Urol Res (2010) 38:187–194
DOI 10.1007/s00240-009-0238-9

ORIGINAL PAPER

Comparison of the effects of L-carnitine and α-tocopherol on acute ureteral obstruction-induced renal oxidative imbalance and altered energy metabolism in rats

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Received: 1 April 2009 / Accepted: 4 November 2009 / Published online: 26 November 2009

Abstract The suppression of renal energy metabolism during ureteral obstruction is a well-known phenomenon; however, its exact responsible mechanism(s) and association with simultaneously induced renal oxidative stress have not been clarified. This study examined the improving effects of L-carnitine, a facilitating cofactor for mitochondrial oxidation of fatty-acids as well as a scavenger of free-radicals, and α-tocopherol as the most potent antioxidant on renal metabolic defect and oxidative stress induced by acute unilateral ureteral obstruction (UUO). The left ureter was ligated in ether-anaesthetised rats, in which L-carnitine, α-tocopherol or their vehicles were intraperitoneally injected in four different groups. After elapsing 24 h of UUO-induction, both kidneys were removed and stored at −80°C. There were also two sham-operated and control groups. The kidney samples were assessed to measure the levels of ferric reducing/antioxidant power (FRAP) and malondialdehyde (MDA) for evaluating their redox state, as well as, their amounts of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by using luciferin–luciferase method. As much as 24 h of UUO in vehicle-treated groups caused increases in MDA and ADP, but decreases in FRAP, ATP, and ATP/ADP of the obstructed kidney with respect to those of the sham group. α-tocopherol normalised the levels of MDA and FRAP but did not affect the altered amounts of energy metabolic indices in the obstructed kidney, while L-carnitine could ameliorate all of them. These findings suggest that oxidative stress may not involve in development of acute UUO-induced suppression of renal aerobic metabolism, and probably reduction of energy substrates is a responsible factor.

Keywords Unilateral ureteral obstruction · Obstructive nephropathy · Oxidative stress · Renal energy metabolism · α-Tocopherol · L-Carnitine

Introduction

Obstructive uropathy represents a blockage of urine outflow due to the occurrence of impedance anywhere along the urinary tract, which causes a rise in pressure proximal to the point of obstruction. High back pressure directly and indirectly damages renal parenchyma and leads to obstructive nephropathy, which is an important cause of end-stage renal disease [1]. The renal levels of reactive oxygen species (ROS) are increased in the setting of ureteral obstruction from the early phase [2, 3], and oxidative stress has been shown to contribute in the development of serious complications in obstructive nephropathy, i.e., reduced renal blood flow and glomerular filtration rate (GFR) [4], apoptosis [5], inflammation, and fibrosis [6, 7]. However, the association between

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ureteral obstruction-induced defect of energy metabolism and oxidative stress in the kidney has not been clarified, and even there have been a few reports concerning renal metabolic alterations in obstructive nephropathy during the past two decades.

The intact kidneys, which constitute <1% of total body weight, account for about 10% of total oxygen consumption, and hence, they receive about 20% of cardiac output. The high rate of renal oxygen consumption is mainly determined by sodium reabsorption rate [8]. Absolute sodium reabsorption rate depends on the filtered load of sodium, and therefore, it is expected the fall of GFR in the obstructed kidney to result in reduction of oxygen consumption [9]. Indeed, fall of oxygen consumption was identified in the kidneys of animals subjected to ureteral obstruction with different durations [10, 11], which was then shown to be associated with reduced renal mitochondrial oxidative phosphorylation [12, 13]. Therefore, it is obvious that ureteral obstruction results in suppression of aerobic metabolism in the kidney, but its exact responsible mechanism is yet to be identified. However, several studies have been recently indicated that the increased generation of ROS during the process of ischaemia/reperfusion in kidney [14], heart [15], and brain [16] played important role in damaging mitochondrial inner membrane integrity and respiratory chain proteins to cause mitochondrial bioenergetic defect, which was protected by using different antioxidants.

Vitamin-E is the most important lipid-soluble antioxidant, and α-tocopherol is its main biological active isoform. It is incorporated into plasma and all intracellular membranes, in which it scavenges the chain-propagating peroxyl radicals and hence effectively inhibits lipid peroxidation [17, 18]. L-carnitine is an amino acid derivative, which is synthesised from L-lysine and L-methionine mainly in liver and kidney, and found in nearly all cells of the body. It is an essential cofactor required for the translocation of long-chain fatty-acids into the mitochondrial matrix, where they undergo *β*-oxidation to produce adenosine triphosphate (ATP) [19]. In addition, the importance of L-carnitine as an antioxidant has attracted increasing attention over the past decade [20], which was also proven in acute [21] and chronic [22] renal failure. Therefore, for the first time, the improving effects of L-carnitine on acute unilateral ureteral obstruction (UUO)-induced renal aerobic metabolic defect and oxidative stress have been investigated in the present study. Furthermore, α-tocopherol has been used as a main antioxidant during acute UUO to determine the inter-relation between changes in redox state and oxidative metabolic alterations in both obstructed and unobstructed kidneys.

Materials and methods

Animal and tissue preparation

Male Sprague-Dawley rats weighing 250–350 g were anaesthetised by ether (Diethylether 99.7%; Merck, Darmstadt, Germany), via putting each animal in a big lidded glass-container having a piece of cotton soaked by about 5 ml of ether. Then, animal was taken out and a small suprapubic incision at left retroperitoneal site was made. Thereafter, the left ureter was identified by using a surgical microscope (SZ2-ET; Olympus, Japan) and ligated by 4–0 silk suture at two points, and finally incision was closed at two layers by 4–0 silk [13]. The total surgical procedure was done in less than half an hour, during which a small container containing ether-soaked cotton was put in front of animal's nose when needed, to maintain anaesthesia. In sham-operated rats, left ureter was similarly exposed and manipulated but not occluded. Rectal temperature was maintained at 37 ± 1°C by using a heated lamp during the entire period of surgical procedure, which was performed under complete sterile condition. Procaine penicillin (40,000 U) and streptomycin (50 mg) were intramuscularly injected [23], and animals were allowed to recover from the anaesthesia prior returning to individual cages. The rats subjected to UUO were divided into four groups of 10 animals each. There were intraperitoneally (i.p.) 1-ml injections of normal saline containing 200 mg/kg L-carnitine hydrochloride (Sigma, Poole, Dorset, UK) in the UUO + LC group and normal saline alone in the UUO group at 15 min before and 12 h after UUO-induction. In the UUO + AT and UUO + OO groups, rats were i.p. given 50 mg/kg α-tocopherol acetate (Sigma) dissolved in olive oil and olive oil alone, respectively, of 1-ml volume at 6 h before and 9 h after UUO-induction. Therapeutic range for the treatment with α-tocopherol varies widely, between 7 to 1,000 mg/kg/day in rats [24], therefore, according to its pharmacokinetics [25] and previous reports using it [16, 26, 27] and also similarly for L-carnitine [21, 22, 28], their doses and times of injections were chosen in order to be effective in encountering with alterations of renal oxidative status and metabolism from the beginning and during 24-h of UUO. Sham-operated rats (sham group, $n = 10$) also received normal saline twice, same as the UUO group. There was also a control group ($n = 10$), in which rats did not experience operational procedure and injections. The rats subjected to UUO and sham operation were re-anaesthetised and control rats were anaesthetised, and all of them underwent midline laparotomy. After elapsing exactly 24 h of ureteral occlusion in the four obstructive groups, equivalent period in the sham group, or immediately in the control group, both kidneys were removed, decapsulated, and longitudinally sectioned on ice, frozen in liquid nitrogen, and

preserved at −80°C. Rats were killed by injecting an overdose of pentobarbital anaesthetic, and all experiments were performed in accordance with the international conventions on animal experimentation and approved by the Ethics Committee of Shiraz University (Medical Sciences).

Ferric reducing/antioxidant power (FRAP) assay

The FRAP was assessed based on the method of Benzie and Strain [29], in which the fresh FRAP reagent was prepared through mixing 50 ml of 300 mM acetate buffer (pH = 3.6), 5 ml of 20 mM $FeCl_3 \cdot 6H_2O$, and 5 ml of tripyridyl-s-triazine (TPTZ; Fluka Chemie, Buchs, Switzerland) solution (10 mM in 40 mM HCl). A set of standard solutions of Fe^{2+} with different concentrations was also made from $FeSO_4 \cdot 7H_2O$ in distilled water.

A piece of each frozen kidney sample was rapidly weighed and transferred into cold sodium phosphate buffer (1:10 W/V) in a test tube, and then homogenised using a Griffin & Gorge homogeniser (Loughborough, UK) in a cover of ice. As much as 50 µl of the tissue homogenate or each of the standard solutions was added to 1.5 ml of prewarmed (37°C) FRAP reagent in a test tube and incubated in the water bath (17AD-9; Precision Scientific, USA) at 37°C for 10 min. The absorbance of the blue coloured complex was read against the reagent blank (1.5 ml FRAP reagent + 50 µl distilled water) using a spectrophotometer (UV/Vis-7500; Spectrolab, Newbury, England) at 533 nm. After constructing the standard curve, the FRAP levels of tissue samples were expressed as µmol of Fe^{3+} reduced to Fe^{2+} per gram of kidney weight (µmol/gKW).

Malondialdehyde (MDA) assay

The levels of renal tissue MDA was determined according to the procedure of Ohkawa et al. [30]. After weighing each kidney sample, it was homogenised in ice-cold phosphate-buffered saline (1:10 W/V, pH = 7.4). As much as 200 µl of homogenate was mixed with 200 µl of 8.1% sodium dodecyl sulphate and 1.5 ml of 20% acetic acid, and then pH was adjusted to 3.5. Thereafter, 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (Merck, NJ, USA) was added to the mixture, and it was kept in water bath at 95°C for 60 min. After cooling, 4 ml of *n*-butanol (Merck, NJ, USA) was added to the mixture and was shaken vigorously by a vortex, and then centrifuged at 4,000 rpm for 10 min. The pink-coloured supernatant was removed and its absorbance was read at 532 nm. Various concentrations of 1,1,3,3-tetramethoxypropane (Sigma) were prepared for constructing the standard curve in order to be used for determining the MDA content of tissue samples in nmol/gKW.

Measurements of adenosine triphosphate (ATP) and adenosine diphosphate (ADP)

Measurements of renal ATP and ADP contents were derived from the method of Lundin et al. [31], which was based on the reaction catalysed by luciferase in the presence of luciferin, ATP, Mg^{2+}, and O_2 to produce bioluminescence. When ATP was the limiting component in this reaction, the intensity of the emitted light was proportional to the concentration of ATP. The *Lampyris turkestanicus* luciferase was expressed in *E. coli* cells, strain *BL21* using pET expression system, and purified with Ni-NTA Sepharose (Qiagen, Hilden, Germany) column to homogeneity. The purity of the luciferase was confirmed by SDS-PAGE [32]. The protein concentration was measured by the method of Bradford [33].

Kidney samples were weighed and homogenised in 10% trichloroacetic acid (1:10 W/V) in separate test tubes, and their pH were adjusted to 7.65–7.75 with phosphate buffer. In a sterile and dry cuvette, 190 µl of Tris buffer, 10 µl of tissue homogenate and 50 µl of luciferin–luciferase mixture (40 µg/ml luciferase and 1 mM luciferin, Sigma) were added and quickly vortexed. Then, it was put in a sirius-single tube luminometer (Berthold Detection Systems GmbH, Germany) to have the reading of ATP after subtracting the background.

To measure ADP concentration, 178 µl of Tris buffer, 10 µl of tissue homogenate, 7 µl of pyruvate kinase (Roche, Mannheim, Germany), and 5 µl of phosphoenolpyruvate (Sigma) were mixed in a test tube, quickly vortexed, and left at room temperature for 6 min in order that all of the ADPs in tissue homogenate to be converted into ATPs. Then, 50 µl of luciferin–luciferase mixture were added to this mixture in a cuvette and its reading was subtracted from the first ATP reading to have reading of ADP.

Serial concentrations of ATP (Sigma) were prepared and after adding luciferin–luciferase and taking their readings by luminometer, the standard curve was constructed for determining the ATP and ADP amounts of tissue samples in µmol/gKW. For better evaluation of mitochondrial oxidative phosphorylation, renal tissue ATP to ADP ratio (ATP/ADP) and total ATP plus ADP (ATP + ADP) amounts were also calculated.

Statistical analysis

Data are presented as mean ± SD. The comparisons between right and left renal values in each group were assessed using the Student's paired *t* test. The values of equivalent kidneys were compared between groups by one-way ANOVA followed by Duncan's post-hoc test, and then LSD test for determining the level of *P* values. All the data

analyses were performed using SPSS 11.5 software and significance was taken at $P < 0.05$.

Results

In the right and left kidneys of sham group, the levels of MDA (38.2 ± 4.3 and 38.6 ± 3.2 nmol/gKW, respectively, Fig. 1a), FRAP (4.34 ± 0.72 and 4.28 ± 0.85 µmol/gKW, respectively, Fig. 1b), ATP (1.99 ± 0.44 and 2.26 ± 0.62 µmol/gKW, respectively, Fig. 2a), ADP (0.45 ± 0.11 and 0.47 ± 0.14 µmol/gKW, respectively, Fig. 2b), ATP + ADP (2.44 ± 0.49 and 2.74 ± 0.68 µmol/gKW, respectively, Fig. 2c), and ATP/ADP (4.66 ± 1.69 and 5.11 ± 1.77, respectively, Fig. 2d) were not different from each other and from those of equivalent kidneys in control group. Hence, sham-operation and saline infusion did not affect renal oxidative balance and metabolism of energy. In addition, there were no differences in the values of indices for oxidative status (Fig. 1) and energy metabolism (Fig. 2) of both obstructed and unobstructed kidneys between rats subjected to 24 h of UUO and received either vehicles of normal saline (UUO group) or olive oil (UUO + OO group).

Effects of α-tocopherol and L-carnitine on UUO-induced changes in renal redox status

Figure 1a shows that 24 h of UUO caused MDA contents in UUO and UUO + OO groups to be increased by 33% and 37% in obstructed kidney, respectively, but not to be changed in unobstructed kidney from those of equivalent kidneys in sham group, and also to become different in opposite kidneys of both groups. Administration of α-tocopherol in UUO + AT group was able to largely reduce MDA levels of both obstructed and unobstructed kidneys in comparison to those of UUO + OO group, so that they became equal to and lower than (13%), respectively, those of sham group, and also different from each other. In L-carnitine-treated (UUO + LC) group, MDA of obstructed kidney was significantly decreased in comparison to UUO group, but it was still higher than those of sham (12%) and UUO + AT groups, as well as its own contralateral kidney. In unobstructed kidney of UUO + LC group, MDA level was lower than that of UUO group and similar to those of sham and UUO + AT groups.

Figure 1b indicates reductions in FRAP of both obstructed and unobstructed kidneys in UUO group (43% and 21%, respectively) and UUO + OO group (38% and 18%, respectively) compared to those of equivalent kidneys in sham group. Of course, FRAP was lower in obstructed kidney than unobstructed kidney in both groups. FRAP of obstructed kidney was elevated in UUO + AT group in comparison to UUO + OO group, and reached to the level of sham group. While, unobstructed kidney of UUO + AT group had higher FRAP value than equivalent kidney of UUO + OO and sham (32%) groups as well as its own contralateral kidney. In UUO + LC group, FRAP of obstructed kidney was larger than that of UUO group and not statistically different from those of sham and UUO + AT groups. In addition, FRAP of unobstructed kidney was increased in UUO + LC group with respect to UUO and sham (26%) groups and its own contralateral kidney, but it was equal to that of UUO + AT group.

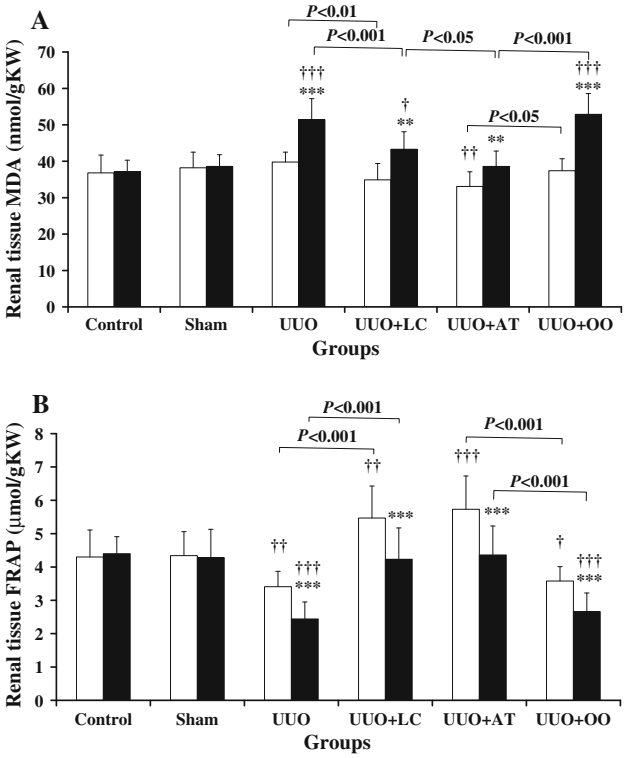

Fig. 1 Effects of L-carnitine and α-tocopherol on UUO-induced alterations in the levels of **a** malondialdehyde (MDA) and **b** ferric reducing/antioxidant power (FRAP) in the right (*open square*) and left (*filled square*) kidneys of rats subjected to 24 h of left unilateral ureteral obstruction (UUO) that received normal saline (UUO group), L-carnitine (UUO + LC group), olive oil (UUO + OO group), or α-tocopherol (UUO + AT group). In the sham group, left ureter was manipulated but not occluded and normal saline was infused, which were not performed in the control group. Data are mean ± SD ($n = 10$ in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for comparison between right and left kidneys in each group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$, versus right or left kidney of the sham group

Effects of α-tocopherol and L-carnitine on UUO-induced changes in renal energy metabolism

Figure 2a shows that 24 h of ureteral obstruction in UUO, UUO + OO, and UUO + AT groups caused ATP contents to be similarly declined by 52–55% in obstructed kidney

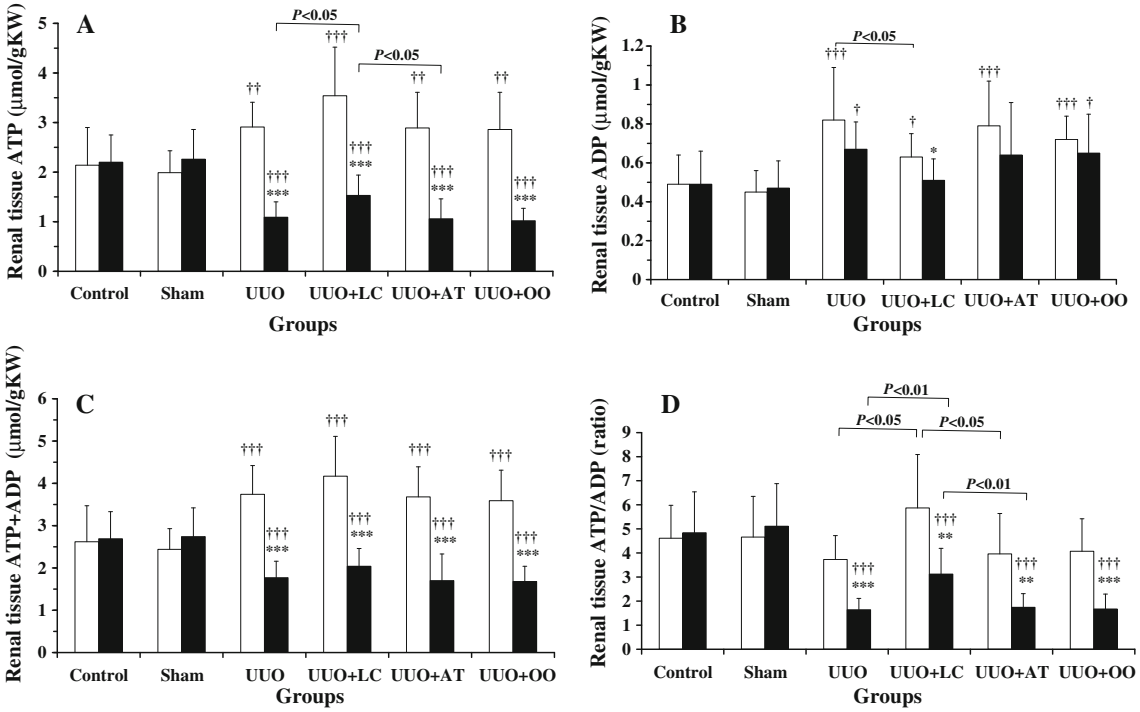

Fig. 2 Effects of L-carnitine and α-tocopherol on UUO-induced alterations in the levels of **a** adenosine triphosphate (ATP), **b** adenosine diphosphate (ADP), **c** total ATP plus ADP (ATP + ADP), and **d** ATP to ADP ratio (ATP/ADP) in the right (*open square*) and left (*filled square*) kidneys of rats subjected to 24 h of left unilateral ureteral obstruction (UUO) that received normal saline (UUO group), L-carnitine (UUO + LC group), olive oil (UUO + OO group), or α-tocopherol (UUO + AT group). In the sham group, left ureter was manipulated but not occluded and normal saline was infused, which were not performed in the control group. Data are mean ± SD ($n = 10$ in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for comparison between right and left kidneys in each group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$, versus right or left kidney of the sham group

and risen by 44–46% in unobstructed kidney with respect to those of equivalent kidneys in sham group, and therefore, there was considerable difference between ATP of the two opposite kidneys in these three groups. Administration of L-carnitine in the UUO + LC group led to elevation of ATP in obstructed kidney compared to UUO and UUO + AT groups, but it was still 32% lower than that in equivalent kidney of sham group. Whereas, ATP in unobstructed kidney of UUO + LC group was not statistically increased with respect to UUO and UUO + AT groups, but it was higher than those in equivalent kidney of sham group (78%) and its own contralateral kidney.

Figure 2b indicates that ADP contents were increased both in obstructed and in unobstructed kidneys in the groups of UUO (43% and 82%, respectively), UUO + OO (38% and 60%, respectively), and UUO + AT (36% not significantly, and 76%, respectively) compared to those of equivalent kidneys in sham group. In addition, levels of ADP were not statistically different between opposite kidneys in UUO, UUO + OO, and UUO + AT groups, and also between equivalent kidneys of these groups. In UUO + LC group, although ADP of obstructed kidney was not statistically reduced in comparison to UUO group, it did not become significantly larger than that of sham group. However, ADP of unobstructed kidney in UUO + LC group was lower than that in UUO group, but it was higher than those of equivalent kidney in sham group (40%) and its own contralateral kidney.

Figure 2c presents that ATP + ADP amounts of the UUO, UUO + OO, UUO + AT, and UUO + LC groups were lower by 35%, 39%, 38%, and 26%, respectively, in obstructed kidney, but higher by 53%, 47%, 51%, and 71%, respectively, in unobstructed kidney than those of equivalent kidneys of sham group. In addition, the contents of ATP + ADP in all four groups were similar between equivalent kidneys and lower in obstructed kidney than unobstructed kidney.

Figure 2d shows that 24 h of UUO caused ATP/ADP to be decreased in obstructed kidney of the UUO, UUO + OO, and UUO + AT groups to the same levels, but not to be significantly changed in their unobstructed kidney with respect to those of equivalent kidneys in sham group. There was also large difference between ATP/ADP values of the opposite kidneys in these three groups. Administration of L-carnitine in UUO + LC group led to improvement of ATP/ADP in both obstructed and unobstructed kidneys compared to those of UUO and UUO + AT groups. However, its values were still lower in the obstructed kidney and not

significantly higher in unobstructed than those of equivalent kidneys in sham group, and they were also different from each other.

Discussion

It is well known that defect in renal energy metabolism occurred in response to ureteral obstruction; however, its exact responsible mechanism has not been fully clarified [1, 9]. Using animal model of UUO for investigating obstructive nephropathy has proven useful for more than 30 years [34] and the model of 24-h UUO was chosen for this study, because it has been shown to be associated with disturbances in renal energy metabolism [12, 13], oxidative balance [2], hemodynamics, and tubular function [35, 36], but without severe structural damages and any fibrosis of the kidney [1, 37]. In addition, the contralateral normal kidney can perform compensatory increase in function [35, 36] to prevent solute retention, volume expansion, and uremia during period of obstruction [12]. Hence, in the present study, contralateral unobstructed kidney in both vehicle-treated groups of UUO and UUO + OO groups produced more ATPs to supply the energy need for its overfunction, the usage of which led them to rise in ADP levels. With regard to increase in ATP + ADP amount and no change in ATP/ADP ratio of unobstructed kidney in both groups, it became obvious that 24 h of UUO resulted in up-regulation of high-energy nucleotides production through normal stimulation of mitochondrial oxidative phosphorylation in contralateral kidney to provide more energy for its compensatory increase in function.

In the obstructed kidney of UUO and UUO + OO groups, decrement of ATP content by about 50% was associated with about 40% increase in ADP level that caused ATP/ADP to be reduced by about 70% from their normal values. Since mitochondria provide roughly 95% of total renal ATP production [38], it can be suggested that 24 h of UUO resulted in suppression of mitochondrial oxidative phosphorylation in obstructed kidney, so that elevated ADPs could not be converted to ATPs. Blondin et al. [12] also indicated that the 51% reduction of ATP level in post-obstructed kidney of rats at few hours after release of 24 h of UUO might be due to the mitochondrial damage observed under electron microscope.

The increased values of MDA, as the final product of lipid peroxidation by ROS [39], and decreases in FRAP levels, as a direct measure of total antioxidant activities [29, 40], in obstructed kidney of UUO and UUO + OO groups indicated that 24 h of ureteral obstruction-induced oxidative stress through not only enhanced generation of ROS but also decreased ability of antioxidant defence system, which is in consistent with other reports [2, 4, 36]. On the other hand, in unobstructed kidney of UUO and UUO + OO groups, the lowered levels of FRAP were associated with no significant changes in MDA values. Since contralateral kidney performed compensatory overfunction during 24 h of UUO, the increased production of ROS was probable in it [36, 41]. However, the overactivities of antioxidant defence mechanisms and their usage, which can be deduced by the lowered level of FRAP, could prevent development of oxidative stress in the unobstructed kidney.

Gulcin [20] showed L-carnitine to be an effective antioxidant in different in vitro assays including reducing power, free-radical scavenging, superoxide anion scavenging, hydrogen peroxide scavenging and etc., which were enhanced with increasing concentration of L-carnitine. However, he found that L-carnitine at comparable concentrations to α-tocopherol had lower levels of effects in these assays. Since the renal tissue concentrations of L-carnitine in the UUO + LC group and α-tocopherol in the UUO + AT group were not measured in the present study, having such comparison was not possible. But, it can be said that α-tocopherol acted against lipid peroxidation better than L-carnitine with the doses used for them in this study, and hence 24 h of UUO-induced oxidative stress in obstructed kidney was prevented completely in UUO + AT group but partially in UUO + LC group. Whereas, the amounts of administrated α-tocopherol and L-carnitine were sufficient to prevent the fall in total antioxidant activity of obstructed kidney and kept its FRAP at the normal level.

It has been shown that acute ischemia/reperfusion-induced oxidative stress in isolated-perfused rat heart [15] and in vivo rat kidney [14] involved in development of defect in mitochondrial ATP production capacity, probably through disturbing mitochondrial membrane integrity. In an elaborate study, Hurtado et al. [16] also demonstrated that 15 min of focal cerebral ischaemia in rat caused significant fall in ATP level of brain cortex, which was prevented by injection of either three mitochondrial accessible antioxidants of α-tocopherol, reduced glutathione (GSH), or MnTBAP as a superoxide dismutase mimetic at 2 h before ischaemia. In contrast, prevention or amelioration of lipopolysaccharide-induced oxidative stress in rat brain [42] and guinea pig kidney [43] by pre-treatment with different antioxidants, did not have any effect on their disturbed mitochondrial energy metabolism. In the present study, administration of α-tocopherol, which completely prevented UUO-induced oxidative stress in obstructed kidney and caused unobstructed kidney to have redox state even better than normal condition, could not affect the alterations, in ATP, ADP, ATP + ADP, and ATP/ADP levels, of both kidneys. Therefore, it seems that the changes in renal ROS levels might not play important role in the altered energy metabolism of obstructed and unobstructed kidneys during 24 h of UUO, and hence

other factors were probably involved in UUO-induced renal metabolic disturbances.

In a rare recent study concerning UUO effect on renal metabolism, Krarup et al. [44] applied microdialysis probes into porcine kidney and found fall in cortical interstitial glucose concentration after 240 min of UUO, and related it to acute ureteral obstruction-induced decreases in tubular glucose reabsorption and gluconeogenesis. Also, it had been demonstrated that 24 h of UUO in rat caused increases in triglyceride synthesis and content of obstructed kidney, which was due to both a decrease in fatty-acid oxidation and an increased release of fatty-acids from phospholipids [45]. In the present study, L-carnitine administration resulted in conversion of more ADPs to ATPs in obstructed and unobstructed kidneys, and hence it decreased ADP and increased ATP contents while kept ATP + ADP amount unchanged and elevated ATP/ADP of both kidneys in the UUO + LC group with respect to UUO group. Therefore, treatment with L-carnitine, as a cofactor required for oxidation of fatty-acids in the mitochondria, did improve ureteral obstruction-induced renal energy metabolic suppression, which was most probably not due to its antioxidant property. Totally, it can be suggested that the deficiency of energy substrates might importantly contribute to disturbed mitochondrial aerobic metabolism in acute ureter-obstructed kidney.

In summary, the results of this study showed that 24 h of UUO induced oxidative stress and suppression of aerobic energy metabolism in obstructed kidney, whereas it resulted in a compensatory increase of function in association with regulated stimulation of energy metabolism and without increased ROS levels in unobstructed kidney. Administration of α-tocopherol prevented the appearance of oxidative imbalance in obstructed kidney but did not alter its indices of energy metabolism, suggesting that development of oxidative stress did not probably contribute in the suppression of renal aerobic metabolism induced by acute ureteral obstruction. However, treatment with L-carnitine during 24 h of UUO, which partially improved redox status, could increase conversion of ADP to ATP and elevate ATP/ADP ratio in obstructed kidney. Therefore, L-carnitine ameliorated disturbed renal energy production, not by its antioxidant effect, but through stimulating β-oxidation of fatty-acids in the mitochondria, which more likely indicated to deficit of energy substrates as one of the major responsible factors for the suppressed renal aerobic metabolism during acute UUO. This will open new perspectives for the use of L-carnitine in the treatment of renal dysfunctions associated with ureteral obstruction.

Acknowledgments The authors acknowledge the Research Council of Shiraz University (Medical Sciences), Shiraz, Iran for the financial support of this study (grant number: 84-2434). The authors state no conflict of interest.

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