Effects of L-Carnitine on Oxidative Stress Responses in Patients with Renal Disease

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ABSTRACT

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Purpose: Hemodialyzed patients demonstrate elevated oxidative stress and reduced functional status. Exercise induces health benefits, but acute exertion up-regulates oxidative stress responses in patients undergoing hemodialysis. Therefore, the aim of the present study was to examine the effect of L-carnitine supplementation on i) exercise performance and ii) blood redox status both at rest and after exercise. **Methods**: Twelve hemodialysis patients received either L-carnitine (20 $mg \cdot kg^{-1}$ i.v.) or placebo in a double-blind, placebo-controlled, counterbalanced, and crossover design for 8 wk. Participants performed an exercise test to exhaustion before and after supplementation. During the test, $\dot{V}O_2$, respiratory quotient, heart rate, and time to exhaustion were monitored. Blood samples, collected before and after exercise, were analyzed for lactate, malondialdehyde, protein carbonyls, reduced and oxidized glutathione, antioxidant capacity, catalase, and glutathione peroxidase activity. **Results**: Blood carnitine increased by L-carnitine supplementation proportionately at rest and after exercise. L-carnitine supplementation increased time to fatigue (22%) and decreased postexercise lactate (37%), submaximal heart rate, and respiratory quotient but did not affect $\dot{V}O_{2peak}$. L-carnitine supplementation increased reduced/oxidized glutathione (2.7-fold at rest, 4-fold postexercise) and glutathione peroxidase activity (4.5% at rest, 10% postexercise) and decreased malondialdehyde (19% at rest and postexercise) and protein carbonyl (27% at rest, 40% postexercise) concentration. **Conclusions**: Data suggest that a 2-month L-carnitine supplementation may be effective in attenuating oxidative stress responses, enhancing antioxidant status, and improving performance of patients with end-stage renal disease.

Key Words: ANTIOXIDANT STATUS, FUNCTIONAL CAPACITY, HEMODIALYSIS, EXERCISE

Oxidative stress is defined as a loss of the normal balance between the reactive oxygen species (ROS) production and the antioxidant system and is considered a major factor in dialysis-induced morbidity and mortality (12). In addition to an excess ROS generation, uremic patients have a reduced antioxidant capacity that predisposes them to cellular oxidative damage, which may contribute to the development of inflammation, infections, anemia, amyloidosis, and ischemic and toxic renal tubular injury and acceleration of atherosclerosis (12). ROS production in patients with end-stage renal disease (ESRD) undergoing hemodialysis is mainly attributed to neutrophil activation during dialysis as a result of interaction of blood with the dialysis membrane and endotoxin-contaminated dialysate, iron overload, alterations in erythrocyte pentose–phosphate shunt activity, abundance of advanced glycation end products, high homocysteine levels, intradialytic cytokine activation, advanced age, high prevalence of diabetes, malnutrition, chronic inflammation, reduced antioxidant capacity, and so forth (33).

L-carnitine, a small water-soluble organic solute, along with its natural esters acylcarnitines, forms a component of the endogenous carnitine pool in humans (29). Carnitine is a cofactor required for transport of long-chain fatty acids into

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the mitochondrial matrix, where they undergo β-oxidation for cellular energy production and for removal of fat metabolism by-products and toxic compounds from the cells (13). Although L-carnitine deficiency is an infrequent problem in a healthy, well-nourished population consuming adequate protein, ESRD patients undergoing hemodialysis may develop metabolic and functional disturbances because of acute removal of carnitine during dialysis, intestinal malabsorption, reduced renal synthesis, altered membrane transport mechanisms, or increased carnitine requirements (13). However, its decline in plasma and muscle has not been consistently confirmed (13).

Hemodialyzed patients (HD) exhibit severely impaired exercise tolerance because of muscle atrophy, renal anemia, inactivity, malnutrition, and impaired muscle energetics (14). Carnitine deficiency in patients with ESRD may cause metabolic and functional disturbances because of acute removal of carnitine during dialysis, thereby contributing to the augmented morbidity, mortality, and deteriorated quality of life of these patients (26). Although acute exercise augments ROS generation and alters antioxidant status (7) in various tissues, regular exercise training evokes numerous health benefits in this patient population (16). Carnitine supplementation may be able to reduce tissue oxidative damage after exercise and aid muscle tissue repair and remodeling (36). Induced ischemia of endothelial cells appears to evoke carnitine release and oxidative injury, a response that was attenuated by subsequent carnitine ingestion, suggesting an anti-inflammatory role for carnitine (6). These observations indicate that carnitine may improve the postexercise inflammatory response, which involves an oxidative burst by immune cells. These claims are extrapolated by findings suggesting that L-carnitine may act as an antioxidant in patients with ESRD and in healthy adults (1,2). However, we have not found another study that examined the effects of L-carnitine supplementation on exercise-induced oxidative stress responses in this patient population.

Because of carnitine's significant physiological function, numerous investigations examined whether its exogenous administration provides an ergogenic effect during exercise, mainly by measuring fatigue, fat oxidation, aerobic capacity, and lactate concentration (13,30). These studies produced equivocal results that are mainly attributed to the inability to raise muscle carnitine concentrations after its supplementation (30). Furthermore, the effects of carnitine supplementation on physical performance of patients undergoing maintenance and peritoneal dialysis have not been adequately investigated, whereas the few clinical studies performed have generated inconsistent results because of differences in the experimental design, patient selection criteria, sample sizes, and carnitine dosage protocols (13).

In the view of the above observations, the aim of the present study was to examine the effect of exogenous L-carnitine supplementation on i) exercise performance and ii) indices of oxidative stress and antioxidant status in patients undergoing hemodialysis both at rest and after an acute bout of exercise.

MATERIALS AND METHODS

Subjects. Twelve male patients undergoing hemodialysis, recruited from our outpatient hemodialysis unit, volunteered to participate in the present study. The procedures were in accordance with the 1975 Declaration of Helsinki, and approval was received from the institutional review board. Written informed consent was signed by all participants. The inclusion criteria were as follows: a) chronic therapy for at least 1 yr before the study; b) 4-h HD sessions, thrice weekly (mean $K_t/V \geq 1.4$), with standard bicarbonate dialysis using biocompatible membranes (low-flux polysulfone); c) hemoglobin levels ≥ 11 g·dL^{-1} (erythropoiesis-stimulating agents were administered to all patients); d) absence of antioxidant supplementation (vitamin E, statins, or any medication for the reduction of uric acid); e) adequate nourishment (total serum protein 6.8 ± 0.5 g·dL^{-1} and serum albumin 4.3 ± 0.2 g·dL^{-1}; f) no residual renal function; g) ability to perform stationary cycling; and h) had not received any L-carnitine treatment in the previous 6 months. Exclusion criteria were as follows: a) the presence of any active infectious/inflammatory disease (serum C-reactive protein levels $\geq 0.5 \pm 0.4$ mg·dL^{-1}); b) uncontrolled hypertension and diabetes mellitus; c) diseases that might interfere with exercise capacity and/or be exacerbated by activity such as ischemic cardiopathy or symptoms related to coronary artery disease, anemia (hemoglobin levels < 11 g·dL^{-1}, hematocrit $< 33\%$), chronic lung disease, and orthopedic disorders; d) use of steroids, immunosuppressives, and psychotropic agents; and e) hospitalization within 3 months before the study. The participants' characteristics are shown in Table 1.

Experimental design. Patients received either L-carnitine or placebo for 8 wk in a counterbalanced fashion (same patients received both L-carnitine and placebo in a random order) using a crossover, double-blind, repeated-measures design. During their first visit for each trial (placebo or carnitine), subjects were taught how to complete a 5-d diet recall questionnaire and had their anthropometric profile measured. In their second visit, subjects returned their diet recall forms and underwent a progressive diagnostic test to exhaustion (GXT) on a stationary cycle ergometer to evaluate their peak oxygen consumption ($\dot{V}O_{2peak}$) while blood was collected before and immediately after testing. Thereafter, participants entered the trial phase, during which they received either

TABLE 1. The physical and clinical characteristics of the subjects.

Age (yr)	53.8 ± 2.3
Height (m)	1.72 ± 0.02
Weight (kg)	78.9 ± 2.1
BMI (kg·m^{-2})	26.5 ± 0.8
Body fat (%)	25.2 ± 1.1
Dialysis vintage (months)	84.9 ± 2.9
Residual urea clearance (mL·min^{-1})	1.3 ± 0.3
Interdialytic weight gain (kg)	2.7 ± 0.8
Predialysis potassium (mmol·L^{-1})	5.3 ± 0.5
Predialysis BUN (mg·100 mL^{-1})	62 ± 4.8
K_t/V	1.4 ± 0.5

Values are presented as means ± SE.
BMI, body mass index; BUN, blood urea nitrogen; K_t/V, dialyzer clearance of urea.

placebo or L-carnitine. Nutrition evaluation, measurement of anthropometric profile, and exercise testing (and blood sampling) was repeated (at the same time of day and in the same order) after the first trial (placebo/L-carnitine) as well as before and after the second trial (placebo/L-carnitine). Testing took place 24 h after the last supplementation session. There was a 3-month washout period between trials. We tried to control for the order effect by using a counterbalanced design, in which half of the participants received the placebo and half the carnitine and then the reverse. Indeed, a paired *t*-test for the presupplementation period in both the rest and the exercise values showed no order effect for any of the dependent variables measured in the present study. The experimental design of the study is illustrated in Figure 1.

Supplementation protocols. L-carnitine (Carnitine Superamin Inj Fl., Vianex, Athens, Greece; purity > 99%) or placebo (saline of equal dose) was administered intravenously after each dialysis session, at a dose of 20 $mg \cdot kg^{-1}$ of dry body weight (as a slow 2- to 3-min bolus) three times per week for 8 wk. Each supplement was dissolved in distilled water, and a week's supply was stored in amber-colored bottles with a coded label and dosing instructions.

Exercise stress testing. Cardiopulmonary exercise testing was performed on an upright stationary cycle ergometer (Monark 834E, Varberg, Sweden) before the HD session. The testing protocol included an initial workload of 10–20 W for 1 min. Thereafter, the workload increased by 5–10 W each minute until exhaustion (7). A 12-lead ECG, perceived exertion, heart rate, and brachial artery cuff pressure were monitored continuously during the test and recovery (7). $\dot{V}O_{2peak}$ was evaluated with a pulmonary gas exchange system (Oxycon Mobile, Yorba Linda, CA). Oxygen uptake was measured continuously via breath-by-breath analysis with the use of a computerized system. Patients stopped the exercise because of general fatigue or leg pain before an objective criterion for exercise cessation occurred. The $\dot{V}O_{2peak}$ was taken as the largest $\dot{V}O_2$ characterized by a plateau achieved during the test while the exercise time to exhaustion was recorded.

Anthropometric measurements. Body weight was measured to the nearest 0.5 kg (Beam Balance 710; Seca, UK), with subjects lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208; Seca). Body mass index was calculated as the ratio of body weight (kg)/height (m^2). Percentage body fat was calculated from seven skinfold measures (Jackson and Pollock equation) using a Harpenden caliper (John Bull, UK) (15).

Diet records. To examine whether dietary habits influenced oxidative stress variables and antioxidant status outcomes, 5-d diet recalls were completed. A trained dietician taught the subjects how to complete diet recall questionnaires and to determine food serving and sizes. Diet records were analyzed using the computerized nutritional analysis system Science Fit Diet 200A (Science Technologies, Athens, Greece).

Blood collection and handling. Blood samples (10 mL) were drawn from a forearm vein with subjects in a seated position. Whole-blood lysates were produced by adding 5% trichloroacetic acid (TCA) to whole blood (1:1 v/v) collected in plain tubes, vortexed vigorously, and centrifuged at 4000*g* for 10 min at 4°C. The supernatants were removed and centrifuged again (28,000*g*, 5 min, 4°C). The clear supernatants were transferred to Eppendorf tubes and were used for reduced (GSH) and oxidized (GSSG) glutathione determination. Another blood portion was collected in plain tubes, left on ice to clot for 20 min, and centrifuged (1500*g*, 10 min, 4°C) for serum separation. Serum was transferred in tubes and was used for the determination of malondialdehyde (MDA), protein carbonyls (PC), catalase, and total antioxidant capacity (TAC). Another blood portion (1 mL) was collected in heparin-coated tubes for the determination of glutathione peroxidase (GPX) activity. Blood samples were stored in multiple aliquots (–70°C) and thawed only once before analysis. A blood aliquot (1 mL) was immediately mixed with EDTA to prevent clotting for hematology. Blood samples were protected from light and auto-oxidation.

Analytical methods. Total carnitine (TC) was measured using a standard spectophotometric rate assay at 405/600 nm (RK-2500; Kainos Laboratories, Inc., Tokyo, Japan). A small quantity of venous blood (200 μL) was immediately added to 400 μL of 5% TCA and centrifuged (2500 rpm, 15 min). The supernatant was removed and frozen at −70°C until analyzed for lactate by an enzymatic method (7), with materials purchased from Sigma Chemical (St. Louis, MO).

Serum malondialdehyde (MDA) levels were measured by reverse-phase high-performance liquid chromatography with fluorimetric detection (excitation 532 nm and emission 550 nm) as previously described (22). PC, GSH, GSSG, TAC, and

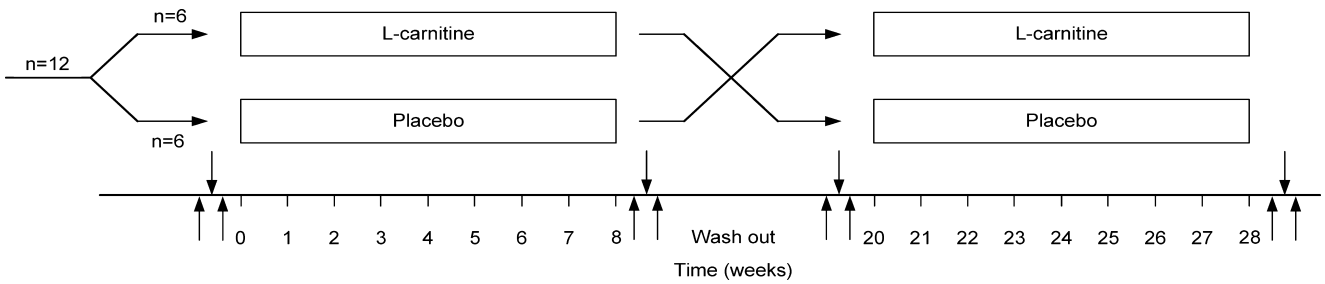

FIGURE 1—The experimental design of the study. Downward arrows indicate an acute exercise session, and upward arrows indicate blood samplings.

catalase were assayed with methods previously described (23). In brief, for PC analysis, 50 μL of serum was added to 50 μL of 20% TCA, incubated in ice bath for 15 min, and centrifuged (15,000*g*, 5 min, 4°C). The supernatant was discarded, and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (in 2.5 N HCL) for the sample or 500 μL of 2.5 N HCL for the blank was added in the pellet. The samples were incubated in the dark at room temperature for 1 h with intermittent vortex every 15 min and centrifuged (15,000*g*, 5 min, 4°C). The supernatant was discarded, and 1 mL of 10% TCA was added, vortexed, and centrifuged at (15,000*g*, 5 min, 4°C). The supernatant was discarded, and 1 mL of ethanol-ethyl acetate (1:1 v/v) was added, vortexed, and centrifuged (15,000*g*, 5 min, 4°C). The washing step was repeated two more times. The supernatant was discarded, and 1 mL of 5 M urea (pH 2.3) was added, vortexed, and incubated (37°C, 15 min). The samples were centrifuged (15,000*g*, 3 min, 4°C), and the absorbance was read at 375 nm. For GSH determination, 20 μL of whole blood treated with TCA was mixed with 660 μL of 67 mM sodium-potassium phosphate (pH 8.0) and 330 μL of 1 mM 5,5′-dithiobis-2-nitrobenzoate. The samples were incubated in the dark at room temperature for 45 min, and the absorbance was read at 412 nm. For GSSG determination, 260 μL of whole blood treated with TCA was neutralized up to pH 7.0–7.5 with NaOH. After the addition of 4 μL of 2-vinyl pyridine, samples were incubated for 2 h at room temperature. Five microliters of whole blood treated with TCA was mixed with 600 μL of 143 mM sodium phosphate (6.3 mM EDTA, pH 7.5), 100 μL of 3 mM NADPH, 100 μL of 10 mM 5,5′-dithiobis-2-nitrobenzoate, and 194 μL of distilled water. The samples were incubated for 10 min at room temperature. After addition of 1 μL of glutathione reductase, the change in absorbance at 412 nm was read for 3 min.

For measurement of catalase activity, 2975 μL of 67 mM sodium-potassium phosphate (pH 7.4) was added to 20 μL of serum, and the samples were incubated (37°C, 10 min) (23). Five microliters of 30% hydrogen peroxide was added in the samples, and the change in absorbance was immediately read at 240 nm for 1.5 min. Whole-blood GPX activity was measured spectrophotometrically at 37°C, using cumene hydroperoxide as the oxidant of glutathione (Ransel RS 505; Randox, Crumlin, UK). For TAC determination, 480 μL of 10 mM sodium-potassium phosphate (pH 7.4) and 500 μL of 0.1 mM 2,2-diphenyl-1 picrylhydrazyl were added in 20 μL serum and incubated in the dark for 30 min at room temperature. The samples were centrifuged (20,000*g*, 3 min), and the absorbance was read at 520 nm (23). Total protein in serum was assayed using a Bradford reagent.

Each assay was performed on the same day to eliminate variation in assay conditions and within 1 month of blood collection. Spectrophotometric assays were performed on a Hitachi 2001 UV/VIS (Hitachi Instruments Inc.) in triplicates. Plasma volume changes postexercise were computed on the basis of hematocrit and hemoglobin values. Intra- and interassay coefficients of variation for all assays ranged from 2.2% to 5.9% and from 2.9% to 7.1%, respectively.

Statistical analysis. Data are presented as mean ± SEM. Normality was examined by the Kolmogorov–Smirnov test and was found not to differ significantly from normal. A time (pre- or postsupplementation) × time (pre- or postexercise) × trial (placebo vs carnitine) ANOVA with repeated measures on all factors was used. If a significant interaction was obtained, pairwise comparisons were performed through Bonferroni correction analysis. Differences in physical characteristics between the placebo and the L-carnitine trials were examined by paired *t*-test. Differences in diet among trials were examined through one-way ANOVA. To check

TABLE 2. Resting and maximal exercise values of performance variables for pre- and postsupplementation period in the two groups.

	Baseline		Supplementation	
	Placebo	L-Carnitine	Placebo	L-Carnitine
Heart rate (beats·min^{-1})				
Rest	78.3 ± 1.5	77.4 ± 1.9	78.9 ± 2.8	78.0 ± 1.7
Postexercise[a]	127.5 ± 2.9[b]	125.2 ± 3.7[b]	124.6 ± 4.8[b]	128.8 ± 4.5[b]
At 50% of $\dot{V}O_{2peak}$	107.1 ± 3.1[b]	105.5 ± 3.8[b]	108.1 ± 2.4[b]	98.5 ± 2.6*,[b]
Lactate (mM)				
Rest	0.92 ± 0.3	0.90 ± 0.3	0.94 ± 0.1	0.93 ± 0.2
Postexercise	3.84 ± 0.8[b]	3.94 ± 1.1[b]	3.76 ± 1.3[b]	2.64 ± 0.7*,[b]
Exercise time to exhaustion (min)	9.8 ± 0.5	9.9 ± 0.7	9.6 ± 0.4	12.4 ± 1.1†
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	14.1 ± 0.8	13.7 ± 0.6	13.9 ± 1.1	14.2 ± 0.9
Mean exercise RER	0.98 ± 0.02	0.99 ± 0.03	0.98 ± 0.01	0.94 ± 0.01‡
Serum TC (μmol·L^{-1})				
Rest	20.4 ± 5.2	22.9 ± 3.1	19.6 ± 4.6	59.7 ± 7.7*
Postexercise	31.8 ± 3.9[b]	35.1 ± 5.1[b]	29.8 ± 4.1*,[b]	92.7 ± 7.3*,[b]
Uric acid (mg·dL^{-1})				
Rest	6.6 ± 1.6	6.7 ± 1.8	6.8 ± 1.5	6.6 ± 1.9
Postexercise	7.8 ± 2.1[b]	7.7 ± 2.0[b]	7.9 ± 2.3[b]	7.9 ± 2.2[b]

Values are presented as means ± SE.
$\dot{V}O_{2peak}$, peak oxygen consumption; TC, total carnitine.
[a] Maximal exercise heart rate.
[b] An exercise effect.
* Significant difference between groups ($P < 0.05$).
† Significant difference with the corresponding placebo trial.
‡ A trend of difference between groups ($P < 0.1$).

for a possible order effect, a paired *t*-test was used for all the dependent variables in the presupplementation period either at rest or after exercise (no significant effects detected for any of the dependent variables examined). Confidence intervals (CI) were calculated for each dependent variable. Statistical significance was considered when $P < 0.05$. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) was used for all analyses. Data are presented as means ± SD.

Power calculations were based on the effect sizes computed between the resting value and the postexercise value at the baseline measurements for each dependent variable using the software G*Power 3 (8). For the variables exercise time to exhaustion, $\dot{V}O_{2peak}$, and mean exercise RER, the effect sizes were computed between the placebo value and the carnitine value either at baseline or after supplementation. The pooled SD of the resting and exercise values and one tail were used for these calculations, whereas the alpha level was set at 0.05. For the performance measurements, the statistical power for the placebo and the carnitine trials, respectively, was 1.00 and 1.00 for heart rate, 1.00 and 1.00 for lactate, 0.09 and 0.97 for exercise time to exhaustion, 0.08 and 0.13 for $\dot{V}O_{2peak}$, 0.05 and 0.29 for mean exercise RER, 0.84 and 0.88 for TC, and 0.14 and 0.12 for uric acid. For the redox status indices, the statistical power for the placebo and the carnitine trials, respectively, was 0.08 and 0.28 for MDA, 0.98 and 0.79 for PC, 0.78 and 0.79 for GSH, 0.31 and 0.92 for GSSG, 0.62 and 0.82 for GSH/GSSG, 0.58 and 0.63 for TAC, 0.98 and 0.73 for catalase, and 0.69 and 0.44 for GPX.

RESULTS

Patient characteristics. The comparison of the data between resting trials revealed no significant interaction and no significant main effect of trial on any of the dependent variables measured, indicating that the washout period between trials was efficient. Supplementation compliance was 100%. Sixteen eligible patients agreed to participate (24% of the entire dialysis cohort available). Two of them were relocated, and two were medically excluded from participation. Primary renal disease included glomerulonephritis (eight patients), arterial hypertension (two patients), and obstructive uropathy (two patients). Eight patients had arteriovenous fistulas, whereas four had grafts (none was using a central vascular catheter). No adverse clinical effect related to L-carnitine supplementation was reported. Subjects were not receiving antioxidant supplements and had similar dietary intakes in both trials (data not shown).

Carnitine responses. TC ($\mu mol \cdot L^{-1}$) (Table 2) levels were similar in the two trials at rest before supplementation (placebo = 19.3 ± 2.6 $\mu mol \cdot L^{-1}$, L-carnitine = 21.6 ± 3.1 $\mu mol \cdot L^{-1}$, CI = 0.507–4.362, $P = 0.907$). Exercise increased TC by approximately 60% in both trials (CI = 3.8–39.4, $P = 0.000$). As expected, L-carnitine supplementation resulted in higher ($P = 0.000$) resting TC levels (placebo = 18.2 ± 5.1 $\mu mol \cdot L^{-1}$, L-carnitine = 64.4 ± 5.7 $\mu mol \cdot L^{-1}$, CI = 28.9–51.2).

Performance responses. Exercise time to exhaustion (Table 2) increased ($P = 0.000$, CI = 1.8–3.1) only in L-carnitine by 22%. $\dot{V}O_{2peak}$ (CI = 1.1–2.1) and heart rate (at rest and at maximal exercise) remained unaffected by L-carnitine administration. However, heart rate at 50% of $\dot{V}O_{2peak}$ and postexercise lactate declined ($P < 0.05$) in the L-carnitine trial. Mean RER value tended ($P < 0.10$) to be lower in the L-carnitine trial.

Oxidative stress responses. Exercise alone elevated MDA ($P = 0.000$, CI = 0.01–0.14) and PC ($P = 0.000$, CI = 0.01–0.4) in both trials (Fig. 2). Nevertheless, L-carnitine supplementation attenuated MDA and PC responses both at rest ($P = 0.000$, CI = 0.02–0.05 for MDA and $P = 0.005$, CI = 0.06–0.26 for PC) and postexercise ($P = 0.000$, CI = 0.05–0.1 for MDA and $P = 0.009$, CI = 0.06–0.32 for PC) compared with placebo.

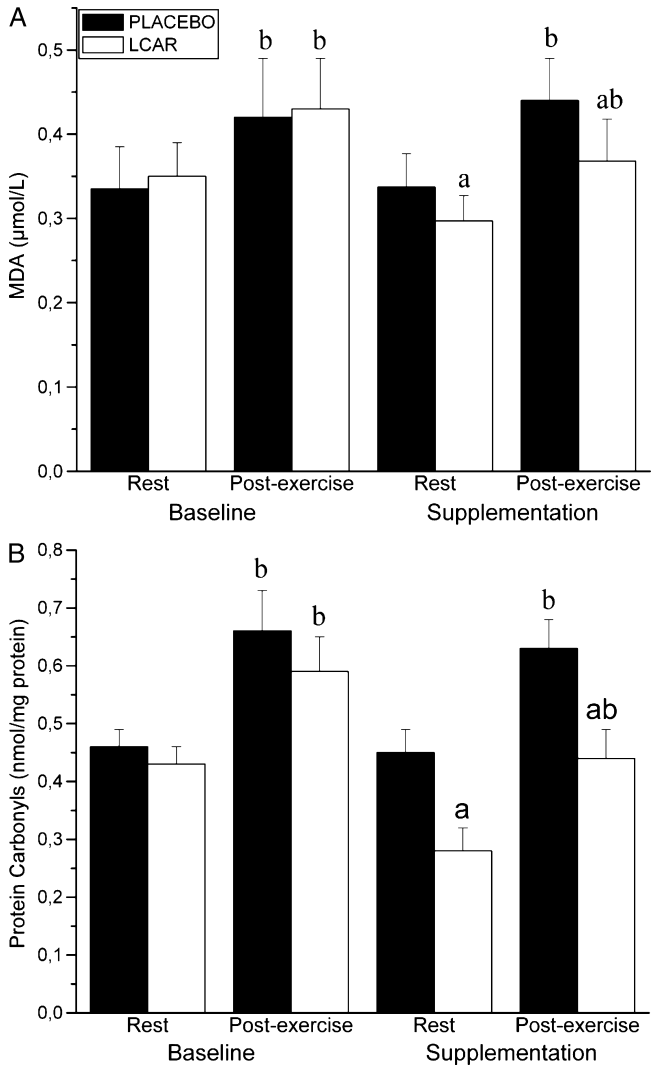

FIGURE 2—Serum MDA (A) and PC (B) at rest and postexercise at baseline and after supplementation (means ± SEM). [a]Significant difference between trials; [b]an exercise effect; MDA, malondialdehyde.

GSH, GSSG, and GSH/GSSG changes are shown in Figure 3. Although acute exercise decreased GSH concentration by 30% in both trials, no statistical differences were recorded (P = 0.17–0.53, CI = 0.04–0.8), possibly because of large variation in GSH values. L-carnitine supplementation elicited a GSH fall of a smaller (P = 0.01, CI = 0.5–1.7) magnitude compared with placebo after exercise. Similarly, despite an 11.5%–19% rise in GSSG postexercise, no statistical differences were detected (P = 0.1–1.0, CI = 3.8–46.8), possibly because of large variation in GSSG values. However, L-carnitine administration induced a GSSG rise of a smaller (P = 0.06, CI = 2.8–74.8) magnitude compared with placebo postexercise. Similarly to GSH and GSSG, their ratio declined by approximately 50% after acute exercise, but this decline did not reach statistical significance (P = 0.1–0.5, CI = 0.01–0.04), possibly because of large variation in GSH/GSSG values. Nevertheless, L-carnitine supplementation maintained GSH/GSSG at higher levels (P = 0.002, CI = 0.01–0.05) compared with placebo postexercise.

TAC, catalase, and GPX changes are shown in Figure 4. TAC increased (P = 0.01–0.04, CI = 1.4–10.9) by exercise in both trials but remained unaffected by L-carnitine supplementation. Acute exercise elevated catalase (P = 0.000–0.01, CI = 0.19–84.9) and GPX activity (P = 0.000–0.04, CI = 33.2–981.1) in both trials. L-carnitine supplementation decreased (P = 0.03, CI = 12.9–173.5) serum catalase activity postexercise but not at rest as compared with placebo. GPX activity was elevated by L-carnitine administration both at rest (P = 0.000, CI = 211.5–763.8) and postexercise (P = 0.000, CI = 211.5–763.8). Exercise increased uric acid concentration (Table 2) (P = 0.000, CI = 0.2–1.7) in both trials, but no differences were detected between trials.

DISCUSSION

In this study, we evaluated the effects of a short-term carnitine supplementation on oxidative stress and antioxidant status markers as well as functional status indices of patients undergoing hemodialysis. The present investigation provides evidence that short-term L-carnitine supplementation may attenuate resting and exercise-induced oxidative stress responses, enhance antioxidant status, and foster functional performance of this population of patients.

Resting TC values of male HD patients range from 17 to 40 μmol·L^{-1}, which is lower than their healthy age-matched counterparts (46–87 μmol·L^{-1}) (4,30). Our results (20–23 μmol·L^{-1}) approach the lower range, which is in line with earlier reports (30), but lower than the concentrations reported by others (4), probably because of participants' long dialysis history (>90 vs 40–60 months). Long-term hemodialysis results in marked alterations of the endogenous carnitine pool (4,30) because of a substantial accumulation of L-carnitine's acylated forms (30). Removal of the longer chain esters across the dialysis membrane is impaired because of the increased molecular weight and lipophilicity accompanying increasing chain length. It was hypothesized that

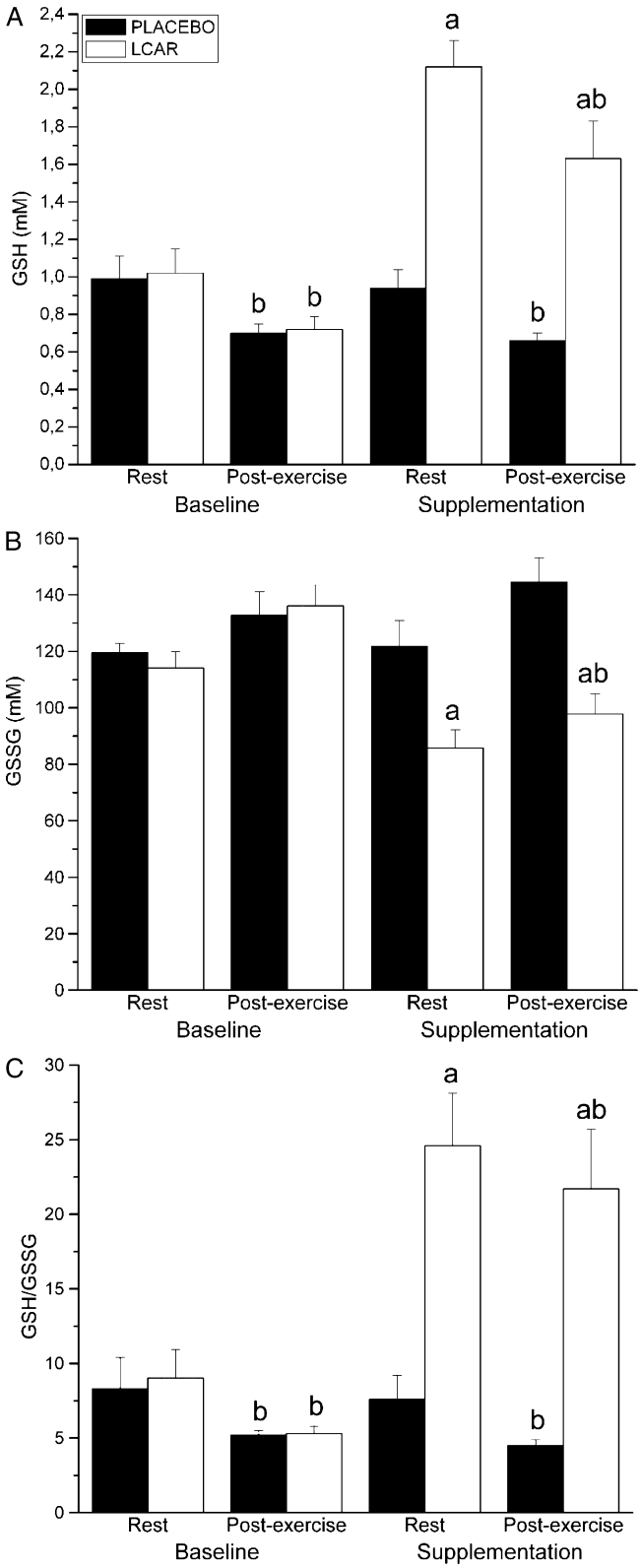

FIGURE 3—Reduced glutathione (A), oxidized glutathione (B), and their ratio (C) at rest and postexercise at baseline and after supplementation (means ± SEM). [a]Significant difference between trials; [b]an exercise effect; GSH, reduced glutathione; GSSG, oxidized glutathione.

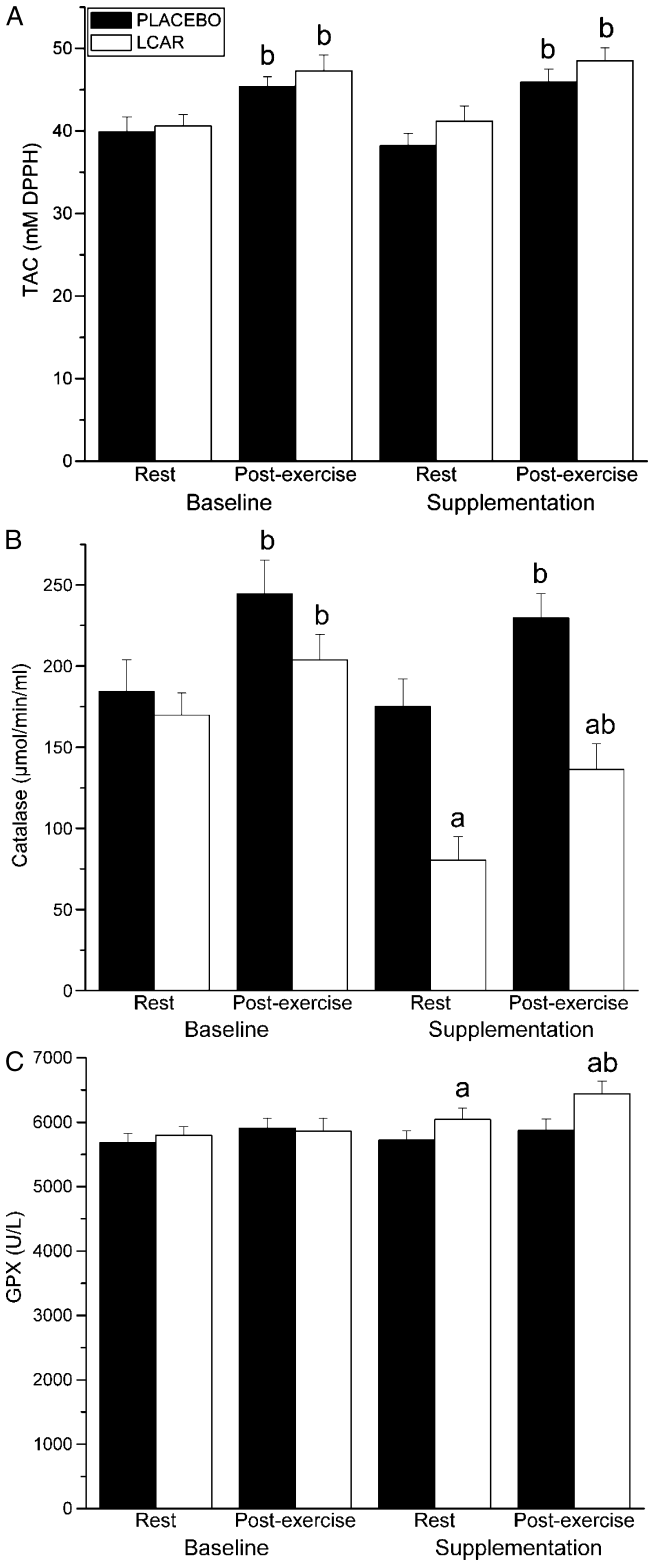

FIGURE 4—TAC (A), catalase activity (B), and GPX activity (C) at rest and postexercise at baseline and after supplementation (means ± SEM). [a]Significant difference between trials; [b]an exercise effect; TAC, total antioxidant capacity; GPX, glutathione peroxidase.

L-carnitine supplementation would cause carnitine accumulation in endothelial cells or prevent deficiency. Circulating TC levels were significantly higher during all time points examined in the present investigation during L-carnitine supplementation, indicating high compliance. Intravenous L-carnitine supplementation (2 $g \cdot d^{-1}$) increased TC (2.5-fold) at rest and postexercise, which is higher than the increase seen in healthy adults (3.5%–70%) with similar dosages (39). Data on exercise effects on circulating carnitine levels in HD patients are unavailable. Exercise increased TC in HD patients by almost 60% independent of supplementation, which coincides with earlier reports in healthy adults (38,39). An exchange of free carnitine and carnitine esters between the muscle and the blood may be taking place during exercise (39).

Participants in the present study demonstrated a severely impaired $\dot{V}O_{2peak}$ corresponding to 45% of the respective values of their age-matched population and substantially lower peak lactate values than those reported for healthy controls (7). Circulating carnitine concentration is positively correlated with strength, fatigue, and exercise tolerance in patients with ESRD (4). Although previous research in healthy humans suggested that L-carnitine supplementation does not affect performance and substrate metabolism (5,39), in rats carnitine consumption improved endurance and delayed fatigue (28). Relative endogenous carnitine deficiency may be required for an effect of L-carnitine supplementation on physical performance because L-carnitine supplementation enhanced endurance and delayed fatigue in clinical states characterized by carnitine deficiency such as cancer (11). In ESRD, L-carnitine supplementation was shown to be both effective (9) and ineffective (25) in improving physical performance. In the present study, L-carnitine supplementation did not affect $\dot{V}O_{2peak}$ but increased exercise time to exhaustion by 22%. Most of carnitine effects during exercise are linked to an enhancement of the long-chain fatty acids transport across the outer and inner mitochondrial membranes, thereby enhancing the metabolic flux through the TCA cycle favoring fat metabolism and reducing carbohydrate utilization during exercise. RER may be used to calculate fuel oxidation at exercise steady state so that an RER reduction would indicate an increase of fat oxidation during exercise. Others have reported a decline (27) or no changes (5,39) of RER during exercise with L-carnitine supplementation in healthy humans. In the present study, L-carnitine administration resulted in RER reduction, suggesting an increase in lipid oxidation and possible glycogen sparing. Theoretically, a rise in free CoA because of an increase in muscle carnitine could enhance 2-oxoglutarate conversion to succinyl CoA, thereby fostering the substrate flux through the Krebs cycle. A decrease in the acetyl CoA/CoA ratio would stimulate pyruvate dehydrogenase activity so that some pyruvate would be diverted from its reduction to lactate to its oxidation to acetyl CoA, thus allowing acetylcarnitine synthesis (5). This is in line with our finding of reduced postexercise lactate accumulation after L-carnitine administration, as previously shown in healthy adults (39). In support of

L-carnitine's ergogenic effect, submaximal exercise heart rate (at 50% $\dot{V}O_{2peak}$) was reduced by L-carnitine supplementation.

In agreement with previous findings (6), exercise increased lipid peroxidation (23%), and protein oxidation (43%), and reduced GSH/GSSG ratio (a valid oxidative stress marker). L-carnitine supplementation at similar dosages (0.4–0.6 $g \cdot kg^{-1}$) for 1–4 months has been shown to have antioxidant properties preventing lipid peroxidation and restoring antioxidant status (3). Although L-carnitine supplementation prevented lipid peroxidation in the venous effluent after reperfusion of isolated perfused kidney samples (24), until today, there are no reports on L-carnitine effects on exercise-induced oxidative stress in patients with ESRD. A protective action by L-carnitine against oxidative injury during chronic renal insufficiency and renal ischemia-reperfusion has been previously published (34). In the present study, L-carnitine supplementation of patients with ESRD for 2 months induced a substantial reduction of lipid and protein oxidation both at rest and postexercise.

A question arises as to whether this lowering of oxidative stress responses after L-carnitine supplementation is linked to an enhanced action of the body's antioxidant reserves. Despite a TAC rise after exercise, there were no differences between trials after carnitine supplementation. This finding suggests that carnitine did not activated body's antioxidant defenses in serum. Uric acid elevation has been estimated to account for nearly one-third of TAC increase (40) and, like TAC, although it increased postexercise in both trials, there were no differences between trials after carnitine consumption.

Other antioxidants might have contributed to the attenuation of oxidative stress responses such as GSH and antioxidant enzymes' activity such as GPX. L-carnitine supplementation in the present study prevented GSH depletion and increased the GSH/GSSG ratio, indicating an improved redox state and reduced oxidant stress. In agreement with our findings, Sener at al. (34) showed that L-carnitine may reverse GSH depletion in nephrectomized rats. Furthermore, L-carnitine supplementation has also been shown to raise glutathione reserves in ischemia and aging, which in turn increases GPX activity, as shown in the present study (17,34). GPX activity increased both at rest and postexercise, coinciding with earlier observations suggesting that L-carnitine supplementation may foster basal antioxidant capacity through antioxidant enzyme activation (3). Therefore, L-carnitine supplementation may raise GSH levels in HD patients, which in turn increases GPX activity and GSH/GSSG ratio, thereby enhancing antioxidant protection. Previous studies have also noticed an increase of catalase activity by L-carnitine supplementation, indicating a more efficient removal of hydrogen peroxides (10). However, in the present study, serum catalase activity was reduced both at rest and postexercise. Catalase has no apparent function in serum because it is an intracellular enzyme. Therefore, its reduced activity after exercise probably indicates a reduced muscle fiber or erythrocyte damage, resulting in its increased leakage into the circulation. In support of this observation, L-carnitine supplementation has been shown to reduce postexercise muscle damage (35).

The mechanisms through which L-carnitine exerts its antioxidant effects are not clear. Volek et al. (38) proposed that L-carnitine administration alters postexercise hypoxanthine and xanthine oxidase concentrations. According to this model, ADP and H^+ accumulation due to high glycolytic flux stimulates the adenylate kinase reaction, resulting in AMP and ATP synthesis. Subsequently, hypoxanthine is produced because of AMP oxidation. Furthermore, calcium ATPase pumps are inhibited because of inadequate ATP supply, resulting in increased intracellular calcium, which then stimulates the calcium-dependent proteases. Part of xanthine dehydrogenase is converted into xanthine oxidase after being cleaved by these proteases. Oxygen is used as an electron acceptor during xanthine oxidase synthesis, resulting in superoxide radicals production during exercise, which then activates the initiation of lipid and protein oxidation that ultimately causes cell disruption or damage. During the postexercise period, L-carnitine supplementation has been shown to enhance recovery by creating a more anabolic environment (18,19,21) and attenuate the increase of hypoxanthine and xanthine oxidase, suggesting a better function of calcium pumps probably because of a higher ATP supply (35,38).

Kraemer et al. (21) offered another plausible explanation in regard to the antioxidant effect of L-carnitine. Accumulation of L-carnitine in the capillary endothelial cells seems to up-regulate oxygen delivery to exercising muscles via a vasodilatory effect on the capillary, thereby reducing local muscular hypoxia usually seen with exercise stress (21,38). This effect may reduce the hypoxia-related tissue damage from free radicals after acute exercise, thereby allowing an improved interaction between anabolic hormones and their cellular receptors that results in increased protein synthesis or reduced anticatabolic effects at the receptor level (20,38). This effect of L-carnitine supplementation is also supported by the observed rise of tissue-level oxygen consumption during the postexercise period, an adaptation that may further explain the L-carnitine-induced attenuation of hypoxic stress markers herein and in previous studies (20,35,38).

In conclusion, this study investigated the effects of L-carnitine supplementation in functional capacity as well as in basal and exercise-induced oxidative stress responses in patients with ESRD. Although the study's sample size is relatively small because of a narrow patient pool size, a counterbalanced, double-blind design was used to offset this problem. Female patients were not included in this investigation (because of an absence of female patients satisfying the eligibility criteria). The results of the present investigation suggest that L-carnitine intravenous infusion for 2 months may be effective in attenuating oxidative stress responses, enhancing antioxidant status, and improving performance of patients undergoing chronic hemodialysis therapy. L-carnitine supplementation effectiveness in patients undergoing hemodialysis was questioned early in this decade (37). However, recent research evidence suggests that

exogenous carnitine administration is safe (31), and it may reduce basal oxidative stress and inflammation not only in patients with ESRD (as shown in this study) but also in healthy adults (2,38). Previous research indicates that patients with ESRD demonstrating a high erythropoietin resistance index (i.e., $\geq 0.02\ \mu g \cdot kg^{-1} \cdot wk^{-1} \cdot g\ Hb^{-1}$) and hence a more greatly disturbed carnitine profile are more likely to respond to L-carnitine supplementation when administered for an improvement in erythropoietin requirements (29). This line of evidence along with other beneficial effects associated with L-carnitine supplementation in patients undergoing hemodialysis (i.e., lower hospitalization rates, reduced left ventricular hypertrophy and protein sparing) (1,32) suggests that its exogenous administration may benefit this patient population. Nevertheless, large-scale randomized clinical trials are needed to confirm any beneficial effects of L-carnitine supplementation.

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Conflict of interest: none.

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