



## Effects of L-carnitine on oxidative stress parameters in oophorectomized rats



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### KEYWORDS

L-carnitine;  
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**Abstract** *Aims:* The aim of this study was to investigate the antioxidant effects of L-carnitine on the oxidative stress parameters in oophorectomized rats.

*Methods:* Twenty-four female albino Wistar rats were used. Rats were divided into four groups: laparotomy-only (LO<sub>SALINE</sub>) group, oophorectomy plus L-carnitine 100 mg/kg/day (OX<sub>L100</sub>) group, oophorectomy plus L-carnitine 500 mg/kg/day (OX<sub>L500</sub>) group, and oophorectomy-only (OX<sub>SALINE</sub>) group. Experimental protocol was started on day 21 post-castration. Various dosage forms of L-carnitine or isotonic saline were administered intraperitoneally for 14 consecutive days. Nitric oxide (NO), malondialdehyde (MDA), total antioxidant status (TAS), total oxidative stress (TOS) and oxidative stress index (OSI) were evaluated in the tissues including kidney, liver and heart, and sera.

*Result(s):* In the heart tissue samples, there was no difference in the levels of NO, OSI and TOS between the groups. However, MDA levels in OX<sub>SALINE</sub> group were significantly higher than OX<sub>L500</sub> group. On the other hand, there was no statistically significant differences between the groups in terms of levels of NO, MDA, TAS, TOS and OSI in liver, kidney and sera samples.

*Conclusion(s):* Levels of MDA in the heart tissue were significantly higher in OX<sub>SALINE</sub> group compared to OX<sub>L500</sub> group. Thus, it may be suggested that L-carnitine reduces oxidative stress at least in the heart of oophorectomized rats.

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### 1. Introduction

Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. A variety of physiological changes occur in the body; some of

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them are related to cessation of ovarian functions, and others are because of the aging process.<sup>1</sup> It is also known that level of lipid peroxide, a marker of free-radical production and cell membrane lipid peroxidation (LPO), increases after menopause.<sup>2</sup> In various studies, it has been shown that the menopause impairs oxidative systems and causes increase in LPO.<sup>2,3</sup> LPO is a process mediated by free oxygen radicals. During this process, the polyunsaturated fatty acids that are the structure of phospholipid, glycolipid, glyceride or sterol present in cell membrane are converted into various noxious products such as peroxides, alcohols, aldehydes, and hydroxy fatty acids. One of the major end products of lipid peroxidation, is malondialdehyde (MDA).<sup>4</sup>

Oxygen-free radicals more generally are known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS), and one of them is also nitric oxide.<sup>4</sup> Harmful effects of oxidative stress are counteracted by the action of antioxidants. It is well known that various estrogens scavenge reactive oxygen species (ROS) efficiently in both aqueous and lipophilic cellular components.<sup>5</sup>

Furthermore, many *in vitro* and animal studies have reported that L-carnitine is a free radical scavenger, which protects antioxidant enzymes from oxidative damage.<sup>6</sup> L-carnitine is a quaternary amine containing seven carbon atoms with a molecular mass of 161.2 g/mol, water-soluble, small, highly polar compound which is widely distributed in nature.<sup>7</sup> Carnitine exists in two isomeric forms: L-carnitine and D-carnitine. Only the L-isomer of carnitine is metabolically active and synthesized primarily in liver also in the kidney. Tissues containing high levels of L-carnitine are unable to synthesize it. Tissues, other than liver and kidney, are dependent on the active uptake of carnitine from blood into tissues.<sup>8,9</sup> It has been reported that L-carnitine is a powerful antioxidant, and L-carnitine exerts its antioxidant effect by preventing LPO.<sup>10-12</sup>

In this study, we aimed at investigating the protective effects of L-carnitine against oxidative stress in various tissues at the surgically menopausal rats.

## 2. Material and method

This study was conducted in the Experimental Medical Research Unit (DETAB, Kırıkkale University Medical Faculty, Kırıkkale, Turkey). The study protocol was approved by Kırıkkale University Animal Research Ethical Committee on 22.08.2011 (Approval Number 11/8-11-210).

### 2.1. Animals

A total of 24 adult female Wistar albino rats weighing 200–250 g were used in the study. The rats were given *ad libitum* access to a standard rat chow and tap water, and were housed as a group per cage under standard controlled temperature (20–22 °C), humidity (40–60%), and light (12 h light/12 h dark) in a facility for each group. All animal procedures complied with the items of Helsinki Final Act (1986) related to experimental animals.

### 2.2. Study protocol

The rats were randomly allocated to 4 groups: laparotomy-only group (LO<sub>SALINE</sub> group; *n* = 6), oophorectomized-only

group (OX<sub>SALINE</sub> group; *n* = 6), oophorectomy plus 100 mg/kg/day L-carnitine group (OX<sub>L100</sub> group; *n* = 6) and oophorectomy plus 500 mg/kg/day L-carnitine group (OX<sub>L500</sub> group; *n* = 6). The rats were anaesthetized with intraperitoneal injections of ketamine 80 mg/kg (Ketalar<sup>®</sup>, 50 mg/ml vial; Pfizer Pharmaceuticals, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun<sup>®</sup>, 100 mg/ml vial; Bayer, Leverkusen, Germany) cocktail. All the interventions were performed under aseptic conditions and the rats were spontaneously breathing during surgical procedure. Surgery on each animal was performed through a ventral midline incision. No intervention other than incision was done in the SL group. The ovaries were exposed and bilateral oophorectomy was performed in the other three groups. Abdominal layers were closed anatomically, using 4/0 atrumatic Vicryl<sup>®</sup> and the animals were allowed to recover from anesthesia.

Experimental protocol was started on day 21 post-castration. In rats, antioxidant dosage recommendations for L-carnitine were 100 mg/kg<sup>13,14</sup> and 500 mg/kg<sup>15,16</sup> in the literature.

LO<sub>SALINE</sub> group was administered 0.12 ml/day of isotonic saline solution, OX<sub>L100</sub> group was administered 100 mg/kg/day of L-carnitine, OX<sub>L500</sub> group was administered 500 mg/kg/day of L-carnitine and OX<sub>SALINE</sub> group was administered 0.12 ml/day of isotonic saline solution, intraperitoneally, for 14 consecutive days. On day 35 post-castration, all the animals were anesthetized again and blood samples were obtained via intra-cardiac puncture before the animals were sacrificed. The blood samples were centrifuged at 10,000 rotations per minute (rpm), at +4 °C for 10 min (Eppendorf; 5804/R, Germany). Sera obtained from blood samples were transferred to polyethylene tubes. After removal the liver, heart and kidney tissue samples were immediately washed with cooled isotonic saline and placed on ice-cold plate. Sera and tissue samples (liver, heart and kidney) were stored at –80 °C until they were used for analysis of nitric oxide (NO), malondialdehyde (MDA), total antioxidant status (TAS), and total oxidative stress (TOS).

Flowchart of the study is presented in Fig. 1.

#### 2.2.1. MDA measurement

The evaluation of MDA was based on the modified version of Yagi's fluorometric method.<sup>17</sup> MDA levels were calculated using a standard curve and values are expressed as nmol/g protein.

#### 2.2.2. NO measurement

Nitrates/nitrites were assayed according to the method of Miranda et al.<sup>18</sup> NO (nitrates/nitrites) levels were calculated using a standard curve and multiplying by the dilution factor. Values are expressed as μmol/g protein.

#### 2.2.3. TOS measurement

Erel's method was used for measuring TOS.<sup>19</sup> TOS was assayed with TOS commercial kit (Rel Assay Diagnostics<sup>®</sup>, Gaziantep, Turkey, 2010) adapted to an Olympus<sup>®</sup> AU 480 autoanalyzer (Fully Automatic Chemistry Analyser [AU480] Olympus Diagnostics, Japan). The results are expressed as micromolar hydrogen peroxide equivalents per g protein (μmol H<sub>2</sub>O<sub>2</sub> equiv/g protein).

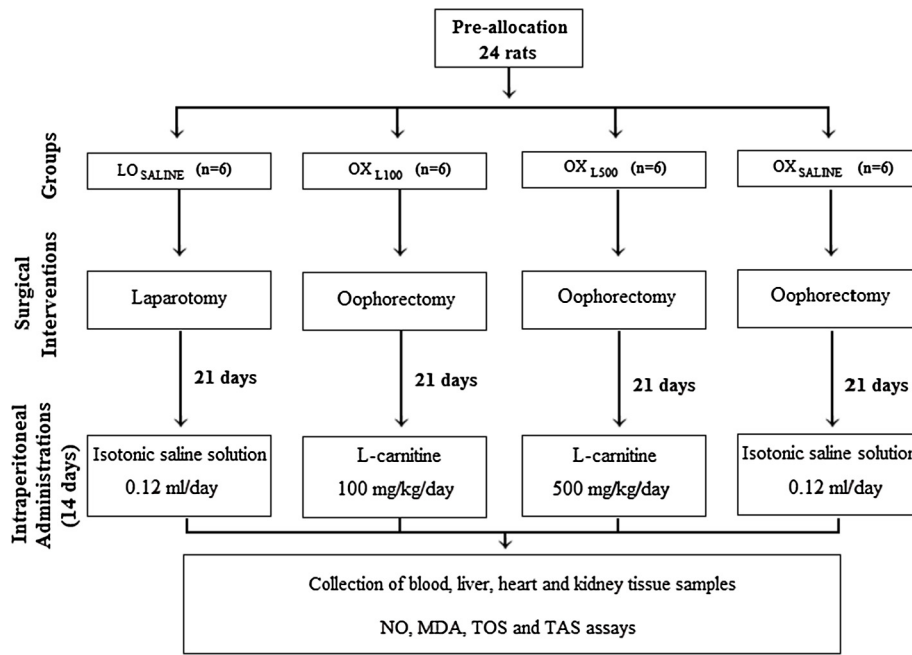


Figure 1 Flowchart of the study.

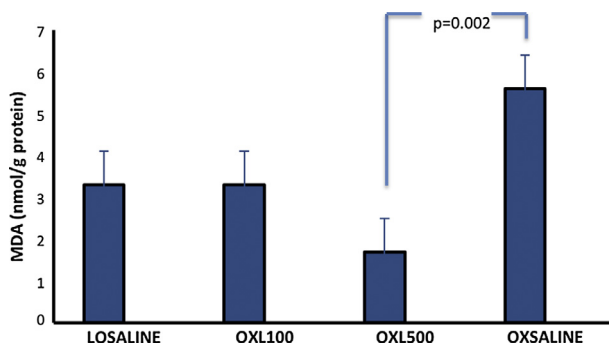


Figure 2 MDA levels in heart tissues in all groups.

#### 2.2.4. TAS measurement

Erel's method was used for measuring TAS.<sup>20</sup> TAS was assayed with TAS commercial kit (Rel Assay Diagnostics®, Gaziantep, Turkey, 2010) adapted to an Olympus® AU 480 autoanalyzer (Fully Automatic Chemistry Analyser [AU480] Olympus Diagnostics, Japan). The results are expressed as micromolar Trolox equivalents per g protein ( $\mu\text{mol Trolox equiv/g protein}$ ).

#### 2.2.5. Calculation of oxidative stress index (OSI)

For calculation, the resulting unit of TAS was converted to  $\mu\text{mol/L}$ , and the OSI value was calculated according to the following formula:  $\text{OSI (arbitrary unit)} = 100 \times \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAS } (\mu\text{mol Trolox equiv/L})$ .<sup>21</sup>

### 3. Statistical analysis

The Statistical Package for Social Sciences (SPSS) 11.5 for Windows software (SPSS Inc., Chicago, IL, United States) was used for statistical analysis. We used the Shapiro Wilk test to test variables for normality of distribution. Levene's test

was used for testing homogeneity of variances. Results of descriptive analysis were presented as mean ( $\pm$  standard deviation) or median (minimum–maximum). The parametric data were statistically analyzed by one-way ANOVA and the inter group comparison of means was conducted using a *post hoc* Tukey's HSD (Honestly Significant Difference) test. The non-parametric data were analyzed by Kruskal-Wallis rank sum tests followed by Conover's multiple comparison tests. Results are considered significant when  $p < 0.05$ .

### 4. Results

In the kidney tissue samples, levels of NO, MDA, and TOS in OXSALINE group were higher (but not significantly) than those in OXL100 and OXL500 groups ( $p > 0.05$  for all comparisons). There was no difference in TAS, and OSI levels of the groups ( $p > 0.05$  for all comparisons) (Table 1).

In the heart tissue samples, there was no difference in the levels of NO, OSI and TOS between the groups ( $p > 0.05$  for all comparisons). MDA levels in OXL500 group were significantly lower than those in OXSALINE group ( $p = 0.002$ ) (Table 2, Fig. 2).

In the liver tissue samples, there was no difference in the levels of NO, MDA, TAS, TOS and OSI between the groups ( $p > 0.05$  for all comparisons) (Table 3).

In the serum samples, levels of NO, MDA, and TOS in OXSALINE group were higher (but not significantly) than those in OXL100 and OXL500 groups ( $p > 0.05$  for all comparisons). There was no difference in TAS and OSI levels of the groups ( $p > 0.05$  for all comparisons) (Table 4).

### 5. Discussion

Estrogen, a natural antioxidant, exhibits radical scavenger properties<sup>22,23</sup> and protects vascular smooth muscle cell

**Table 1** Levels of NO, MDA, TOS, TAS, and OSI in the kidney tissue samples.

	LO <sub>SALINE</sub>	OX <sub>L100</sub>	OX <sub>L500</sub>	OX <sub>SALINE</sub>	P
NO (μmol/g protein)	51.2 ± 29.1	74.1 ± 23.4	70.4 ± 34.9	80.6 ± 17.5	0.297 <sup>a,*</sup>
MDA (nmol/g protein)	2.9 (2.1–6.3)	3.6 (0.7–6.1)	2.6 (1.1–3.3)	6.1 (2.4–7.9)	0.145 <sup>b,*</sup>
TAS (mmol TroloxEqv./g protein)	3.2 ± 1.6	3.2 ± 1.0	3.3 ± 1.0	4.5 ± 1.9	0.389 <sup>a,*</sup>
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eqv/g protein)	6.4 ± 3.7	5.8 ± 4.8	4.6 ± 2.8	9.3 ± 3.4	0.199 <sup>a,*</sup>
OSI	0.25 ± 0.06	0.25 ± 0.04	0.26 ± 0.03	0.25 ± 0.04	0.954 <sup>a,*</sup>

NO, TAS, TOS, and OSI values are expressed as mean ± standard deviation and MDA values as median (minimum–maximum).

<sup>a</sup> One-way ANOVA.

<sup>b</sup> Kruskal Wallis test.

\*  $p > 0.05$ .

**Table 2** Levels of NO, MDA, TOS, TAS, and OSI in the heart tissue samples.

	LO <sub>SALINE</sub>	OX <sub>L100</sub>	OX <sub>L500</sub>	OX <sub>SALINE</sub>	P
NO (μmol/g protein)	56.2 ± 19.7	64.6 ± 33.1	68.3 ± 39.6	47.5 ± 36.4	0.705 <sup>b,*</sup>
MDA (nmol/g protein)	3.3 ± 1.1	3.3 ± 1.6	1.7 ± 0.5 <sup>a</sup>	4.6 ± 1.3 <sup>d</sup>	0.004 <sup>b,**</sup>
TAS (mmol TroloxEqv./g protein)	2.1 (1.6–2.4) <sup>b,c,d</sup>	2.5 (1.5–5.4) <sup>b,e,f</sup>	3.1 (1.7–4.6) <sup>a,c,e</sup>	5.6 (2.2–9.3) <sup>a,d,f</sup>	0.030 <sup>b,**</sup>
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eqv/g protein)	6.5 ± 3.6	8.3 ± 3.4	7.5 ± 5.0	8.9 ± 5.6	0.804 <sup>b,*</sup>
OSI	0.16 (0.01–0.36)	0.27 (0.21–0.28)	0.28 (0.19–0.30)	0.26 (0.19–0.29)	0.282 <sup>b,*</sup>

NO, MDA and TOS values are expressed as mean ± standard deviation and TAS and OSI values as median (minimum–maximum).

<sup>a</sup> Statistically significant differences between OX<sub>L500</sub> and OX<sub>SALINE</sub> groups ( $p < 0.01$ ).

<sup>b</sup> Statistically significant differences between OX<sub>L100</sub> and LO<sub>SALINE</sub> groups ( $p < 0.001$ ).

<sup>c</sup> Statistically significant differences between OX<sub>L500</sub> and LO<sub>SALINE</sub> groups ( $p < 0.001$ ).

<sup>d</sup> Statistically significant differences between OX<sub>SALINE</sub> and LO<sub>SALINE</sub> groups ( $p < 0.001$ ).

<sup>e</sup> Statistically significant differences between OX<sub>L500</sub> and OX<sub>L100</sub> groups ( $p = 0.030$ ).

<sup>f</sup> Statistically significant differences between OX<sub>L100</sub> and OX<sub>SALINE</sub> groups ( $p < 0.001$ ).

<sup>g</sup> One-way ANOVA.

<sup>h</sup> Kruskal Wallis test.

\*  $p > 0.05$ .

\*\*  $p > 0.05$ .

**Table 3** Levels of NO, MDA, TOS, TAS, and OSI in the liver tissue samples.

	LO <sub>SALINE</sub>	OX <sub>L100</sub>	OX <sub>L500</sub>	OX <sub>SALINE</sub>	P
NO (μmol/g protein)	50.9 ± 27.0	77.6 ± 27.0	70.1 ± 16.2	73.2 ± 35.0	0.361 <sup>a,*</sup>
MDA (nmol/g protein)	2.8 ± 1.1	3.1 ± 1.4	3.4 ± 2.8	4.8 ± 2.3	0.334 <sup>a,*</sup>
TAS (mmol TroloxEqv./g protein)	2.2 (1.7–3.2)	2.9 (1.6–8.7)	2.7 (1.9–3.2)	4.2 (1.9–6.0)	0.307 <sup>b,*</sup>
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eqv/g protein)	9.9 (5.8–13.3)	7.8 (1.4–11.8)	4.3 (0.9–10.0)	10.0 (3.9–25.9)	0.066 <sup>b,*</sup>
OSI	0.21 (0.01–0.24)	0.24 (0.19–0.28)	0.26 (0.22–0.29)	0.26 (0.20–0.29)	0.109 <sup>b,*</sup>

NO and MDA values are expressed as mean ± standard deviation and TAS, TOS and OSI values as median (minimum–maximum).

<sup>a</sup> One-way ANOVA.

<sup>b</sup> Kruskal Wallis test.

\*  $p > 0.05$ .

membrane phospholipids against peroxidation.<sup>24</sup> After menopause, the level of estrogen decreases in sera. Menopause impairs oxidative system, and causes increase in LPO and oxidative stress.<sup>2,3</sup>

In our study, we found as expected that MDA levels of oophorectomized groups were higher compared to LO<sub>SALINE</sub> group, although these differences were not statistically signifi-

cant level. Bednarek-Tupikowska et al. found that the level of lipid peroxide increases after menopause and can be reduced by Hormone Replacement therapy (HRT).<sup>2</sup> And also in other study, it has been determined that oxidative stress markers (MDA, glutation) and protein's redox level increase in the brain, liver and plasma of oophorectomized rats and all these processes could be prevented by HRT.<sup>5</sup> It seems to be HRT

**Table 4** Levels of NO, MDA, TOS, TAS, and OSI in the serum samples.

	LO <sub>SALINE</sub>	OX <sub>L100</sub>	OX <sub>L500</sub>	OX <sub>SALINE</sub>	P
NO (μmol/g protein)	3.4 ± 1.1	3.1 ± 1.2	2.8 ± 1.3	4.4 ± 1.9	0.235 <sup>a,*</sup>
MDA (nmol/g protein)	20.1 ± 7.4	18.8 ± 5.0	18.5 ± 3.0	23.6 ± 4.4	0.335 <sup>a,*</sup>
TAS (mmol TroloxEqv./g protein)	1.4 ± 0.5	1.4 ± 0.5	1.2 ± 0.2	1.3 ± 0.3	0.876 <sup>a,*</sup>
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eqv/g protein)	2.4 (2.3–4.3)	2.1 (1.3–5.9)	2.2 (1.3–3.4)	4.0 (1.5–6.1)	0.163 <sup>b,*</sup>
OSI	0.18 ± 0.06	0.16 ± 0.03	0.19 ± 0.02	0.18 ± 0.06	0.628 <sup>a,*</sup>

NO, MDA, TAS and OSI values are expressed as mean ± standard deviation and TOS values as median (minimum–maximum).

<sup>a</sup> One-way ANOVA.

<sup>b</sup> Kruskal Wallis test.

\*  $p > 0.05$ .

might be protective oxidative stress in menopause. However, sometimes we cannot use these drugs and we are supposed to find other choices.

It has been shown that exogenous L-carnitine is a free-radical scavenger and it protects cells against oxygen radicals.<sup>10,11</sup> To our knowledge, there is no study in the literature that assessed the effects of L-carnitine on oxidant and antioxidant status in sera or other tissues, particularly in menopause or in experimental/surgical menopause.

In our study, NO, MDA, TAS, TOS and OSI levels in the various tissue specimens and sera of surgically menopause rats were detected. These oxidative stress markers were mostly increased in the kidney tissue specimens of OX<sub>SALINE</sub> group when compared with the other groups, although these differences were not significant level. Oxidative stress parameters' levels were similar in OX<sub>L100</sub>, OX<sub>L500</sub> and LO<sub>SALINE</sub> groups. Contrary to our hypothesis, we could not find protective effect of L-carnitine in kidney tissues. In the literature, there is no study on the effects of carnitine on antioxidant system and LPO about kidney during menopause. However, Fatouros et al. have investigated the effects of L-carnitine on oxidative stress response in patients with renal disease and they have observed that L-carnitine reduces exercise-induced oxidative stress, increases levels of antioxidants, and improves performance in patients with end-stage renal failure.<sup>25</sup>

Furthermore when we examined liver tissue specimens, NO, MDA, TAS, TOS and OSI levels were mostly increased in OX<sub>SALINE</sub> group when compared with the other groups. However these differences were not significant as in the kidney specimens. Oxidative stress parameters' levels were similar in L-carnitine treated groups and the LO<sub>SALINE</sub> group. In reviewing of the literature, it could be seen that there are no menopause-specific liver changes. However, it was known that oxidative stress and LPO have important roles in occurrence of liver injury. L-carnitine exhibits antioxidant effect by reducing metabolic stress and the use of L-carnitine has recently come into question in the treatment of many diseases such as liver disease.<sup>25</sup> While there is no study on menopause and the use of L-carnitine, there are studies that investigate the effects of carnitine on liver disease in the literature. In a study investigating the protective effects of L-carnitine against hydrogen peroxide cytotoxicity in hepatocytes, it has been reported that L-carnitine that exhibits antioxidant effects by reducing MDA and increasing catalase (CAT) and superoxide dismutase (SOD), reduces cytotoxic effect of hydrogen peroxide.<sup>26</sup>

It is accepted that menopause is an important risk factor for cardiovascular disease. In cases of estrogen deficiency such as

menopause, deterioration in oxidative system resulted in increasing of lipoprotein oxidation which causes cardiovascular disease.<sup>27</sup> In this study, we found that MDA (marker of LPO) levels in heart tissue were significantly lower in OX<sub>L500</sub> group compared to OX<sub>SALINE</sub> and this situation supports our hypothesis that high dose L-carnitine reduces oxidative stress in heart tissue. Conversely, there were differences in TAS levels between the groups. This suggests that high oxidative stress may be suppressed by antioxidants which increase reactively or distinct antioxidant mechanism. Suppression of oxidative stress by L-carnitine may play an important role in the heart. These results may be related to treatment duration or dose. In the literature, carnitine and its derivative have been subjected to numerous studies which have shown promising results.<sup>28–32</sup> Carnitine that exerts cardio-protective effect against hypoxia and oxidative stress, is a specific treatment choice.<sup>30</sup> In experimental studies, it has been determined that carnitine administration before or during acute ischemia increases ATP levels, while reducing oxidative stress, improves hemodynamic parameters, and reduces the degree of ST segment elevation.<sup>13,28,31,32</sup> In a rat study conducted by Gomez-Amores et al., it has been shown that chronic L-carnitine treatment reduces systemic oxidative stress related to hypertension and strengthens antioxidant defense.<sup>10</sup>

According to our study, NO, MDA, TAS, TOS and OSI levels in the sera were similar between all the groups. In parallel with the literature, serum levels of NO and MDA were higher in the OX<sub>SALINE</sub> group compared to the LO<sub>SALINE</sub> group, and serum levels of NO, MDA and TOS were lower in the L-carnitine treated groups compared to the OX<sub>SALINE</sub> group, although these differences were not statistically significant. This can be explained by the rapid uptake of exogenous carnitine via active transport from plasma to tissues.<sup>33</sup> Gumral et al. have examined the effects of carnitine and selenium on oxidative stress in blood of rat induced by 2.45-GHz electromagnetic radiation, and they have found that LPO levels were higher in the radiation exposure group compared to controls and also glutathione levels were lowest in the L-carnitine receiving group. They have concluded that L-carnitine exhibited protective effect via its free radical scavenging activity in the blood, but no such effect has been demonstrated for selenium.<sup>34</sup>

In accordance with our hypothesis, the level of MDA (a LPO product) in the heart tissue was higher in OX<sub>SALINE</sub> group when compared to the OX<sub>L500</sub> group. We couldn't find similar results in other tissues and sera. These results may be related to tissue-specific antioxidant effect of L-carnitine.

Moreover, Topcuoglu et al. have reported similar tissue-specific antioxidant effect for estrogen.<sup>5</sup> Probably, L-carnitine can act in different ways on different tissues. In our study, L-carnitine exhibits statistically significant reduction in MDA levels in heart tissue in surgically menopausal rats. This effect may be dose dependant.

In conclusion, L-carnitine 500 mg/kg/day exhibits antioxidant effect in the heart by reducing oxidative stress. L-carnitine may be an alternative for menopausal supportive treatment, because it is naturally occurring in human body, easy-to-obtain, easy-to-administrate, cheap and has low incidence of adverse events. However, there is a need for large scale, prospective and long-term studies that evaluate the effects of carnitine in menopause.

### Conflict of interest

There is no conflict of interest.

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