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Effect of chronic L-carnitine supplementation on carnitine levels, oxidative stress and apoptotic markers in peripheral organs of adult Wistar rats

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ABSTRACT

This study investigated the effects of L-carnitine supplementation on carnitine levels, oxidative stress and apoptotic markers in the stomach, kidney, liver and testis tissues in adult rats. Rats were randomized to control and L-carnitine supplemented (LCAR) groups. Control group received distilled water for 7 months by intragastric gavage and the LCAR group was given 50 mg/kg/day L-carnitine via intragastric intubation for the same period. L-carnitine concentrations and caspase-3 activity were measured by fluorometric methods while cleaved caspase-3 was determined by Western blot analysis. Bcl-2 associated X protein (Bax) and B-cell lymphoma/leukemia-2 (Bcl-2) were quantified by enzyme immunoassay and Western blot analysis. Oxygen/nitrogen species (ROS/RNS) and total antioxidant capacity (TAC) were analyzed by colorimetric assay. Tissue L-carnitine concentrations were significantly increased in the LCAR group compared to controls. Anti-apoptotic Bcl-2 levels were significantly increased while pro-apoptotic Bax was significantly decreased in LCAR group rats compared to controls. Tissue caspase-3 was significantly alleviated in the LCAR group compared to controls. L-carnitine supplementation increased TAC and decreased ROS/RNS generation in the kidney, liver, stomach and testis tissues compared to controls. Obtained data suggests that L-carnitine supplementation can potentially be used to lessen both oxidative and apoptotic progression in peripheral organs.

1. Introduction

Carnitine (3-hydroxy-4-N-trimethyl-ammonio-butanoate) is a quaternary ammonium compound biosynthesized from lysine and methionine. It is required for fatty acid transport into the mitochondrial matrix via the carnitine/acylcarnitine shuttle, where β-oxidation occurs. Acetate is generated and utilized in the tricarboxylic acid (TCA) cycle for the generation of energy (Adeva-Andany et al., 2017). In mammals, consumed L-carnitine is taken in from the small intestine by passive diffusion and active transport (Bremer, 1983). Once absorbed, L-carnitine is integrated into the body carnitine pool which is composed of uncharged L-carnitine and acylcarnitines known as short chain carnitine esters. L-carnitine can also be produced in the liver and kidneys via methylation of L-lysine (Rebouche, 1992). Red meat is a rich source of lysine and thus a stringent vegetarian diet may lead to L-carnitine deficiency (Rebouche, 1992). L-carnitine can buildup in liver, skeletal muscle, heart, brain and testis (Rebouche, 1992).

Carnitines are considered as conditionally-essential nutrients (Post et al., 2019) and their ingestion is generally regarded as an effective way to support endurance and shorten post-workout recovery (Kendler, 2006). In peripheral tissues, it facilitates β-oxidation by transporting medium and long chain fatty acids into the mitochondria (Mingorance et al., 2011). L-carnitine is also important in preserving cell membrane stability through its participation in acetylation of membrane phospholipids and amphiphilic actions (Adeva-Andany et al., 2017). Recent studies have observed that L-carnitine has antioxidant properties and may protect cells from toxic reactive oxygen species in some metabolic disorders (Mescka et al., 2015).

It is widely accepted that oxidative stress caused by formation of free radicals and oxidants is balanced by antioxidant defense

Abbreviations: Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma/leukemia-2; DCF, dichlorodihydrofluorescein; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase; LCAR, L-carnitine supplemented; MPT, mitochondrial permeability transition; NO, nitric oxide; ONOO-, peroxynitrite anion; ROO, peroxyl radical; ROS, reactive oxygen species; ROS/RNS, Oxygen/nitrogen species; TAC, total antioxidant capacity; TCA, tricarboxylic acid

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mechanisms that eliminate ROS and reduce oxidative cell injury (Tan et al., 2018). Disorders in systemic and intracellular signals result in apoptosis (Tower, 2015). A number of cysteine proteases, called caspases, are activated in sequence during apoptosis (Shalini et al., 2015). Oxidative stress is an important regulator of apoptosis which can be induced by two major pathways. The extrinsic pathway involves binding of TNF-α and Fas ligand to membrane receptors leading to caspase-8 activation, while the intrinsic pathway participates in stress-induced mitochondrial cytochrome *c* release (Westphal et al., 2014).

Antioxidants obtained as nutritional supplements can neutralize ROS stress and protect against apoptosis (He et al., 2017). L-carnitine scavenges free oxygen radicals and also acts as a protective agent against mitochondria (Vanella et al., 2000). This study has evaluated the effects of chronic L-carnitine supplementation on oxidative stress and apoptotic markers in peripheral organs of adult rats.

2. Experimental procedures

2.1. Animals

All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University Medical School. Male Wistar rats weighing 350–450 g were housed in stainless steel cages and given food and water ad libitum. Animals were maintained at 12 h light-dark cycles and a constant temperature of 23 ± 1 °C at all times. Twenty male Wistar rats, aged 3 months were randomized to control and LCAR groups. Vials containing 1 g L-carnitine (Sigma-tau, Utrecht, Netherlands) were diluted in distilled water and the LCAR group was given 50 mg/kg/day L-carnitine via intragastric intubation for 7 months. The control group received distilled water for the same period by intragastric gavage. At the end of the 7 month treatment period, rats were anesthetized intraperitoneally with a mixture of ketamine (100 mg/kg, Richter Pharma AG, Wels, Austria) and xylazine hydrochloride (10 mg/kg, Bioveta, Czech Republic). An incision on the abdominal skin was performed to expose the abdominal cavity. Animals were sacrificed by exsanguination via the abdominal aorta. Dissected liver, kidney stomach and testis were flash frozen in liquid nitrogen and stored at −80 °C until analyzed.

2.2. Tissue measurement of l-carnitine

Tissue L-carnitine levels were measured by a fluorometric assay kit (BioVision catalog #K642-100; CA, USA). Tissues were homogenized in assay buffer supplied with the kit and centrifuged to 13,000 g for 10 min. Supernatants were transferred to an ultrafiltration unit and centrifuged through a 10-kDa molecular mass cut-off filter (Amicon, Millipore Corporation, Bedford, MA) to remove protein. The utilized method measures free L-carnitine by transferring an acetyl group from CoA to carnitine and the free CoA formed is further processed with subsequent oxidation of the Oxi-Red probe to give fluorescence (Ex/Em 535/587 nm). Background control measurements were performed for each sample and the measured values were subtracted from the sample reading to correct for levels of acyl-CoA or free Coenzyme A in the sample. The net fluorescence values were compared with a known carnitine standard curve. Results were expressed as nmol L-carnitine per gram tissue protein.

2.3. Enzyme immunoassay for measurement of Bcl-2 and Bax

Tissue Bcl-2 and Bax protein was measured by a commercial enzyme-linked immunosorbent assay (ELISA) test kit (YL Biotech; Bcl-2 catalog #YLA0086RA, Bax catalog #YLA0122RA, Shanghai, China). A standard curve of absorbance values of known Bcl-2 and Bax standards was plotted as a function of the logarithm of standard concentrations (μg/ml) using the GraphPad Prism Software program for windows version 5,03. (GraphPad Software Inc). Bcl-2 and Bax concentrations in the samples were calculated from their corresponding absorbance values via the standard curve. Results were expressed as ng per mg tissue protein.

2.4. Caspase-3 activity

Caspase-3 activity in tissues was measured via a fluorometric assay kit (Abcam, catalog # ab39383, Cambridge, UK). Tissues were homogenized in lysis buffer supplied with the kit and incubated on ice for 10 min. After centrifugation for 20 min at 10,000 g, supernatants were transferred to clean tubes and assayed for caspase activity based on fluorometric detection (Ex/Em 400/505 nm) of free 7-amino-4-trifluoromethyl coumarin (AFC). Fold increase in caspase 3 activity was determined by comparing with control and enzyme activity was defined as % of control.

2.5. Western blot analysis

Tissues were homogenized in 2 ml ice-cold homogenizing buffer (50 mM K_2HPO_4, 80 μM leupeptin (Sigma-Aldrich, Steinheim, Germany), 2.1 mM Pefabloc SC (SERVA, Heidelberg, Germany), 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich), 1 μg/ml aprotinin (SERVA; pH 7.4). Homogenates were centrifuged (40,000 g, 30 min, 4 °C) and supernatants were stored at −80 °C until analyzed. Proteins were denatured at 100 °C in sample buffer (BioRad Laboratories Inc. USA) and separated on 12% miniprotean TGX precast electrophoresis gels (BioRad Laboratories Inc. USA). Resolved proteins were transferred to nitrocellulose membranes and incubated with either rabbit polyclonal anti-Bcl-2 antibody (1/1000 dilution Abcam, # ab59348 Cambridge, UK); rabbit polyclonal anti-Bax antibody (1/1000 dilution Abcam, # ab53154 Cambridge, UK); rabbit monoclonal cleaved caspase-3 antibody (1/1000 dilution #9664, Cell SignalingTechnology, Danvers, MA, USA) and anti-actin (1:1000, #AANO1, Cytoskeleton Inc. Denver, CO, USA). Primary antibody incubations were for 1 h at room temperature. Horseradish peroxidase–conjugated goat anti-rabbit IgG (1:10,000 dilution; Zymed Laboratories, San Francisco, CA) was used as a secondary antibody, and immunoreactive proteins were visualized by chemiluminescence via ECL reagent (Amersham Pharmacia Biotech, Buckinghamshire, UK). All Western blots were quantified by densitometric analysis using NIH ImageJ 1.44p software.

2.6. Total antioxidant capacity measurement

Tissue samples were homogenized in cold PBS and centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatants were stored at −80 °C for protein determination and TAC assay. Total antioxidant capacity was measured by OxiSelect Total Antioxidant Capacity Assay Kit (Cell Biolabs, Inc. San Diego,CA, USA). The utilized method is based on the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with an absorbance at 490 nm. The net absorbance values of antioxidants were compared with a known uric acid standard curve. Results were expressed as mM uric acid equivalents (UAE) per g/ml tissue protein.

2.7. Reactive oxygen and nitrogen species measurement

Tissue samples were homogenized in cold PBS (10–50 mg/mL) and centrifuged at 10,000 × *g* for 5 min. The supernatants were stored at −80 °C for protein determination and ROS/RNS assay. Reactive oxygen species and reactive nitrogen species were measured by OxiSelect *in vitro* ROS/RNS assay kit (Cell Biolabs, Inc. San Diego,CA, USA). The method uses the fluorogenic probe, dichlorodihydrofluorescin DiOxyQ (DCFH-DiOxyQ), which is a specific ROS/RNS probe. The DCFH-DiOxyQ probe can react with hydrogen peroxide (H_2O_2), peroxyl

radical (ROO·), nitric oxide (NO), and peroxynitrite anion (ONOO-). These free radical molecules are representative of both ROS and RNS, thus allowing for measurement of the total free radical population within a sample. Samples were measured fluorometrically against a DCF standard by using a fluorescence microplate reader (Synergy Mx, Bio-Tek Instruments Inc. Vermont, USA). The free radical content in unknown samples was determined by comparison with the pre-determined DCF standard curve and expressed as DCF nM per mg/ml tissue protein.

2.8. Protein measurements

Protein concentrations were measured at 595 nm by a modified Bradford assay using Coomassie Plus reagent with bovine serum albumin as a standard (Pierce, Thermo Fisher Scientific, Roskilde, Denmark).

2.9. Statistical analysis

Statistical analysis was performed using SigmaStat statistical software version 2.0 (Sigma, St. Louis, MO, USA). Statistical analysis for each measurement is described in figure and table legends. Statistical analysis for each measurement is described in figure and table legends. To compare the groups via the SigmaStat statistical sofware, we first performed a normality test. A test that passed indicated that the data matched the pattern expected if the data was drawn from a population with a normal distribution. If the sample data were not normally distributed, the normality test failed. In such case the software performed a nonparametric test.

3. Results

3.1. Changes in body weight

Body weight was analyzed before and after the experimental period of 7 months. Changes in body weight within each group before and after the experimental period was analyzed by Paired *t*-test. There was a significant increase in body weight after 7 months in both control and LCAR groups (Fig. 1). Statistical analysis of body weight between control and LCAR groups before and after the experimental period were analyzed by *t*-test. No significant difference was found in body weight among the experimental groups before and after 7 months.

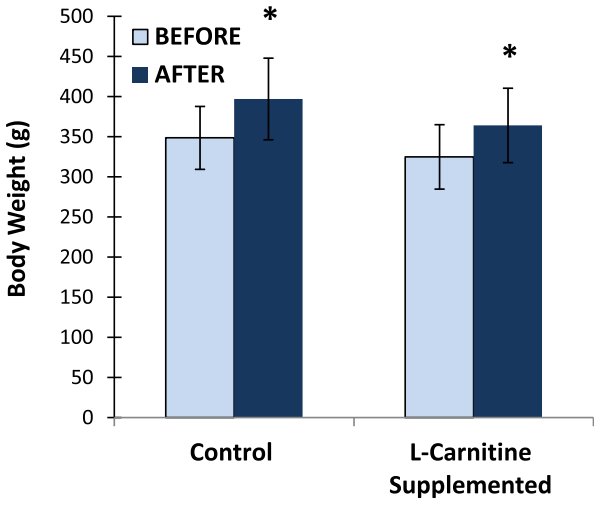

Fig. 1. Changes in body weight during the treatment period. Data are mean ± SD (n = 10). Body weights were measured before and after the experimental period. *, p < 0,001, vs. before the experimental period within each group. Statistical analysis was done by paired-t-test.

Table 1
L-Carnitine concentrations in rat peripheral organs.

L-Carnitine (nmol/g protein)	Control (*10*)	L-Carnitine Supplemented (*10*)
Liver	26,15 ± 8,39	66,80 ± 18,30*
Kidney	75,85 ± 11,39	108,40 ± 15,60*
Stomach	108,06 ± 17,49	181,83 ± 33,71*
Testis	21,54 ± 5,94	61,74 ± 14,50*

Values are mean ± SD. n for each measurement is italicized in parentheses. Statistical analysis was done by *t*-test in liver, kidney and stomach and by Mann-Whitney Rank Sum Test in testis measurements. *, p < 0.001.

3.2. L-carnitine levels

L-carnitine levels in rat peripheral organs are shown in Table 1. A significant increase was observed in tissue L-carnitine levels in the L-carnitine supplemented group compared to control.

3.3. Bcl-2 levels

Bar graph data of tissue Bcl-2 levels are shown in Fig. 2A. Bcl-2 protein (mean ± SD) measured in liver, kidney, stomach and testis (n = 10) was significantly increased in L-carnitine supplemented rats compared to controls. Liver values in LCAR group rats vs. controls was 172,25 ± 29,32 vs. 130,75 ± 14,53 ng/mg protein, respectively. Kidney values in LCAR group vs. control was 206,14 ± 13,82 vs. 159,26 ± 27,55 ng/mg protein, respectively. Stomach values in LCAR group vs. control was 506,72 ± 69,48 vs. 348,39 ± 41,23 ng/mg protein, respectively. Testis values in LCAR group vs. control was 301,07 ± 41,11 vs. 225,93 ± 54,43 ng/mg protein, respectively. Western blot analysis of Bcl-2 protein confirmed ELISA measurements. L-carnitine supplementation significantly increased Bcl-2 levels in liver, kidney, stomach and testis as shown in Fig. 3A and 3B.

3.4. Bax levels

Bar graph data of tissue Bax levels are shown in Fig. 2B. Bax protein (mean ± SD) measured in liver, kidney, stomach and testis (n = 10) was significantly decreased in L-carnitine supplemented rats compared to controls. Liver values in LCAR group rats vs. controls was 151,69 ± 14,41 vs. 204,17 ± 58,51 ng/mg protein, respectively. Kidney values in LCAR group vs. control was 179,85 ± 44,97 vs. 265,48 ± 29,08 ng/mg protein, respectively. Stomach values in LCAR group vs. control was 402,59 ± 64,88 vs. 670,50 ± 109,67 ng/mg protein, respectively. Testis values in LCAR group vs. control was 340,14 ± 30,96 vs. 445,52 ± 90,74 ng/mg protein, respectively. Western blot analysis of Bax protein confirmed ELISA measurements. L-carnitine supplementation significantly decreased Bax levels in liver, kidney, stomach and testis as shown in Fig. 3A and C.

3.5. Caspase-3 activity

A significant decrease was found in caspase-3 activity in L-carnitine supplemented rats compared to controls (Fig. 2C). Western blot analysis of cleaved caspase-3 confirmed activity measurements. L-carnitine supplementation significantly decreased cleaved caspase-3 protein in liver, kidney, stomach and testis as shown in Fig. 3A and D.

3.6. Total antioxsidant capacity and ROS/RNS

L-carnitine supplementation significantly increased total

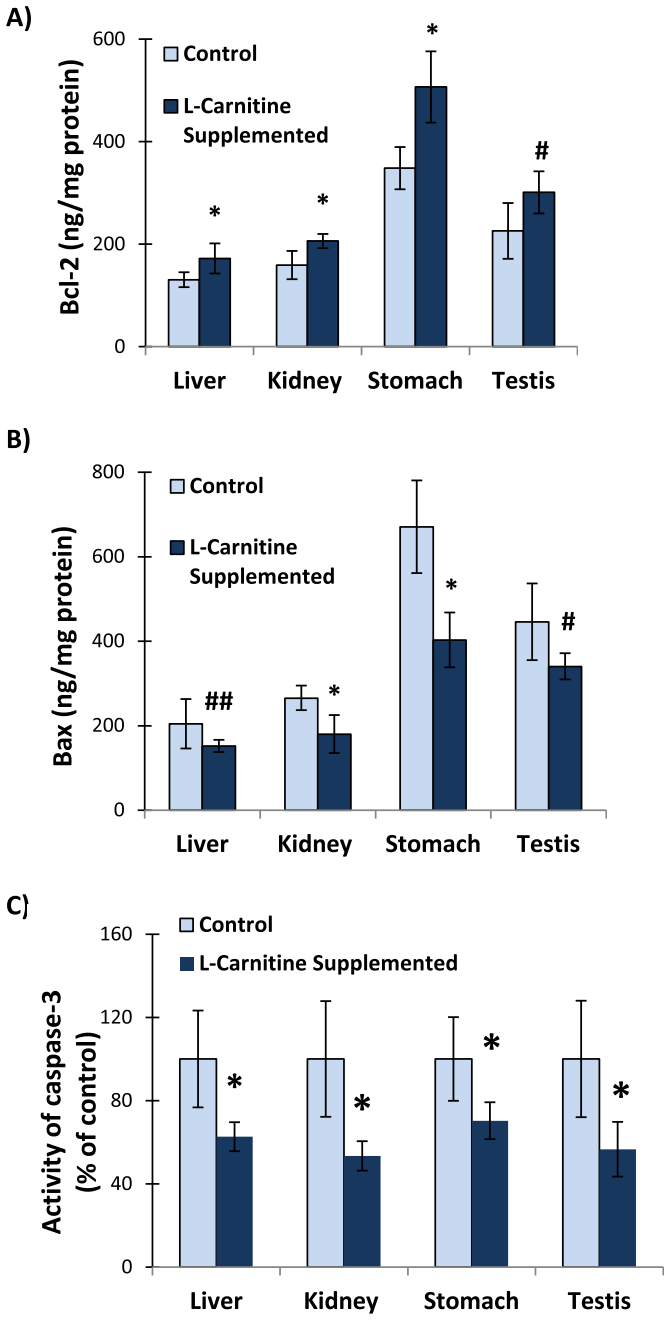

Fig. 2. Apoptotic markers in rat peripheral organs. A) Bcl-2 levels. Values are mean ± SD. n = 10 for each group. Statistical analysis was done by *t*-test. #, p = 0.003 and *, p < 0.001 vs. control. B) Bax levels. Values are mean ± SD. n = 10 for each group. Statistics was done by *t*-test in kidney, stomach, testis analysis and by Mann-Whitney Rank Sum Test in liver analysis. #, p = 0.003, ##p = 0.002 and *,p < 0.001 vs. control. C) Caspase 3 activity. Values are mean ± SD. n = 10 for each group. Statistics was done by *t*-test in testis and by Mann-Whitney Rank Sum Test in liver, kidney and stomach. *,p < 0.001 vs. control.

antioxidant capacity in liver, kidney, stomach and testis compared to controls (Fig. 4A). Total ROS and RNS in liver, kidney, stomach and testis was significantly decreased in L-carnitine supplementation compared to controls. (Fig. 4B).

4. Discussion

The LCAR group was given 50 mg/kg/day L-carnitine via intragastric intubation for 7 months. In a 50 kg human this dose would be compatible to 2500 mg per day. In one review of L-carnitine's safety, it was reported that the observed safe level (OSL) or highest observed intake (HOI) risk assessment method indicated that the evidence of safety is strong at intakes up to 2000 mg/day L-carnitine equivalents for chronic supplementation. Although much higher levels have been tested without adverse effects and may be safe, the data for intakes above 2000 mg/day are not sufficient for a confident conclusion of long-term safety (Hathcock and Shao, 2006).

For most humans, 2000 mg L-carnitine or less per day is reported to be comparatively safe and free from any serious side effects. In one study, people who took 3000 mg of L-carnitine L-tartrate every day for 21 days experienced no negative effects (Rubin et al., 2001). Serum samples were analyzed for markers of liver and renal function along with various minerals and electrolytes. In addition, whole blood was analyzed for a complete blood count. It was determined that there were no statistically significant differences between the L-carnitine and the placebo conditions for any of the variables examined. The results of this study suggested that L-carnitine when used as a dietary supplement, has no adverse effects on metabolic and hematological safety variables in normally healthy men.

Studies have shown that L-carnitine levels tend to decline with age and that older adults may benefit from L-carnitine supplementation (Nagai et al., 2017). In one study, 2000 mg of L-carnitine reduced fatigue and increased muscle function in older adults (Pistone et al., 2003). Other research reveals that 2000 mg acetyl-L-carnitine may also help boost brain health and function as we age (Malaguarnera et al., 2007). However, it is important to mention that L-carnitine supplements may raise blood levels of trimethylamine-N-oxide (TMAO) over time (Vallance et al., 2018). High levels of TMAO are linked to an increased risk of atherosclerosis (Koeth et al., 2013). More studies on the safety of L-carnitine supplements are needed.

Rats did not show a positive effect of L-carnitine supplementation on weight loss. Although the cellular mechanism of L-carnitine suggests it could benefit weight loss, its effects, if present at all, are small. The results of L-carnitine supplementation in both human and animal studies have shown that it does not aid weight loss. Rats endogenous carnitine synthesis was reported to be adequate to ensure efficient beta-oxidation of fatty acids during the catabolic phase (Brandsch and Eder, 2002). In an eight-week study in 38 women who exercised four times per week, there was no difference in weight loss between those who took L-carnitine and those who didn't (Villani et al., 2000). Another human study monitored L-carnitine's effect on fat burning during a 90-min stationary bicycle workout. Four weeks of taking supplements did not increase fat burning (Broad et al., 2005). More research is needed to confirm the benefits of L-carnitine in older population.

We have seen a significant increase in liver, kidney, stomach and testis L-carnitine levels in rats following long term L-carnitine supplementation. Our observation is in agreement with a previous study which has shown that long term L-carnitine supplementation restores decreased tissue carnitine levels in 22 months old rats (Tanaka et al., 2004).

Bcl-2 and Bax proteins are involved in regulating the mitochondrial permeability transition (MPT) pore complex. Bax can open the MPT pore resulting in the release of cytochrome *c* into the cytosol, while Bcl-2 can stabilize and inhibit the opening of the MPT pore and protect against cytochrome *c*-release. Consequently, the ratio of anti-apoptotic Bcl-2 to proapoptotic Bax can show the susceptibility of cells to apoptosis by regulating mitochondrial integrity and preventing the apoptotic cascade activation (Wu and Bratton, 2013). Our results showed that L-carnitine supplementation in rats can upregulate Bcl-2 and downregulate Bax protein compared with non-treated controls. Our findings are in agreement with previous studies that have shown similar effects of L-carnitine (Keshavarz-Bahaghighat et al., 2018; Tousson et al., 2014). Due to the role of Bcl-2 and Bax proteins in the apoptotic cascade, treatment with L-carnitine can inhibit downstream apoptotic

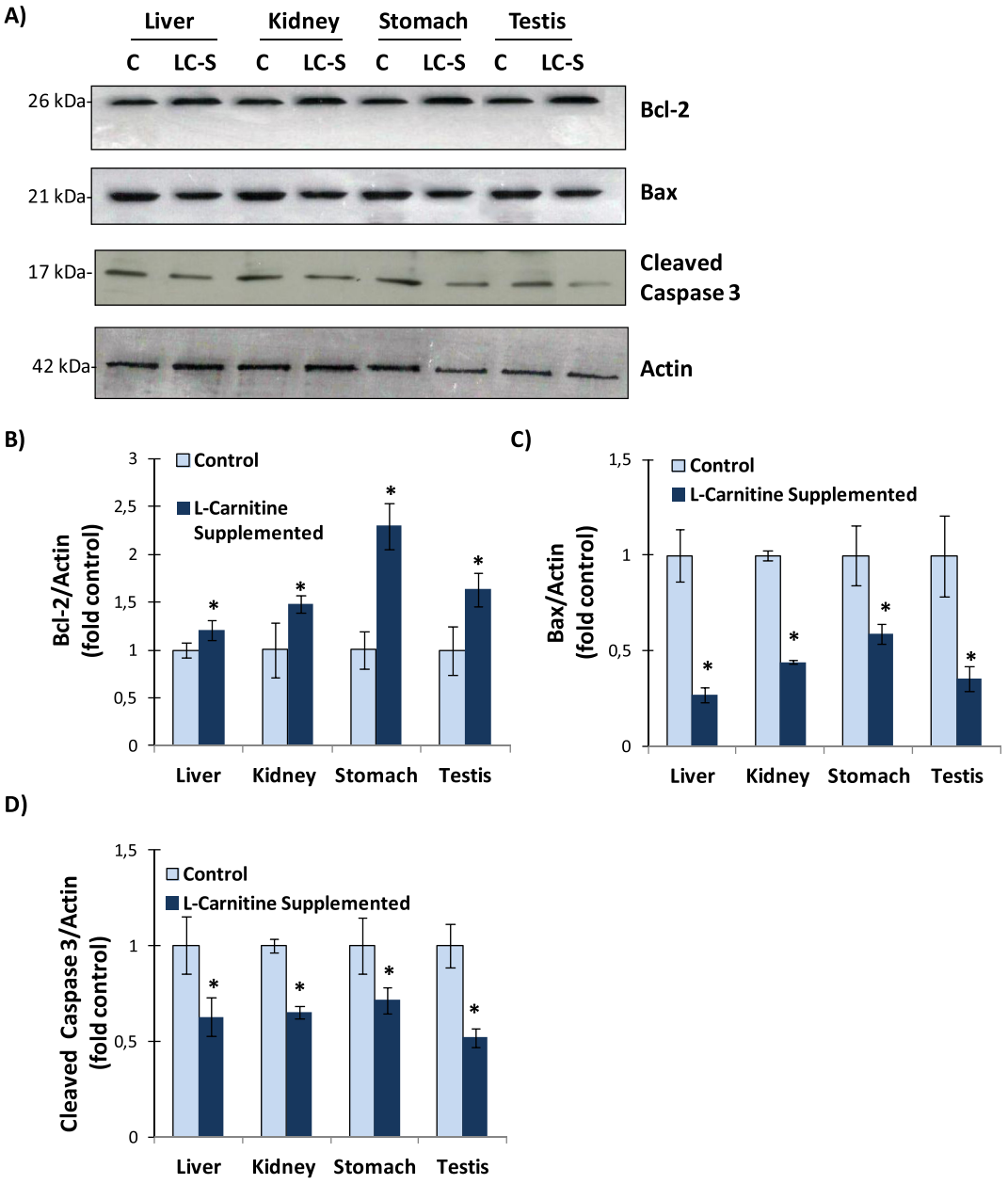

Fig. 3. A) Representative western immunoblots for Bcl-2, Bax and cleaved caspase-3 in the liver, kidney, stomach and testis tissues. C, control. LC-S, L-Carnitine supplemented. **B)** The band densities of Bcl-2 estimated by ImageJ software. Data shown are representative of 3 separate experiments and values are given as mean ± SD. Statistical analysis was performed by *t*-test. *, $p < 0.05$ vs. control of the same organ. **C)** The band densities of Bax estimated by ImageJ software. Data shown are representative of 3 separate experiments and values are given as mean ± SD. Statistical analysis was performed by *t*-test. *, $p < 0.05$ vs. control of the same organ. **D)** The band densities of cleaved caspase-3 estimated by ImageJ software. Data shown are representative of 3 separate experiments and values are given as mean ± SD. Statistical analysis was performed by *t*-test. *, $p < 0.05$ vs. control of the same organ.

signaling pathways and ultimately prevent cellular apoptosis. Our results support the hypothesis that the tissue protective effect of L-carnitine on apoptosis may be, at least partly, mediated by regulating the expression of Bax and Bcl-2.

Cytochrome *c* release from mitochondria is a critical point in the apoptotic cascade and activation of downstream caspases such as caspase-3 (Shalini et al., 2015). In the present study, the results showed that treatment with L-carnitine induced a significant decrease in caspase-3 activation and cleaved caspase-3 protein levels compared with untreated rats. Our results are in agreement with previous studies that have also shown that L-carnitine supressed caspase-3 activity (Ye et al., 2010; Xiang et al., 2013; Moosavi et al., 2016). It was previously shown that L-carnitine has a stabilizing activity on the outer mitochondrial membrane, which can prevent the efflux of cytochrome C into the cytosol (Di Cesare et al., 2007). Since caspase-3 can be activated by ROS, the suppressive effect of L-carnitine on caspase-3 activity suggests that the inhibitory effect could also be related to the antioxidant property and mitochondrial protection of L-carnitine.

We have shown that L-carnitine increases total antioxidant capacity in the kidney, liver, stomach and testis of adult rats. Our results are in agreement with previous studies that have shown that L-carnitine suppresses oxidative damage (Arockia and Panneerselvam, 2001). Used as a safe and efficient nutritional supplement, the protective effect of L-carnitine on renal, hepatic, testicular and stomach tissues has also been reported in models involving oxidative stress (Salmanoglu et al., 2016; Estaphan et al., 2015; Khushboo et al., 2018; Adefisayo et al., 2018). It

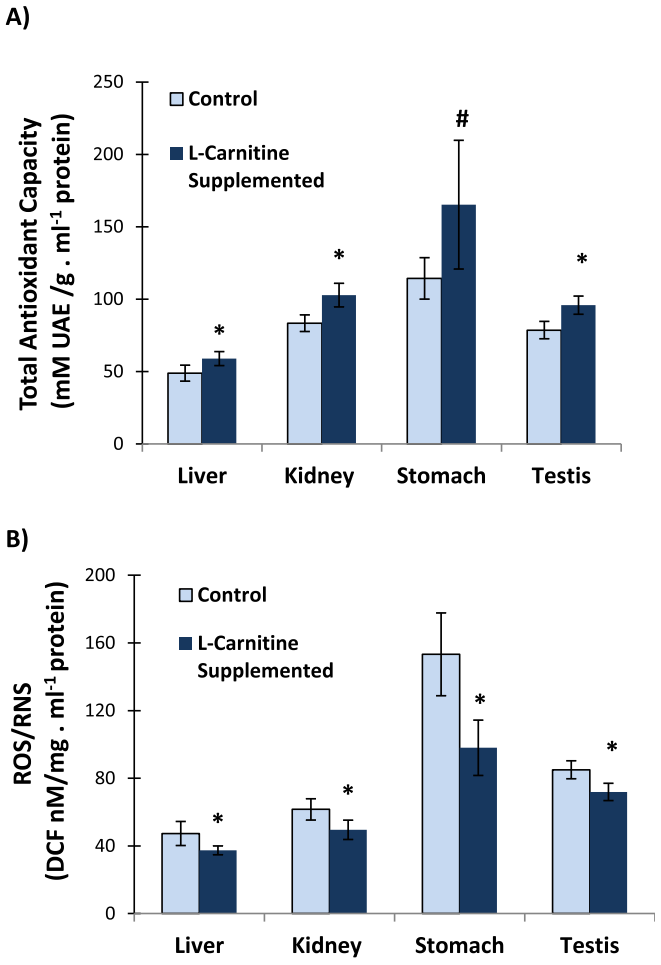

Fig. 4. Total antioxidant capacity and in rat peripheral organs. A) Total antioxidant capacity is reported as uric acid equivalent. Values are mean ± SD. n = 10 for each group. Statistical analysis was done by *t*-test. #, p = 0.003 and *, p < 0.001 vs. control. B) Total reactive oxygen species (ROS) and reactive nitrogen species (RNS). Values are mean ± SD. n = 10 for each group. Statistical analysis was done by *t*-test. *, p < 0.001 vs. control.

was shown that L-carnitine had an effective free radical scavenging activity compared to α-tocopherol and trolox as reference antioxidants (Gülçin, 2006). Carnitine can also act as a chelator by decreasing the concentration of redox activite cytosolic iron (Arockia and Panneerselvam, 2001). Knowing the antioxidant function of L-carnitine, we further examined the effect of L-carnitine on intracellular ROS production in the liver, kidney, stomach and testis tissues. We observed that L-carnitine supplementation caused an apparent decrease in intracellular ROS production compared to non treated controls.

In conclusion, we have provided evidence that the tissue protective effect of L-carnitine in adult rats were mediated, at least, through scavenging oxygen free radicals, enhancement of total antioxidant capacity, reduction of caspase-3 activity and regulation of apoptosis related protein expression of Bcl-2 and Bax. Our results show that the antioxidant effect may be a major mechanism for L-carnitine-mediated tissue protection.

Author contributions

S.E.K. conducted and designed the experiments as well as drafted the manuscript. E.A. carried out laboratory analysis of all measured parameters. G.A. carried out animal preparation and tissue dissections. B. D. carried out animal preparation. and tissue dissections. M.A. designed the experiments and contributed to the writing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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