

# The Effects of Exogenous L-Carnitine on Lipid Peroxidation and Tissue Damage in an Experimental Warm Hepatic Ischemia-Reperfusion Injury Model

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## ABSTRACT

**Background:** L-Carnitine is the essential endogenous factor for the transport of long-chain fatty acids from the cytoplasm to within the mitochondrion where the  $\beta$ -oxidation process takes place. L-Carnitine is a superoxide scavenger and an antioxidant that possesses an anti-ischemic action and a stabilizing effect on cell membranes. It may be of help in liver ischemia reperfusion injury. Results regarding the effects of L-carnitine on liver ischemia and reperfusion injury are few and conflicting.

**Objective:** The aim of this study was to investigate the efficacy of exogenous L-carnitine on lipid peroxidation and protecting liver at different stages of experimental total warm hepatic ischemia-reperfusion (TWHIR) procedure in rats.

**Methods:** This experimental study in healthy, weanling, male Wistar rats (weighing 180–200 g) was conducted at the Experimental Animal Research Laboratory of the Faculty of Medicine of Mersin University, Mersin, Turkey. Rats were randomly divided into 5 groups: (A) Control group; (B) TWHIR procedure only; (C) L-carnitine administered 2 hours before the TWHIR procedure; (D) L-carnitine administered just before the TWHIR procedure; and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure. Total warm hepatic ischemia (via the Pringle maneuver) and reperfusion were performed for 45 and 30 minutes, respectively. L-Carnitine (200 mg/kg) was administered intravenously. At the end of each procedure a blood sample

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was drawn and total hepatectomy was performed following reperfusion. Malondialdehyde (MDA) and myeloperoxidase (MPO) levels of both plasma and liver tissue, total antioxidant capacity (TAOC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in plasma, and histopathologic examination were analyzed to assess lipid peroxidation and damage in liver tissue.

**Results:** Thirty-four rats (mean [SD] age, 59.26 [1.2] days; mean [SD] weight, 194.1 [5.1] g) were used in the study. There was a significant difference observed between groups A (n = 5) and B (n = 5) for all evaluation parameters. The TWHIR procedure performed in group B was associated with significant increases versus baseline in ALT, AST, MDA, and MPO in plasma, and MDA and MPO in liver tissue, but a significant decrease of TAOC in plasma. ALT, AST, serum and liver MDA, and MPO levels of group B were significantly higher than all groups administered L-carnitine. L-Carnitine administration between total warm hepatic ischemia and reperfusion was associated with a significant attenuation in all parameters. The liver MDA levels of groups C (n = 8) and D (n = 8) were significantly lower than that of group E (n = 8) (mean [SD]: C, 16.53 [3.32] and D, 18.28 [1.67] vs E, 23.05 [3.52];  $P = 0.001$  and  $P = 0.016$ , respectively). The mean (SD) liver MPO level of group C (1.09 [0.16]) was significantly lower than that of groups D (2.12 [0.25]) and E (2.11 [0.28]) (both,  $P = 0.001$ ). The TAOC of group B (0.77 [0.12]) was significantly lower than that of groups C (1.34 [0.19]) and D (1.08 [0.20]) ( $P = 0.001$  and  $P = 0.015$ , respectively). The TAOC of group C was significantly higher than that of the other L-carnitine groups (E, 0.94 [0.13]) ( $P = 0.023$  vs group D; and  $P = 0.001$  vs group E). Histopathologic scores of groups A, C, and E were significantly lower than that of group B, but the difference between groups B and D was not statistically significant.

**Conclusions:** In this experimental study, administration of exogenous L-carnitine was associated with significantly decreased lipid peroxidation in plasma and liver tissue when administered prior to a TWHIR procedure. In addition, L-carnitine seemed to be more effective with regard to decreasing lipid peroxidation in liver tissue when administered before warm hepatic ischemia. L-Carnitine was associated with significantly decreased leukocyte sequestration in plasma and liver tissue. A significant increase in TAOC was associated with L-carnitine administered prior to ischemia. These observations suggest that L-carnitine might have a protective effect against ischemia-reperfusion injury in rat liver tissue. (*Curr Ther Res Clin Exp.* 2007; 68:32–46) Copyright © 2007 Excerpta Medica, Inc.

**Key words:** L-carnitine, experimental, liver, ischemia, reperfusion.

## INTRODUCTION

Ischemia-reperfusion (IR) injury is a phenomenon whereby cellular damage in a hypoxic organ is accentuated following the restoration of oxygen delivery.<sup>1</sup> Injury associated with total warm hepatic ischemia reperfusion (TWHIR) is relevant clinically with regard to hepatic surgery, liver transplantation, hypo-

volemic shock, types of toxic liver injury, veno-occlusive disease, and Budd-Chiari syndrome.

Reperfusion of ischemic tissue is believed to cause cellular damage through the production of free oxygen radicals.<sup>2</sup> One of the most popular hypotheses to explain injury by reactive oxygen species is the damage of membranes by lipid peroxidation.<sup>3</sup>

There is a need to increase the ischemic time period without damaging the liver when performing major hepatic surgery and liver transplantation. Among the currently tested therapeutic strategies, ischemic preconditioning or pharmacologic interventions that prevent the formation of reactive oxygen species and/or promote their detoxification have the greatest potential to eliminate the postischemic oxidant stress and reduce IR injury.<sup>3</sup> L-Carnitine is one agent being tested for the prevention of liver damage induced by IR.<sup>4-6</sup>

L-Carnitine is the essential endogenous factor for the transport of long-chain fatty acids (acyl-CoA) from the cytoplasm to within the mitochondrion where the  $\beta$ -oxidation process takes place. It increases the oxidation of glucose during the reperfusion phase by performing transportation of long-chain fatty acids to the mitochondrial matrix for  $\beta$ -oxidation.<sup>7</sup> L-Carnitine is a superoxide scavenger, antioxidant, and DNA cleavage protector.<sup>8</sup> It also has an anti-ischemic action and a stabilizing effect on cell membranes.<sup>2</sup>

L-Carnitine has been studied in experimental IR injury models regarding the heart, kidney, skeletal muscle, spinal cord, and skin flaps. Experimental studies in animals concerning the heart have suggested that L-carnitine possesses an anti-ischemic action.<sup>2</sup> L-Carnitine has been found to be of value for preventing decline in renal function that occurs during IR.<sup>9</sup> It has been reported as effective for reducing the IR injury in skeletal muscle<sup>7</sup> and has also been found to protect the motor neuron cells from ischemic spinal cord injury.<sup>10</sup> L-Carnitine has a dose-dependent effect to increase flap survival in random skin flaps.<sup>11</sup> A literature search was performed utilizing Entrez PubMed (no time or language limits; key terms: *total warm hepatic ischemia reperfusion, carnitine, and human*). Despite the existence of several experimental studies, there appear to be no well-designed human studies of L-carnitine treatment administered for TWHIR injury; this is a limitation of the available literature.

There are few studies regarding the effect of exogenous L-carnitine on liver IR injury. A trial by Yonezawa et al<sup>4</sup> could not demonstrate a protective effect of L-carnitine to warm hepatic IR injury. But the study by Atila et al<sup>5</sup> found that exogenous L-carnitine might be helpful in preventing free oxygen radical damage and inflammatory reactions in liver tissue related to warm hepatic IR. Puetz et al<sup>6</sup> found that L-carnitine might be suitable to protect fatty livers from IR injury.

The aim of our study was to investigate the efficacy of exogenous L-carnitine on lipid peroxidation and liver damage when administered at different stages of experimental TWHIR procedure in rats. We expect this study to supply more evidence to the existing literature about the effects of L-carnitine by using myeloperoxidase (MPO) and total antioxidant capacity (TAOC) in addition to

malondialdehyde (MDA) to evaluate the damage in liver tissue. This study might also help determine the timing of L-carnitine administration to provide better results in decreasing lipid peroxidation and liver damage.

## **MATERIALS AND METHODS**

After study design approval from the institutional ethical committee, 34 healthy, weanling, male Wistar rats weighing 180 to 200 g were obtained from the experimental animal producing center of the institution (Mersin University, Mersin, Turkey). Study animals were kept in standard cages and had access to water and rat chow ad libitum. All experiments were carried out in accordance with the guidelines of the National Animal Welfare Law<sup>12</sup> and with the Helsinki Declaration of Animal Rights.<sup>13</sup>

### **Study Design**

Rats were randomly divided into 5 groups: (A) control group; (B) TWHIR procedure only; (C) L-carnitine administered 2 hours before the TWHIR procedure; (D) L-carnitine administered just before the TWHIR procedure; and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure. Before surgical procedures, all rats were anesthetized with ketamine 100 mg/kg intramuscularly. After laparotomy, total liver ischemia was achieved by clamping the hepatic pedicle (Pringle maneuver) with a microvascular clamp for 45 minutes. Reperfusion was performed by removing the clamps for 30 minutes. IV L-carnitine 200 mg/kg was administered to rats in groups D and E over 1 minute through infrahepatic vena cava during laparotomy. In group C, L-carnitine was administered through the tail vein of the rats before laparotomy. At the end of the reperfusion period, blood samples were drawn from the suprahepatic vena cava by a fine needle. A total hepatectomy was performed before the rats were euthanized. Each liver tissue sample was divided into 2 pieces of equal size for histopathologic and biochemical evaluations. The rats in group A (control) received hepatic pedicle preparation but no clamping. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, TAOC of plasma, and MDA and MPO levels of both plasma and liver tissue were studied to evaluate the damage in liver tissue.

The comparison of group A with group B was made to determine if TWHIR caused an injury in the liver tissue. Group B was compared with groups C, D, and E to determine whether L-carnitine decreased liver tissue damage or not. Groups C, D, and E were also compared with each other to evaluate at which stage of the TWHIR procedure L-carnitine was most effective.

### **Plasma and Liver Tissue Analyses**

Blood taken from the rats was centrifuged for 5 minutes at 3000 rpm and the plasma obtained was stored at  $-20^{\circ}\text{C}$  until analysis.

### **Plasma ALT and AST**

ALT and AST levels of plasma were analyzed using a Cobas Integra 700 biochemical analyzer (Roche Diagnostics, GmbH, Mannheim, Germany).

### **MDA in Plasma and Liver Tissue**

Liver tissues were homogenized in 0.15% potassium chloride and then centrifuged for 10 minutes at 300 rpm. Liver tissue supernatant and plasma MDA levels, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction according to the procedures used by Yagi.<sup>14</sup> The principle of MDA determination in plasma and tissue homogenates depends on measurement of the color produced by the interaction of barbituric acid with MDA as a result of lipid peroxidation. The color-reactive 1,1,3,3-tetraethoxypropane was used as the primary standard.

### **MPO in Serum and Liver Tissue**

MPO is a heme-containing enzyme within the azurophil granules of neutrophils, and MPO activity was measured as a simple quantitative method of detecting leukosequestration. A 300-mg tissue sample was homogenized in 0.02 M ethylenediaminetetraacetic acid (pH = 4.7) in a Teflon Potter homogenizer (Braun, Melsungen, Germany). Homogenates were centrifuged at 20,000g for 15 minutes at 4°C. Afterward, the pellet was rehomogenized in 1.5 mL 0.5% hexadecyltrimethylammonium and centrifuged at 20,000g for 15 minutes at 4°C. The principle of MPO determination in plasma and tissue homogenates depends on the fact that it reduces *o*-dianisidine. The *o*-dianisidine was measured at 410 nm by spectrophotometer (Cary 1, Varian Australia Pty Ltd, Clayton South, Australia).<sup>15</sup>

### **Total Antioxidant Status**

ABTS (2,2'-Azino-di-[3-ethylbenzthiazolinesulfonate]) is incubated with a peroxidase (methemoglobin) and hydrogen peroxide to produce the radical cation ABTS<sup>+</sup>. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree, that is proportional to their concentration.<sup>16</sup>

### **Histopathologic Examination**

The liver tissues were fixed in a 10% formalin solution for 24 hours. Standard dehydration was then performed and the samples were embedded in paraffin wax. Hematoxylin and eosin (H-E) stained slides were prepared by using standard methods.<sup>17</sup> Light microscopic analysis of the liver tissues was performed by a researcher (C.T.) blinded to allocation group. H-E stained slides were investigated at  $\times 40$ ,  $\times 100$ , and  $\times 200$  magnifications.

Portal and lobular inflammations were scored between 0 and 3. The scoring system of histopathologic changes was: 0 = no inflammatory cells in portal or lobular space, and no hepatocyte necrosis; 1 = minimal focal inflammation or hepatocyte necrosis in portal or lobular space; 2 = moderate focal inflammation

or hepatocyte necrosis in portal or lobular space; and 3 = severe focal inflammation or hepatocyte necrosis in portal or lobular space.<sup>5</sup>

### Statistical Analyses

The differences between the 5 groups were determined using analysis of variance. The comparison of the L-carnitine groups (C–E) with the TWHIR-only group (B) was performed by Dunnett's test. Comparison between groups C, D, and E was performed by Tukey test. ALT and AST were analyzed with a non-parametric test, the Kruskal-Wallis test. The Dunn multiple comparison test was used to compare histopathologic scoring.

Data for ALT and AST were presented as median with 25th to 75th percentiles, as a descriptive statistic; for other parameters mean (SD) were determined, and 95% CI values were reported in the figures. A value of  $P < 0.05$  was considered statistically significant. All statistical analyses were performed with SPSS version 11.5 (SPSS Inc., Chicago, Illinois).

## RESULTS

Thirty-four rats (mean [SD] age, 59.26 [1.2] days; mean [SD] weight, 194.1 [5.1] g) were randomly divided into 1 of 5 groups. The values of evaluation parameters in group A ( $n = 5$ ) represented the baseline values of rats. The values in group B ( $n = 5$ ) presented the results of rats injured after hepatic ischemia and reperfusion procedure. The values obtained in groups C ( $n = 8$ ), D ( $n = 8$ ), and E ( $n = 8$ ) reflected the effect of exogenous L-carnitine at different stages of the ischemia and reperfusion procedure.

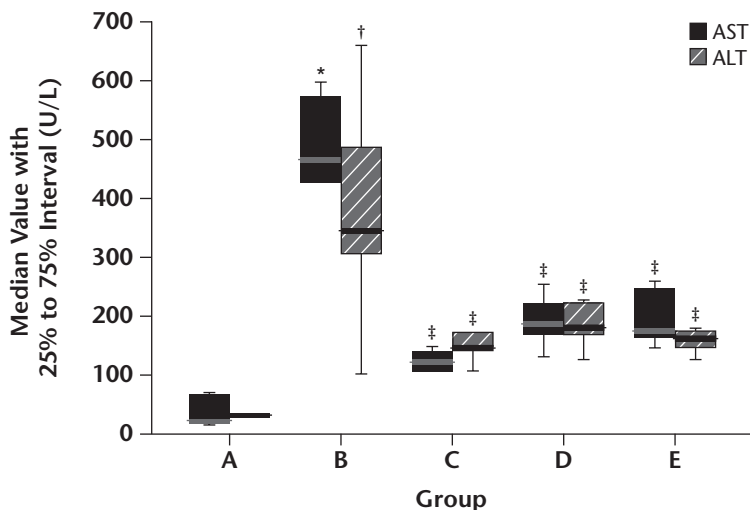
### Plasma ALT and AST

Median plasma ALT levels with 25th to 75th percentiles were the following: A, 31 (31–33); B, 345 (204–574); C, 146 (139–170); D, 178 (157–221); and E, 161 (147–177). Median plasma AST levels with 25th to 75th percentiles were the following: A, 21 (17–69); B, 468 (288–586); C, 121 (107–140); D, 186 (162–232); and E, 172 (161–249). Plasma ALT and AST levels of group B were significantly higher than those of group A ( $P = 0.020$  and  $P = 0.001$ , respectively).

The descriptive statistics of plasma ALT and AST for the TWHIR groups (B–E) are shown in **Figure 1**. L-Carnitine administered in groups C, D, and E was associated with a reduction in plasma ALT and AST levels when compared with group B ( $P = 0.001$  for all comparisons). There was not a significant difference between the L-carnitine groups for comparison of plasma ALT and AST.

### MDA and MPO of Plasma and Liver Tissue

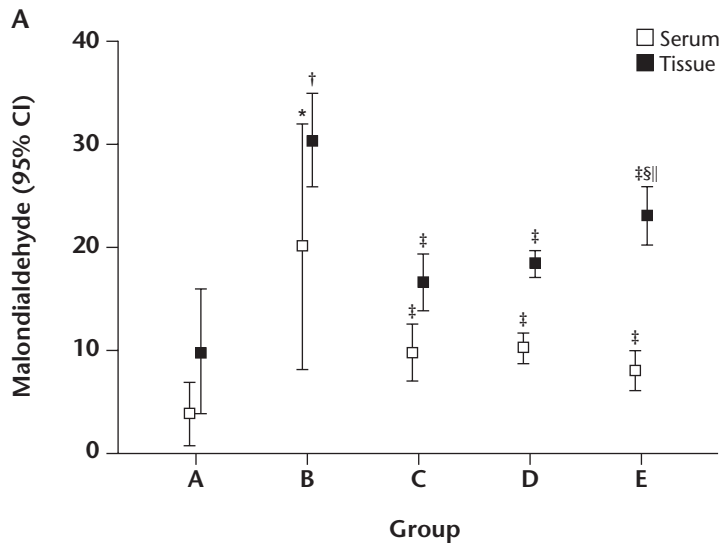
Mean (SD) nmol/mL of plasma MDA were as follows: A, 3.66 (2.44); B, 20.04 (9.66); C, 9.65 (3.31); D, 10.17 (1.94); and E, 7.94 (2.24). Mean (SD) nmol/g of liver tissue MDA were: A, 9.75 (4.90); B, 30.47 (3.61); C, 16.53 (3.32); D, 18.28 (1.67); and E, 23.05 (3.52). Plasma and liver tissue MDA levels of group B were signifi-



**Figure 1.** Median aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values with the 25% to 75% interval in the 5 study groups (A–E). (A) Control (n = 5); (B) total warm hepatic ischemia-reperfusion (TWHIR) procedure only (n = 5); (C) L-carnitine administered 2 hours before the TWHIR procedure (n = 8); (D) L-carnitine administered just before the TWHIR procedure (n = 8); and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure (n = 8). \* $P = 0.020$  versus group A; † $P = 0.001$  versus group A; ‡ $P = 0.001$  versus group B.

cantly higher than group A ( $P = 0.017$  and  $P = 0.001$ , respectively). The descriptive statistics of both plasma and liver tissue MDA for TWHIR groups are shown in **Figure 2A**. Significantly lower levels of plasma MDA were observed in the L-carnitine groups (C, D, and E) when compared with group B ( $P = 0.001$  for each comparison). The difference in plasma MDA levels between the L-carnitine groups was not statistically significant. Liver MDA generation in the L-carnitine groups was significantly lower than that observed in group B ( $P = 0.001$  for all comparisons). When the L-carnitine groups were compared with each other, liver MDA generation in groups C and D were significantly lower than group E ( $P = 0.001$  and  $P = 0.016$ , respectively).

Mean (SD) U/L of plasma MPO activity were as follows: A, 0.27 (0.04); B, 1.22 (0.10); C, 0.80 (0.22); D, 0.83 (0.37); and E, 0.59 (0.16). Mean (SD) U/g of liver tissue MPO activity were as follows: A, 0.92 (0.13); B, 2.65 (0.23); C, 1.09 (0.16); D, 2.12 (0.25); and E, 2.11 (0.28). Plasma and liver tissue MPO activity levels of group B were significantly higher than group A ( $P = 0.001$  and  $P = 0.001$ , respectively). The descriptive statistics of both plasma and liver tissue MPO activity for the groups that underwent TWHIR are shown in **Figure 2B**. Plasma MPO activity for the groups administered L-carnitine (C, D, and E) was significantly lower than for group B ( $P = 0.016$ ,  $P = 0.022$ , and  $P = 0.001$ ,



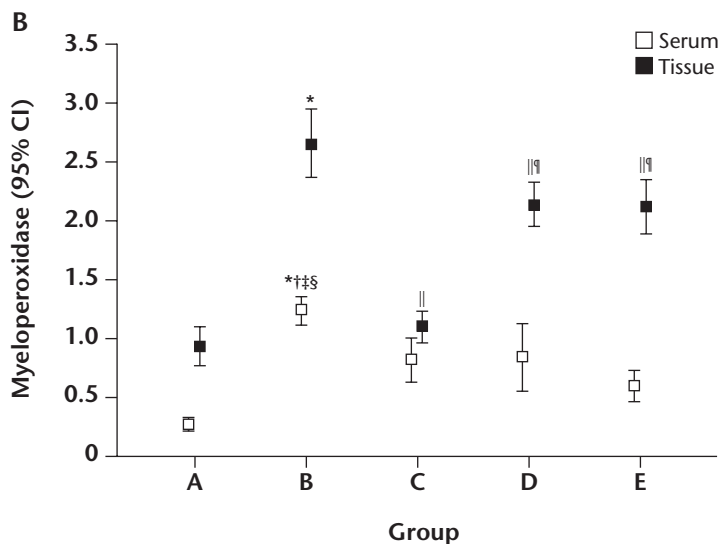
**Figure 2A.** Malondialdehyde (95% CI) values of both serum and liver tissue in the 5 study groups (A–E). (A) Control (n = 5); (B) total warm hepatic ischemia-reperfusion (TWHIR) procedure only (n = 5); (C) L-carnitine administered 2 hours before the TWHIR procedure (n = 8); (D) L-carnitine administered just before the TWHIR procedure (n = 8); and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure (n = 8). \* $P = 0.017$  versus group A; † $P = 0.001$  versus group A; ‡ $P = 0.001$  versus group B; § $P = 0.001$  versus group C; || $P = 0.016$  versus group D.

respectively). However, there was not a significant difference between the L-carnitine groups with regard to plasma MPO activity level. Liver tissue MPO activity for all of the L-carnitine groups was significantly lower than for group B (all,  $P = 0.001$ ). When these groups were compared with each other, liver tissue MPO activity in group C was significantly lower than groups D and E (both,  $P = 0.001$ ).

### Total Antioxidant Status

Mean (SD) mmol/L of plasma TAOC were as follows: A, 1.94 (0.21); B, 0.77 (0.12); C, 1.34 (0.19); D, 1.08 (0.20); and E, 0.94 (0.13). TAOC of group B was significantly decreased when compared with group A ( $P = 0.001$ ). The descriptive statistics of TAOC for groups B through E are shown in **Figure 3**. Administration of L-carnitine in groups C and D was associated with significantly increased TAOC when compared with group B ( $P = 0.001$  and  $P = 0.015$ , respectively). When the groups administered L-carnitine were compared with each other, the TAOC of group C was significantly higher than those of groups D and E ( $P = 0.023$  and  $P = 0.001$ , respectively).



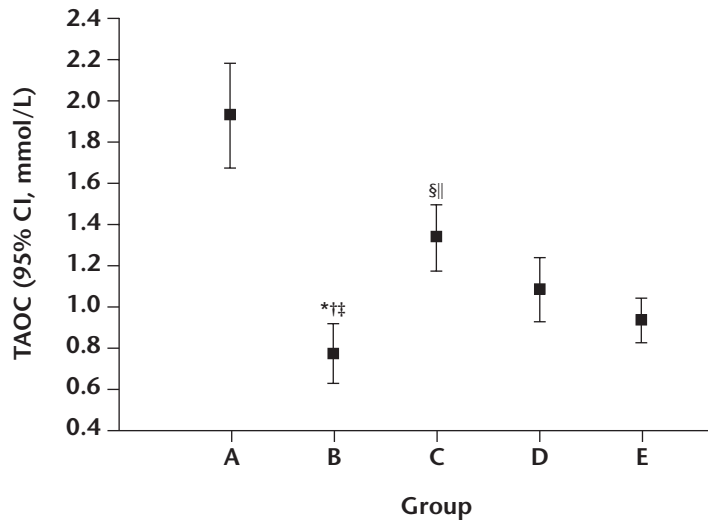


**Figure 2B.** Myeloperoxidase (95% CI) values of both serum and liver tissue in the 5 study groups (A–E). (A) Control (n = 5); (B) total warm hepatic ischemia-reperfusion (TWHIR) procedure only (n = 5); (C) L-carnitine administered 2 hours before the TWHIR procedure (n = 8); (D) L-carnitine administered just before the TWHIR procedure (n = 8); and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure (n = 8). \* $P = 0.001$  versus group A; † $P = 0.016$  versus group C; ‡ $P = 0.022$  versus group D; § $P = 0.010$  versus group E; || $P = 0.001$  versus group B; ¶ $P = 0.001$  versus group C.

### Histopathologic Scoring

Normal histologic findings were detected after the histopathologic examination of the liver tissue of the rats in the control group (median [25%–75% interval], 0 [0–0.5]). Severe focal inflammation or hepatocyte necrosis was observed in the portal or lobular space besides reactive Kupffer cells as were fibrin deposits in the sinusoids of group B rats (3.0 [2.0–3.0]). Minimal focal inflammation or hepatocyte necrosis in the portal or lobular space was detected in all 3 carnitine groups. Median score and 25% to 75% interval range for groups C, D, and E were as follows: 1.0 (1.0–1.0), 1.5 (1.0–2.0), and 1.0 (1.0–1.7), respectively.

The median score of group B was significantly higher than that of the control group ( $P = 0.008$ ), indicating damage caused by the TWHIR procedure. The median score of groups C and E were significantly lower than that of group B ( $P = 0.012$  and  $P = 0.036$ , respectively). This suggests that L-carnitine administration reduced inflammation and hepatocyte necrosis. Although the median score of group D was lower than group B, the difference was not statistically significant. The comparison of carnitine groups with each other revealed no statistical significance.



**Figure 3.** Total antioxidant capacity (TAOC) values (95% CI) for all 5 study groups (A–E). (A) Control (n = 5); (B) total warm hepatic ischemia-reperfusion (TWHIR) procedure only (n = 5); (C) L-carnitine administered 2 hours before the TWHIR procedure (n = 8); (D) L-carnitine administered just before the TWHIR procedure (n = 8); and (E) L-carnitine administered after total warm hepatic ischemia but just before the reperfusion procedure (n = 8). \* $P = 0.001$  versus group A; † $P = 0.001$  versus group C; ‡ $P = 0.015$  versus group D; § $P = 0.023$  versus group D; || $P = 0.001$  versus group E.

## DISCUSSION

Operative blood loss has been recognized as the main factor associated with morbidity and mortality in liver resections. Occlusion of the hepatic inflow by clamping of the hepatic pedicle is the oldest method to reduce bleeding from a cut or torn liver tissue. Pringle first used it in patients with liver trauma and reported it with the results of his experimental observations.<sup>18</sup> The normal liver can tolerate continuous pedicular clamping for up to 60 minutes. The Pringle maneuver is a useful technique employed in controlling bleeding during the repair of extensive liver wounds, and when performing hepatic resection and liver transplantation. However, if the period of clamping the hepatic vasculature is too long, TWHIR injury may result.<sup>1</sup> Therefore, a need is evident to increase the ischemic time period to perform more complicated hepatic surgery without damaging the liver.

There are 2 distinct phases of liver injury after TWHIR.<sup>19</sup> The initial phase (<2 h after reperfusion) is characterized by oxidant stress, where production and release of reactive oxygen species appears to directly result in hepatocellular injury.<sup>1</sup> One of the most popular hypotheses to explain injury by reactive oxygen species is the damage of membranes by lipid peroxidation. This mechanism

could certainly explain a loss of ion homeostasis, cell swelling, and necrotic cell death, which are prominent features of reperfusion injury.<sup>3</sup> Endogenous antioxidants usually limit damage by free oxygen radicals; however, studies on models of hepatic IR have suggested that their levels fall during the reperfusion period.<sup>20,21</sup> This is probably the result of degradation of the antioxidants due to the increased free radical activity.<sup>22</sup> Many antioxidant interventions (eg, allopurinol, vitamin E, glutathione, superoxide dismutase, catalase) have been associated with reduced lipid peroxidation and reperfusion injury.<sup>23</sup>

The Pringle maneuver was used in the present experimental study to perform total warm hepatic ischemia. Because of its antioxidant and free oxygen radical scavenger effects, exogenous L-carnitine was used in this study. The same time periods of total warm ischemia and reperfusion were used by Kobayashi et al<sup>24</sup> to maintain liver damage in rats. As the initial (early) phase of liver IR injury was defined as the time period <2 hours after reperfusion,<sup>1</sup> the results of this study reflected the early phase of hepatic injury. Hepatic IR injury, if severe enough, may result in pericentral liver hypoxia, which leads to ischemic hepatitis, characterized by a dramatic rise in serum aminotransferase in the absence of other causes of hepatic necrosis.<sup>1</sup> Significantly higher levels of ALT and AST in group B when compared with group A suggested that our experimental model of TWHIR injury caused liver damage. The decrease in serum aminotransferases in all 3 groups administered L-carnitine indicated attenuation of the liver damage. This effect of L-carnitine was observed when administered at any stage of TWHIR procedure. Although the methods and dosage of carnitine administered were different, Atila et al<sup>5</sup> and Puetz et al<sup>6</sup> reached similar results.

It is understood that oxygen-free radicals are associated with tissue damage that occurs on reperfusion after an ischemic period.<sup>25</sup> Reactive oxygen species damage the cell membranes by lipid peroxidation. Lipid peroxidation products may also be involved in the amplification and continuation of the inflammatory response.<sup>3</sup> Lipid peroxidation process has been found to cause liver cell damage during IR.<sup>26</sup> The MDA is a result of lipid peroxidation. The concentration of free oxygen radicals is usually estimated in connection with the production of MDA.<sup>27</sup> In our study, a significantly higher level of MDA both in serum and liver tissue in group B suggested that TWHIR was the cause of lipid peroxidation. In accordance with the results of the present study, there was an increase of plasma and liver MDA concentrations observed in the study by Su et al.<sup>28</sup> The decrease in concentrations of both plasma and liver MDA in groups C, D, and E suggested that exogenous L-carnitine administration maintained an attenuation in MDA levels (**Figure 2**). This effect of L-carnitine in this study is thought to be associated with the cell membrane stabilizing ability, which accounts for its anti-ischemic action,<sup>2</sup> and superoxide scavenger and antioxidant effects.<sup>8</sup> On the other hand, the study by Yonezawa et al<sup>4</sup> used partial hepatic ischemia and demonstrated that carnitine did not significantly decrease lipid peroxidation. Although different in method, the effect of L-carnitine for preventing lipid peroxidation in experimental acute hepatic

damage induced by carbon tetrachloride<sup>29</sup> and valproic acid<sup>28</sup> was similar to the present study. In these 2 experiments, liver damage was induced by a method other than ischemia.

In the present study, administration of L-carnitine at different time periods of the ischemia-reperfusion procedure did not make a difference in plasma MDA levels. However, L-carnitine administered before ischemia (groups C and D) was significantly more effective for decreasing lipid peroxidation in the liver tissue (**Figure 2**) than when it was administered after ischemia (group E). This may be associated with the limited efficacy of L-carnitine during acute emergency situations following an ischemic episode.<sup>2</sup>

Polymorphonuclear leukocytes may preferentially accumulate in the endothelium during ischemia, and such accumulation may be markedly accelerated following reperfusion. Subsequently, polymorphonuclear leukocytes infiltrate the ischemic lesions and cause damage.<sup>30</sup> The activation of complementary and generation of primary cytokines and chemokines during reperfusion are responsible for the recruitment of neutrophils into the liver. A neutrophil-derived oxidant stress is important in the pathophysiology of reperfusion injury in the liver.<sup>3</sup> The study by Vollmar et al<sup>31</sup> demonstrated that there was a leukocyte flux to liver after hepatic IR. MPO activity is used as a simple quantitative method of detecting leukosequestration.<sup>15</sup> The significantly high MPO activity of both serum and liver tissue in group B demonstrated that IR caused an increase of leukocyte flux. Concordant with the results of the present study, Liu et al<sup>26</sup> found that MPO activity in liver tissue was high in an experimental hepatic IR model reflecting polymorphonuclear leukocyte infiltration after reperfusion. The increase of hepatic leukocyte flux after ischemia reperfusion may be the result of both the manifestation of a systemic inflammatory response and the increase of local chemoattractant activities, such as the production and release of the cytokine-induced neutrophil chemoattractant of the interleukin-8 family.<sup>31</sup> The low MPO activities in carnitine groups reflected the attenuation of leukocyte accumulation both in plasma and liver tissue. There was not a difference between the L-carnitine groups with regard to decreasing plasma MPO activity. However, L-carnitine administered 2 hours before ischemia was observed to be significantly more effective in decreasing leukocyte accumulation in the liver tissue (**Figure 2**). To our knowledge there is no other study that has used MPO activity to evaluate the effects of L-carnitine in hepatic ischemia and reperfusion procedure. However, the study by Gorur et al<sup>32</sup> found that L-carnitine was associated with decreased inflammation and neutrophil infiltration in kidney tissue in a rat kidney IR injury model, which supports the findings of the present study.

TAOC of body fluids expresses a cooperative interaction between various antioxidants and is crucial for the maximum suppression of a free radical reaction in extracellular compartments.<sup>33</sup> TAOC was significantly decreased during hepatic IR in the present study. In accordance with the present study, TAOC was decreased after hepatic IR in an experimental study by Su et al.<sup>28</sup> The adminis-

tration of L-carnitine was associated with increased TAOC in the present study. However, the increase of TAOC was not significant in group E when compared with group B. L-Carnitine administered after ischemia (group E) was not associated with a significant increase in TAOC. The greatest increase in TAOC was associated with administration of L-carnitine 2 hours before ischemia (**Figure 3**). To our knowledge, no other study has compared TAOC while evaluating the effect of L-carnitine in hepatic IR; but there is an agreement between the results of the present study and a study of valproic acid-induced liver damage in which L-carnitine was associated with increased TAOC.<sup>34</sup> The effect of L-carnitine to increase TAOC is related to its antioxidant effect, which is a result of the scavenging capacity of reactive oxygen species.<sup>8</sup> In our study, L-carnitine administered after hepatic ischemia (group E) did not increase TAOC significantly. Again, this may be associated with the limited efficacy of L-carnitine during acute emergency situations after an ischemic episode.<sup>2</sup>

When the histopathologic changes were taken into consideration, there was accordance with the findings of Atila et al<sup>5</sup> and the present study; carnitine was associated with a protective effect in liver tissue. Liver tissue appeared normal in the control group and damaged in the group that received only the TWHIR procedure (group B). This damage was characterized by severe inflammatory changes in lobular and portal spaces, and hepatocyte necrosis. There was an improvement in the histopathologic scores of the L-carnitine groups, reflecting an attenuation of liver tissue damage. Also, the result of histopathologic scoring was compatible with the improved results of all other biochemical evaluation parameters obtained after administration of carnitine.

### **Study Limitations**

The administration of carnitine through the tail vein in group C was a limitation of this study design. However, this alternate route of administration was preferred to standardize the time period of procedure starting with laparotomy and finishing at the end of reperfusion, and prevent repeated administration and greater amount of anesthetic agent. The small number of rats in each arm of the study made the interpretation of histopathologic results difficult.

### **CONCLUSIONS**

In this experimental study, administration of exogenous L-carnitine was associated with significantly decreased lipid peroxidation in plasma and liver tissue when administered prior to a TWHIR procedure. In addition, L-carnitine seemed to be more effective with regard to decreasing lipid peroxidation in liver tissue when administered before warm hepatic ischemia. L-Carnitine was associated with significantly decreased leukocyte sequestration in plasma and liver tissue. A significant increase in TAOC was associated with L-carnitine administered prior to ischemia. These observations suggest that L-carnitine might have a protective effect against IR injury in rat liver tissue.

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