, that is 0.6% of the whole body content (21gr)[7]. Muscle cells are not able to biosynthesize carnitine, thus exogenous carnitine is essential for muscle metabolism[8].

The reduction of ischemia-induced myocardial injury after L-carnitine administration provided scientific evidence of the effectiveness of carnitine administration to patients suffering from cardiovascular disease[9]. Reports on the protective or regenerative effect of carnitine on striated muscle cells are sparse. Similarly, little is known about the protective role against epithelial cell degeneration. However, a few studies report on a protective effect against vascular atherosclerosis and endothelial cell dysfunction[10].

The scope of this study is two-fold: 1) to investigate degenerative changes in the epithelium of the aging rat tongue, and 2) to test the hypothesis that L-carnitine attenuates epithelial cell degeneration.

Material and Methods

Animals

The study was approved by the Bioethics Committee of the Medical School of the Aristotle University of Thessaloniki. Fifteen Wistar rats, 5-, 12- and 18- month old, weighing approximately 500 mg, were used in the experiment. Rats were housed in stainless steel cages, with a maximum of two rats per cage, 12h light-dark cycle, relative humidity and temperature control. They were fed

normal diet, 21% proteins, 6.2% fatty acids, 4.5% fibres, vitamins, minerals and anorganic compounds.

The animals were divided into three groups according to age (Table 1).

L-carnitine (Carnitor, Sigma-Tau, Maryland, USA) was administered at a dose of 300mg/kg b.w./day intraperitoneally for 35 days. After euthanasia, samples of the tongue were obtained both from experimental and control groups and the tissues were processed for electron microscopy.

Transmission Electron Microscopy

Tongue tissue samples were sectioned into <1 cm^3 pieces. They were placed into glutaraldehyde 2.5% for 2 hours and then into osmium tetraoxide 1% for 1 hour. Staining was performed with uranyl acetate 1% (16 hours) and dehydration with advancing ethanol concentrations. Samples were embedded into Epon resin.
Ultra-thin sections (600–900 Å) were taken and stained with Reynolds's stain. Samples were observed under a JEOL transmission electron microscope.

Sample observation

Two sections from each sample were processed for ultrustructural analysis by two observers. Assessment of epithelial cell degeneration included following points: 1) nuclear morphology, 2) interepithelial junctions preservation, 3) mitochondrial cristae preservation, 4) intramitochondrial vacuole formation, 5) mitochondrial swelling, and 6) lipid droplets formation.

Point 1 was answered with normal or abnormal, points 3, 4, 5 and 6 with yes or no. Regarding point 2, it was assessed whether there is preservation of interepithelial junctions, moderate destruction or interepithelial edema.

No differences were found between the two observers regarding the assessment of the samples. The characterization of the samples into normal, moderate or profound degeneration was done according to the number of pathologic alterations found. In particular, the sample was characterized as normal when there was no more than one pathologic alteration. Moderate degeneration was present when two alterations were identified and profound degeneration when more than two changes were present.

Results

Ultrastructural analysis (Table 2)

Alterations of the epithelial cells were present in all groups (A, B and C). Controls from Group A (A-NLC), showed moderate destruction of interepithelial junctions and lipid droplets formation (Figure 1), while the samples from experimental group A (A-LC), showed regeneration of the epithelium and normal interepithelial junctions (Figure 2).

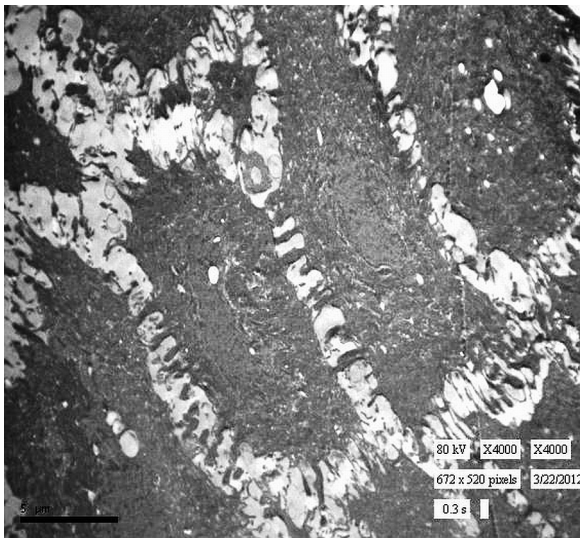

Figure 1: Electron Microscopy image showing moderate destruction of interepithelial junctions in the rat tongue, in 5-month old rats (Group A), that did not receive L-carnitine.

Table 1: The table presents the experimental and control groups in which the rats were divided, and the number of animals that were included to each group.

Groups	L-Carnitine administration groups	Control groups
Group A (5 months)	A^LC* (n=4)	A-NLC#(n=1)
Group B (12 months)	B-LC (n=4)	B-NLC (n=1)
Group C (18 months)	C-LC (n=4)	C-NLC (n=1)

^A, B and C: refer to the age groups, *LC: L-Carnitine, #NLC: Not L-Carnitine

Table 2: Results from the observation of rat tongue samples with electron microscopy. The table presents the number of animals that belong to each category of degeneration, also divided according to age group and L-carnitine administration or not.

Grade of degeneration*	L-carnitine administration groups			Control groups		
Age group (months old)	5-months	12-months	18-months	5-months	12-months	18-months
Normal appearance#	4					
Moderate degeneration^		4	3	1		
Profound degeneration[a]			1		1	1

* Epithelial cell degeneration assessment with electron microscopy according to our proposed six-point system, # Normal appearance: 0 points or 1 point, ^moderate degeneration: 2 points, [a] profound degeneration: >2 points.

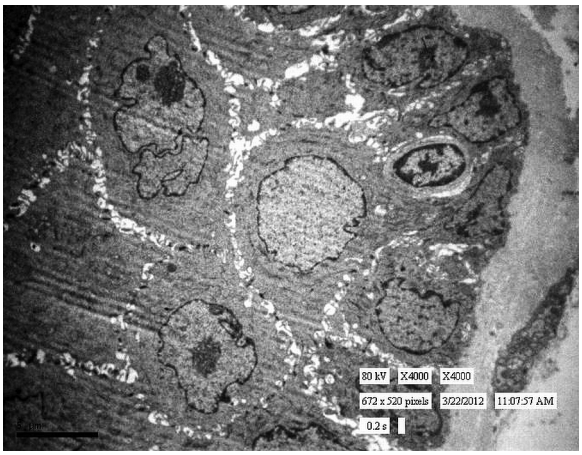

Figure 2: Electron Microscopy image that reveals regeneration of interepithelial junctions in the rat tongue, in 5-month old rats (Group A), that received L-carnitine.

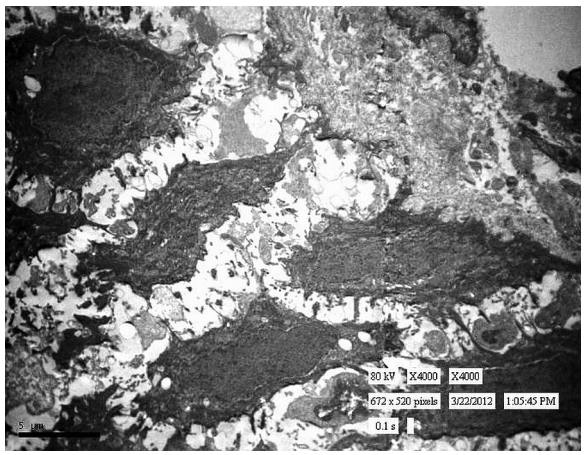

Figure 3: Electron Microscopy image showing interepithelial edema in the rat tongue, in 12-month old rats (Group B) that did not receive L-carnitine.

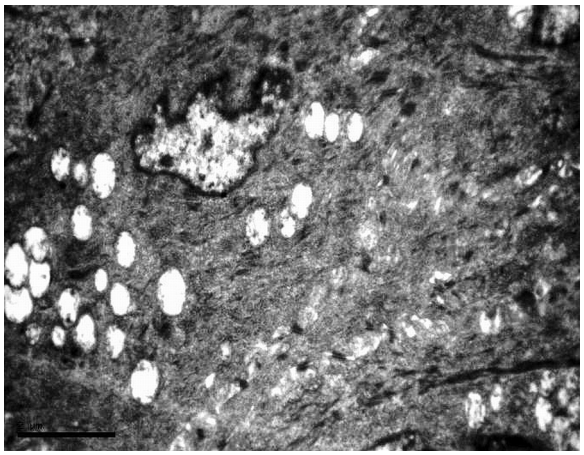

Figure 4: Electron Microscopy image showing atypical nuclear morphology of epithelial cells in the rat tongue, in 12-month old rats (Group B) that did not receive L-carnitine.

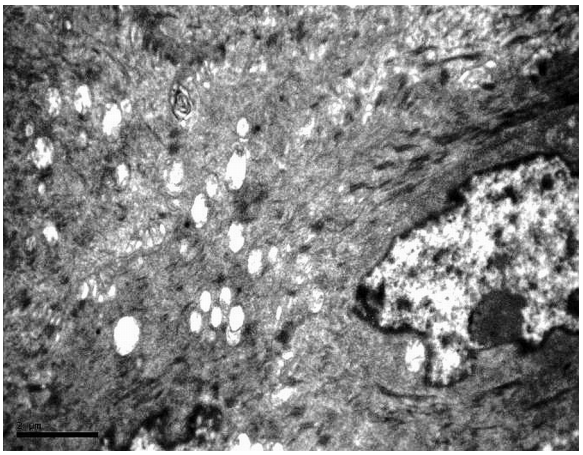

Figure 5: Electron Microscopy image that reveals moderate mitochondrial degeneration in epithelial cells of the rat tongue and partial preservation of mitochondrial cristae, in 12-month old rats (Group B) that received L-carnitine.

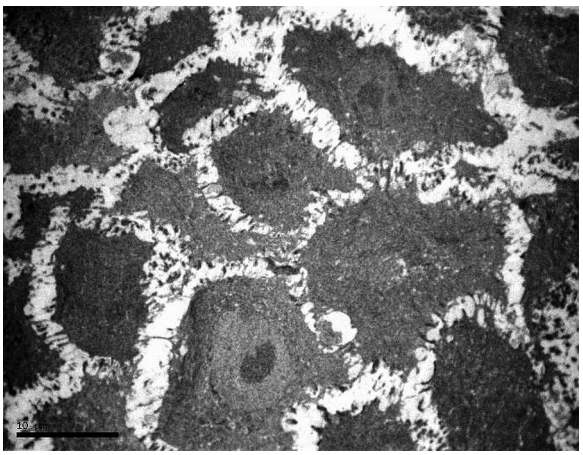

Figure 6: Electron Microscopy image that reveals moderate destruction of interepithelial junctions in the rat tongue, in 12-month old rats (Group B) that received L-carnitine.

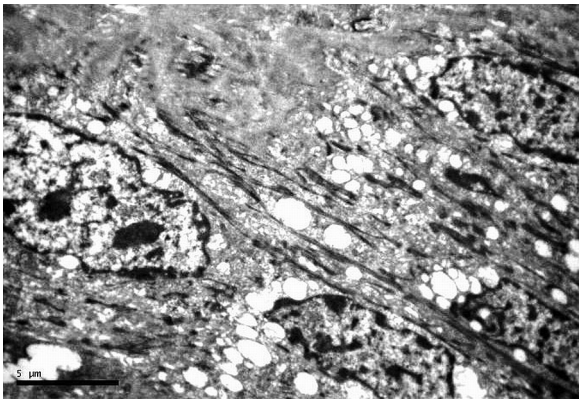

Figure 7: Electron Microscopy image that shows profound degeneration of epithelial cells in the rat tongue, moderate interepithelial junction destruction, lipid droplets formation, mitochondrial swelling, in 18-month old rats (Group C) that did not receive L-carnitine.

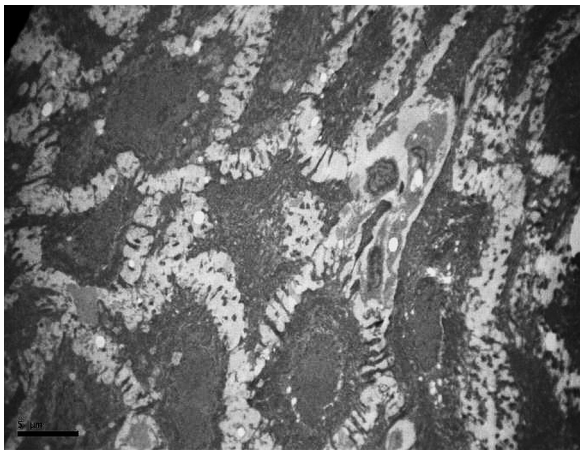

Figure 8: Electron Microscopy image that shows moderate interepithelial junction destruction in the rat tongue, in 18-month old rats (Group C) that received L-carnitine.

Controls from Group B (B-NLC) revealed destruction of interepithelial junctions, interepithelial edema and profound degeneration (Figure 3). In this age group, alterations of the nuclear morphology of the epithelial cells provided evidence of the gradual accumulation of atypical cells during aging (Figure 4). Carnitine administration resulted in partial regeneration in samples of experimental group B (B-LC). Moderate mitochondrial degeneration and an overall maintenance of the interepithelial junctions were present (Figures 5, 6). Partial mitochondrial cristae preservation was also counted as no cristae preservation.

Controls from Group C (C-NLC), revealed profound interepithelial edema and mitochondrial degeneration (Figure 7). Experimental Group C (C-LC), similarly to Group B-LC, revealed moderate mitochondrial degeneration and a partial only destruction of the interepithelial junctions (Figure 8). Only samples from animal Nr. 9 did not reveal any regeneration after L-carnitine administration.

No sample revealed intramitochondrial vacuole formation.

Discussion

Original studies that investigate the effects of L-carnitine on epithelial cells have not been conducted, yet. However, studies exist that report on the effects of L-carnitine on the endothelium. In particular, attention was paid on this action after the first reports on the protective effect of L-carnitine against vascular atherosclerosis and endothelial cell dysfunction[10]. However, the mechanism remains unclear. Elevated levels of free fatty acids (FFA) that reduce the concentration of the cytoplasmic free coenzyme A and increase the production of arachidonic acid metabolites, have been linked to impaired endothelial function[11]. This commences 2 to 3 hours after the experimental rise of FFA plasma levels[10]. L-carnitine administration restores the dysregulation of the endothelium, probably by reducing the FFA plasma levels, that is the result of the improvement of insulin resistance after carnitine administration[10,12]. Another possible mechanism for the attenuation of endothelial cell dysfunction is related to the enhancement of endothelium-derived NO production, that could explain the partial restoration of the endoneurial blood flow caused by L-carnitine in diabetic rats[13]. However, there has been a lot of controversy regarding this issue, in view of the divergent results on the relationship between carnitine deficiency and glycose intolerance[14].

Protective effects of L-carnitine on renal epithelial cells were reported by Ozsoy et al[15]. They found only moderate damage to the renal epithelium in rats receiving a combination of lead acetate and L-carnitine, in contrast to those that were given only lead acetate[15]. Similar protective effects were detected in the germinal epithelium of the seminiferous tubules in rat testes, after treatment with etoposide[16]. This may be an important therapeutic approach in order to preserve fertility.

Degenerative changes in the aging rat tongue have not been presented in the literature, yet. Our study reveals for the first time the gradual accumulation of degenerative changes in the epithelial cells of rat tongue. 5-month old rats, already showed signs of degeneration, mostly moderate destruction of interepithelial junctions and lipid droplets formation. Carnitine administration achieved complete remission of these alterations. The other two age groups (12- and 18- month old rats), revealed profound degeneration in the control group, in particular interepithelial edema, lipid droplets formation, atypical nuclear morphology, mitochondrial swelling and no cristae preservation were present. Carnitine resulted into partial regeneration and samples showed moderate degeneration mainly characterized by partial cristae preservation, lipid droplets formation and moderate interepithelial junction destruction. However, in the experimental Group C, sections from animal Nr. 9 did not show signs of regeneration.

These results show that carnitine administration may result in complete regeneration of epithelial cells with moderate degeneration as it was described in our study. However, even cells with profound degeneration may benefit from carnitine administration, because a partial regeneration as it was presented here, may be beneficial for cell metabolism and survival.

The first studies that investigated the effects of carnitine on cell degeneration aimed at investigating possible protective effects on the cardiac muscle. L-propionylcarnitine was reported to restore contraction of myocardial muscle cells and of cardiac output after ischemia in dog myocardium[17]. A very important effect of carnitine administration on the heart muscle is in case of congestive heart failure (CHF), where attenuation of myocyte apoptosis, caused by the reduction of proapoptotic caspases and TNF-a commences[18].

The protective effect of carnitine on the cardiovascular system is multidimensional and involves: anti-oxidative and anti-inflammatory actions, through the release of NO by endothelial cells[19], decrease in the concentration of FFAs, because of the enhancement of metabolism[10] and vasodilatory effects as a result of the synthesis of ei-

ther prostaglandins or arachidonic acid[20,21].

It is still unclear, whether carnitine possesses anti-atherosclerotic properties. In a previous study, we showed that carnitine did not alter CD4 (used as a marker of early atherosclerotic changes) staining in intimal lesions in aged rats after carnitine administration[22].

Only a few reports exist that investigate the effects on other striated muscles. Carnitine administration was reported to result into enhancement of mitochondrial enzymatic activity in muscle cells, restoration of L-carnitine levels and reduction of abdominal fat mass[23,24].

The discovery of the reduction of muscle strength in postmenopausal women gave rise to investigations on the possible protective role of carnitine on muscles. In animal models of menopause, carnitine was proven to lessen the degeneration of the quadriceps femoris muscle[25].

Our study provides for the first time- to our knowledge- evidence of the protective effect of L-carnitine against epithelial cell degeneration in the aging rat tongue. Limitations as well as possible flaws in the design that must be taken into account include: 1) the intraperitoneal administration of L-carnitine, that may achieve concentrations that are not equal to those after oral administration, 2) the small number of animals used in the study, 3) the assessment of mitochondrial degeneration only by investigating changes in morphology using electron microscopy and not according to mitochondrial enzyme activities that could be investigated with functional assays, 4) the preparation of the samples for electron microscopy could have caused morphological and dimensional changes to mitochondria that could have been attributed to degenerative changes, 5) differences between animals and humans regarding mitochondrial degeneration and activity may not enable extrapolation of the results to humans, 6) the extrapolation of the results of a small tongue section to the whole tongue may be prone to errors.

To conclude, our findings reveal for the first time the profound degeneration of the epithelial cells of the tongue during aging. This process starts early in life and lesions accumulate while aging. L-carnitine administration was shown to completely eliminate the degenerative changes when administered to young rats, while in the older rats regeneration was only partial for epithelial cells.

Conflict of Interest

None declared by Authors.

References

1. Vaz FM, Wanders RJ. Carnitine biosynthesis in mammals. Biochem. 2002; 361: 417-429.
2. Rebouche CJ. Carnitine function and requirements during the life cycle. FASEB J. 1992; 6: 3379-3386.
3. Fritz IB, Marquis NR. The role of acylcarnitine esters and carnitine palmityltransferase in the transport of fatty acyl groups across mitochondrial membranes. Proc Natl Acad Sci U S A. 1965; 54: 1226-1233.
4. Rebouche CJ, Engel AG. Kinetic compartmental analysis of carnitine metabolism in the human carnitine deficiency syndromes. Evidence for alterations in tissue carnitine transport. J Clin Invest. 1984; 73: 857-867.
5. Rebouche CJ, Engel AG. Tissue distribution of carnitine biosynthetic enzymes in man. Biochim Biophys Acta. 1980; 630: 22-29.
6. Engel AG, Rebouche CJ. Carnitine metabolism and inborn errors. J Inherit Metab Dis. 1984; 7 Suppl 1: 38-43.
7. Brass EP. Pharmacokinetic considerations for the therapeutic use of carnitine in hemodialysis patients. Clin Ther. 1995; 17: 176-185, discussion 175.
8. Martinuzzi A, Vergani L, Rosa M, Angelini C. L-carnitine uptake in differentiating human cultured muscle. Biochim Biophys Acta. 1991; 1095: 217-222.
9. Ferrari R, Merli E, Cicchitelli G, Mele D, Fucili A, Ceconi C. Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. Ann N Y Acad Sci. 2004; 1033: 79-91.
10. Shankar SS, Mirzamohammadi B, Walsh JP, Steinberg HO. L-carnitine may attenuate free fatty acid-induced endothelial dysfunction. Ann N Y Acad Sci. 2004; 1033: 189-197.
11. Reddy TS, Bazan NG. Kinetic properties of arachidonoyl-coenzyme A synthetase in rat brain microsomes. Arch Biochem Biophys. 1983; 226: 125-133.
12. Capaldo B, Napoli R, Di Bonito P, Albano G, Saccà L. Carnitine improves peripheral glucose disposal in non-insulin-dependent diabetic patients. Diabetes Res Clin Pract. 1991; 14: 191-195.
13. Cameron NE, Cotter MA. Neurovascular effects of L-carnitine treatment in diabetic rats. Eur J Pharmacol. 1997; 319: 239-244.
14. Ringseis R, Keller J, Eder K. Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency. Eur J Nutr. 2012; 51: 1-18.
15. Ozsoy SY, Ozsoy B, Ozyildiz Z, Aytekin I. Protective effect of L-carnitine on experimental lead toxicity in rats: a clinical, histopathological and immunohistochemical study. Biotech Histochem. 2011; 86: 436-443.
16. Okada FK, Stumpp T, Miraglia SM. Carnitine reduces testicular damage in rats treated with etoposide in the prepubertal phase. Cell Tissue Res. 2009; 337: 269-280.
17. Paulson DJ, Traxler J, Schmidt M, Noonan J, Shug AL. Protection of the ischemic myocardium by L-propionylcarnitine: effects on the recovery of cardiac output after ischemia and reperfusion, carnitine transport and fatty acid oxidation. Cardiovasc Res. 1986; 20: 536-541.
18. Vescovo G, Ravara B, Gobbo V, Sandri M, Angelini A, Della Barbera M, et al. L-Carnitine: a potential treatment for blocking apoptosis and preventing skeletal muscle myopathy in heart failure. Am J Physiol Cell Physiol. 2002; 283: C802-C810.
19. Calò LA, Pagnin E, Davis PA, Semplicini A, Nicolai R, Calvani M, et al. Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. Int J Cardiol. 2006; 107: 54-60.
20. Bertelli A, Bertelli AA, Galmozzi G, Giovannini L, Mian M. Thrombosis induced by endothelin (ET-1) and carrageenin in rats treated with indomethacin and propionyl carnitine. Drugs Exp Clin Res. 1999; 19: 75-78.
21. Christopherson BO, Norseth J. Arachidonic acid synthesis studied in isolated liver cells:" effect of (-)-carnitine and of (+)-decanoylcarnitine. FEBS Lett. 1981; 133: 201-204.
22. Papamitsou T, Barlagiannis D, Dietrich EM, Koumourtzis M, Batziou N, Papaioannidou P, et al. Histological investigation of the effect of L-carnitine on rat aorta. Basic Clin Pharmacol Toxicol. 2011; 109 Suppl 1: 105.
23. Karanth J, Jeevaratnam K. Effect of carnitine supplementation on mitochondrial enzymes in liver and skeletal muscle of rat after dietary lipid manipulation and physical activity. Indian J Exp Biol. 2010; 48: 503-510.
24. Bernard A, Rigault C, Mazue F, Le Borgne F, Demarquoy J. L-carnitine supplementation and physical exercise restore age-associated decline in some mitochondrial functions in the rat. J Gerontol A Biol Sci Med Sci. 2008; 63: 1027-1033.
25. Moustafa AM, Boshra V. The possible role of L-carnitine on the skeletal muscle of ovariectomized rats. J Mol Histol. 2011; 42: 217-225.