Effects of Four Weeks L-Carnitine L-tartrate Ingestion on Substrate Utilization During Prolonged Exercise

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In a randomized, placebo-controlled, double-blind crossover design, 15 trained males undertook exercise trials during two 4 wk supplementation periods, with either 3 g L-Carnitine L-tartrate (LCLT) or 3 g placebo (P) daily. Total carbohydrate and fat oxidation during 90 min steady state cycling were not different between 0 or 4 wk within LCLT or P trials (mean ± standard deviation: carbohydrate oxidation P₀ 99 ± 36, P₄W 111 ± 27, LCLT₀ 107 ± 33, LCLT₄W 112 ± 32 g, respectively; fat oxidation P₀ 99 ± 28, P₄W 92 ± 21, LCLT₀ 94 ± 18, LCLT₄W 90 ± 22 g, respectively). Subsequent 20 km time trial duration was shorter after P (P₀ 31:29 ± 3:50, P₄W 29:55 ± 2:58 min:s, P < 0.01), with no significant change over LCLT (LCLT₀ 31:46 ± 4:06, LCLT₄W 31.19 ± 4.08 min:s). Four weeks LCLT supplementation had no effect on substrate utilization or endurance performance.

Key Words: carnitine, time trial, cyclists, fat oxidation, carbohydrate oxidation

L-Carnitine (LC) is a vitamin-like nutrient, essential for energy production and fat metabolism through its role as a transporter of long chain fatty acids into mitochondria. It is produced naturally in the body, as well as being ingested by way of meat products, with a typical non-vegetarian diet providing 23 to 135 mg of LC daily (12). L-Carnitine supplementation has been shown to increase fatty acid oxidation at rest in healthy and overweight individuals using an assessment of an exhaled breath ¹³CO₂ tracer (22, 39) and has been studied widely in the hope that it could increase fat utilization during exercise, thereby sparing muscle glycogen and prolonging endurance exercise (5, 38). Studies have produced mixed results for endurance exercise (> 40 min), however, with some studies showing reduced respiratory exchange ratio (RER) and reduced heart rate at submaximal exercise intensities (16, 17, 19, 35, 40) and others showing no effect on these parameters (7, 10, 19, 24, 25, 37). These differences might be due to variations in study design, including: differences in exercise mode; exercise intensity and duration; failure
to control prior diet or exercise training; variations in the duration and amount of supplementation; and differences in training status of subjects (6).

A second role of LC is the buffering of intramuscular acetyl-CoA accumulation during exercise (8), which could benefit moderate- to high-intensity exercise by relieving inhibition of the pyruvate dehydrogenase enzyme complex (PDC), thereby increasing glycolytic flux (34). Several researchers have found that the accumulation of acetyl-CoA during exercise does not inhibit PDC during exercise above 50% VO\textsubscript{2max} (28). It has also been shown, however, that CHO oxidation during prolonged exercise at a moderate-high intensity (71% VO\textsubscript{2max}) is increased by acetyl-carnitine accumulation and full PDC activation using the PDC kinase inhibitor, dichloroacetate (27). Hence, it might be that increased availability of acetyl-carnitine alone (or “stockpiling” of acetyl groups) supports increased oxidative glycolytic flux during exercise. A previous study in our laboratory showed a significant increase in CHO oxidation during 1 h of steady-state (SS) cycling at 60% VO\textsubscript{2max} in males following 2 wk of daily supplementation with 3 g of L-Carnitine L-tartrate (LCLT; 14). It is possible that these changes in CHO oxidation following 2 wk of supplementation could be further enhanced by a longer period of supplementation. Furthermore, extending the duration of exercise to include a time trial could enable consideration of the impact of a possible increase in CHO oxidation on subsequent high intensity efforts conducted at a point where muscle glycogen stores might become limiting.

Therefore, the aim of this study was to investigate the metabolic and performance effects of 4 wk of supplementation with LCLT in endurance-trained athletes during 90 min of steady-state cycle exercise followed by a 20 km time trial.

**Methods**

Fifteen non-vegetarian male athletes actively involved in endurance training were recruited. The subjects’ characteristics are shown in Table 1. All subjects were given written and verbal information about the study and underwent pre-participation screening consisting of medical history and physical activity questionnaires. The study was undertaken during the preparation phase of the cycling and triathlon

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32 (20–46)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.4 (169.4–186.2)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>72.3 (55.3–81.7)</td>
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<tr>
<td>VO\textsubscript{2max} (L/min)</td>
<td>4.6 (3.9–5.8)</td>
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<tr>
<td>Training history (y)</td>
<td>7.3 (2–20)</td>
</tr>
<tr>
<td>Current cycle/training (h/wk)</td>
<td>5.9 (1.0–12.5)</td>
</tr>
<tr>
<td>Average workload for steady state ride (W)</td>
<td>205 (140–270)</td>
</tr>
<tr>
<td>% VO\textsubscript{2max} during steady state exercise</td>
<td>64.8 (48–86)</td>
</tr>
<tr>
<td>Sum of 7 skinfolds (mm)</td>
<td>60.2 (33.7–108.7)</td>
</tr>
</tbody>
</table>
Effects of L-Carnitine L-tartrate Ingestion on Substrate Utilization

competitive season (November through March) to ensure consistent endurance-based training was being undertaken and to avoid a training effect that might influence the outcome of the study. No subject was suffering from any metabolic disorder and none was taking any medication or nutritional supplements other than vitamins/minerals or commercial sports drinks (containing only carbohydrate, sodium, and potassium). All experimental procedures were approved by the university ethics of research committee, and all subjects were free to withdraw from the study at any time without obligation.

The study was undertaken using a double blind, placebo-controlled, cross-over design. Subjects attended the laboratory on nine occasions over a period of 13 to 17 wk for measurement of VO2max, completion of two familiarization trials, and four experimental trials interspersed with intermediate visits half way through each supplementation period. The first visit was used to determine VO2max and body composition using the sum of seven skinfold measures (bicep, tricep, subscapular, supraspinale, abdomen, mid thigh, calf; mean of duplicate measures) by a trained anthropometrist following International Society for the Advancement of Kinanthropometry protocols (23). The maximal test was undertaken on a mechanically braked cycle ergometer (Monark Exercise AB, Vansbro, Sweden) in a laboratory where temperature was maintained between 20 to 21 °C. Subjects began cycling at 80 W and the power output increased by 40 W every minute until subjects could no longer maintain a cadence of 80 rpm. Expired gas was measured throughout the test using an online gas analysis system (Sensormedics Vmax 29, Holland) calibrated against known gas concentrations prior to each test. Heart rate was recorded at each workload (PolarVantage NV, Kempele, Finland). The initial two workloads were used as warm up, and subjects were given 2 min on each.

All subsequent exercise trials were undertaken on a KingCycle ergometer (version 6.7, 1997, Kingcycle, High Wycombe, UK), with the athletes using their own bicycle, on the same day of the week and same time of day. Tire pressures were checked and maintained according to stated parameters of the individual tires on each visit. All subjects undertook two familiarization trials at the same time of day as their trial tests. These familiarization trials were undertaken to ensure the correct power output had been selected for the initial period of steady-state cycling, and to familiarize subjects with all testing procedures including the time trials.

Treatment

After the familiarization trials, subjects were allocated to a treatment group in a counter-balanced, double blind, cross-over fashion. Treatments consisted of four capsules taken twice daily with food (i.e., eight capsules per day) for a total of 28 d. The active supplement capsule consisted of 373 mg L-Carnitine L-tartrate (LCLT, L-Carnipure, Lonza Ltd., Basel, Switzerland), equivalent to 2 g LC per day. The placebo capsule consisted of the same amount of L-tartaric acid as the supplement capsules, mixed with methyl cellulose to make a total weight of 373 mg per capsule (P). Only a 2 wk supply was given at any one time and subjects’ compliance to the treatment was encouraged using frequent contacts from the laboratory. Compliance with the treatment was confirmed by changes in plasma carnitine concentration and urinary carnitine excretion. A minimum of 2 wk (actual 2 to 6 wk) washout period separated the two treatment blocks.
Dietary and Exercise Controls

Subjects recorded food intake and exercise over the 2 d prior to the familiarization trials, and were requested to replicate this before each of the experimental trials. Two-day diet and exercise diaries were recorded on each occasion to verify compliance. There was no control for habitual dietary intake between subjects, nor was training controlled over the period of the study, although subjects were requested to maintain as consistent a training regimen as possible to minimize any training effect throughout the study. Only very light exercise was allowed in the 24 h prior to each trial.

The Trials

An outline of the procedures for trials is shown in Figure 1. For each supplementation period, the subjects attended the laboratory on three occasions: baseline experimental trial before treatment commenced (0) and an experimental trial at the end of the treatment period (4 wk) for P and LCLT trials, hereafter denoted as $P_0$, $P_4W$, $LCLT_0$, and $LCLT_4W$, respectively. The third occasion was at 2 wk of supplementation where the full exercise trial was undertaken to sustain subject compliance and familiarity with procedures. Each trial was undertaken 3 h following a standardized meal containing 1 g/kg body mass of carbohydrate (bread and jam). Nude body mass was taken upon entry to the lab, and a heart rate monitor supplied. Subjects then rested in a supine position while a cannula (20 gauge, SSS Healthcare) was inserted into an antecubital vein. Following 5 min of seated rest, a blood sample was drawn without stasis, along with a free flowing capillary sample from a pre-heated hand for analysis of pH (Radiometer model ABL500, Copenhagen, Denmark). The cannula was kept patent at all times using a saline flush of 1 mL following sample collections. Subjects then mounted their bicycle and undertook a standard run-down calibration process involving two short bursts of exercise. Subjects then began cycling for 90 min at a constant power output (mean ± standard deviation 64.8 ± 9.2% VO$_{2\text{max}}$) at a self-selected pedal cadence with power output increased progressively over the first 5 min to the desired level, as means of a warm up. Heart rate (HR, every minute), expired gas (4 min collection every 15 min) and rating of perceived exertion (RPE, Borg 15-point scale every 10 min; 4) were recorded from time zero throughout the steady state period. Blood was drawn at rest and 15, 30, 60, and 90 min. Water was provided throughout the trial, with encouragement to achieve sufficient fluid intake to prevent dehydration, based on volume consumed and the change in body mass observed in the familiarization trials. Subjects were cooled with a fan throughout all trials. Following completion of the 90 min SS, subjects commenced a 20 km time trial on the same bicycle, with ad libitum fluid intake. Verbal encouragement was provided by the same individual in a standardized manner for each trial. Subjects were allowed to listen to music during the time trial, and the type and loudness of music was standardized for each individual. Performance was assessed according to the time taken to complete the distance, but no feedback other than percent of distance completed was provided to the subjects during any of the trials (i.e., subjects were not aware of their HR, TT performance, or power outputs achieved) until completion of the entire study to limit pacing. Capillary and venous blood samples were taken 5 min after the
completion of the time trial. Following completion of the time trial, subjects rested while the cannula was removed, then towel dried and undertook another nude body mass measurement. Urine was collected in the 24 h prior to each 4 wk trial for each supplementation period. If a subject needed to empty his bladder at any point during the 90 min SS he was allowed 2 min to do so; this did not alter the total duration of activity conducted by the subjects. All subjects were allowed 2 min to void following the steady state exercise prior to commencing the time trial. All urine was volumed and included in calculations of fluid loss over the trial.

**Blood and Urine Analysis**

Prior to, and at 15, 30, 60, and 90 min of the SS exercise and after the TT, duplicate 100 μL aliquots of whole blood were immediately deproteinized in 1 mL chilled 0.4 M perchloric acid, shaken vigorously and kept on ice until centrifugation at 10,000 rpm for 3 min. Samples were subsequently frozen at –20 °C until analysis. Blood lactate and glycerol were measured by fluorimetric procedures (Jenway 6200 fluorimeter, Jenway Ltd., Essex, UK) as outlined previously (3, 20). The remaining blood was mixed well in EDTA tubes and duplicate samples were drawn into capillary tubes which were sealed and centrifuged at 10,000 rpm prior to hematocrit measurement (Hawksley Microhematocrit reader, Hawksley & Sons, Sussex, UK). A further 1.5 mL portion of the blood sample was centrifuged before duplicate aliquots of plasma were drawn off for later glucose and free fatty acid (FFA) analysis. Plasma glucose (Sigma Diagnostics, Sigma-Aldrich, Dorset, UK), plasma FFA (Wako Chemicals, Neuss, Germany) and hemoglobin (cyanmethemoglobin method) were assayed within 3 h of blood draws using a Novaspec II spectrophotometer (Amersham Pharmacia, Sweden), using standard reagent kits. Blood and plasma volume changes were calculated from hematocrit and hemoglobin using standard equations (11).

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**TIME (MINS)**

<table>
<thead>
<tr>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enter lab, body mass, cannulation, mount cycle on ergo</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Steady state ride, 65% ( \text{Vo}_{2}\text{max} )</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>20km time trial</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood draws</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>R</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 1 — Overview of exercise trial procedures.
Additional blood was collected into lithium heparin tubes at rest, 60, and 90 min SS exercise, centrifuged at 5000 rpm at 4 °C for 10 min, with plasma extracted into duplicate tubes and frozen at −60 °C until analysis. Noradrenaline and adrenaline analysis was undertaken by HPLC with electrochemical detection using the methodology outlined by Goldstein et al. (15). Plasma carnitine fractions were determined by radiometric methods using liquid scintillation as outlined by McGarry and Foster (21).

Urinary carnitine excretion was determined in each treatment period by means of 24 h urine collections at the end of each supplementation period. A 5 mL sample of mixed urine was collected and frozen at −60 °C until analysis, and the total volume of urine excreted over the 24 h period measured to the nearest milliliter. Urinary carnitine fractions were subsequently analyzed (21).

Statistics

All data were assessed to ensure there was no sequential effect present in the results due to a training or habituation response. Data were checked for normality and analyzed by two-way repeated measures ANOVA, with subject as a random factor (Minitab version 14.1, Minitab, Inc., Coventry, UK, and SPSS version 11.0.0, SPSS, Inc., Chicago, IL). Any non-normal data were transformed using a log transformation prior to analysis. Where only one time point was present, data were analyzed by paired samples t-test. Significance was accepted at $P < 0.05$, and where significance was found, a Tukey post hoc analysis was undertaken to determine where significance lay. All results are expressed as means ± standard deviation.

Results

Pre-trial training and dietary controls were effective in ensuring that there were no differences in pre-exercise dietary energy or CHO intake, plasma glucose, or body mass. Blood and plasma volume fell in the first 15 min of steady state exercise (mean [range] of all trials −5.1 [−4.7 to −5.8] % and −7.8 [−7.4 to −8.6] %, respectively), but did not differ between trials and did not change over the remaining exercise time in any trial. Further, no differences were found between trials for body mass change (mean ± standard deviation: $P_{0W}$ −130 ± 520, $P_{4W}$ −340 ± 570, LCLT$_{0W}$ −230 ± 630, LCLT$_{4W}$ −380 ± 580 g) or fluid intake ($P_{0W}$ 1897 ± 585, $P_{4W}$ 1773 ± 498, LCLT$_{0W}$ 1777 ± 365, LCLT$_{4W}$ 1688 ± 344 mL) over the total exercise period, therefore changes in hydration status over exercise were minimal and the same in each trial. Steady-state exercise HR ($P_{0W}$ 138 ± 9, $P_{4W}$ 140 ± 13, LCLT$_{0W}$ 138 ± 13, LCLT$_{4W}$ 137 ± 13 bpm) and RPE (12 ± 2 all trials) did not differ between trials.

Substrate Metabolism

No differences were found in $V_{O2}$, VE, $V_{CO2}$, RER, total CHO oxidation, or total fat oxidation (Table 2). Rate of CHO oxidation and rate of fat oxidation (Figure 2) over the 90 min of SS exercise was not different within or between P and LCLT periods. There were significant ($P < 0.01$) increases over time during SS exercise in $V_{O2}$, VE, rate of fat oxidation, HR, and RPE, and significant decreases in RER and rate of CHO oxidation, reflecting expected cardiovascular drift and changes in substrate metabolism.
Table 2  Mean RER, VO₂, Total Grams of Carbohydrate and Fat Oxidized Over 90 Min Steady State Exercise and 20 Km Time Trial (TT) Performance Times

<table>
<thead>
<tr>
<th>Trial</th>
<th>RER</th>
<th>VO₂ (L/min)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>TT time (min:sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀</td>
<td>0.79 ± 0.04</td>
<td>3.02 ± 0.51</td>
<td>99 ± 36</td>
<td>99 ± 28</td>
<td>31:29 ± 3:50</td>
</tr>
<tr>
<td>P₄W</td>
<td>0.80 ± 0.03</td>
<td>2.97 ± 0.49</td>
<td>111 ± 27</td>
<td>92 ± 21</td>
<td>29:55 ± 2:58*</td>
</tr>
<tr>
<td>LCLT₀</td>
<td>0.79 ± 0.03</td>
<td>2.98 ± 0.43</td>
<td>107 ± 33</td>
<td>94 ± 18</td>
<td>31:46 ± 4:06</td>
</tr>
<tr>
<td>LCLT₄W</td>
<td>0.80 ± 0.03</td>
<td>2.94 ± 0.46</td>
<td>112 ± 32</td>
<td>90 ± 22</td>
<td>31:19 ± 4:08</td>
</tr>
</tbody>
</table>

Note. Values are means ± standard deviation, n = 15; *P < 0.02 from P₀, LCLT₀ and LCLT₄W.

Figure 2—Rate of carbohydrate (panel A) and fat (panel B) oxidation over 90 min steady-state exercise during LCLT supplementation (n = 15).
Carnitine Status and Hematological Data

There was a significant treatment and trial effect, and a significant treatment × trial interaction for all plasma carnitine fractions. Plasma total carnitine (Figure 3, panel A) and free carnitine (Figure 3, panel B) were elevated at all exercise time points at LCLT_{sw} (P < 0.01) compared to P_{0} and LCLT_{0}. Plasma acyl-carnitine (Figure 3, panel C) was elevated at rest, 60, and 90 min LCLT_{sw} compared to LCLT_{0} (P < 0.05). Plasma total carnitine was higher at all time points for P_{0} than LCLT_{0}, at rest, and 60 min for free carnitine, and at 60 and 90 min for acyl-carnitine (P < 0.05). Plasma total carnitine and acyl-carnitine increased from rest to 60 and 90 min exercise in all trials (P ≤ 0.01) except LCLT_{sw}, where the increase from rest to 90 min was not significant. The mean change in total carnitine (9.3%) was similar to the change in plasma volume at 90 min exercise (8.6%), whereas the increase in acyl-carnitine (58%) was greater than the plasma volume change. There was no change over exercise duration for free carnitine. Urinary carnitine excretion (Figure 4) was significantly elevated after 4 wk LCLT compared to P (P < 0.01 for all fractions).

No significant differences were found in plasma noradrenaline, adrenaline, FFA, glucose, or blood lactate, glycerol, or pH responses to exercise across trials (Table 3).

Time Trial Performance

Mean TT performances for the four trials are presented in Table 2, with no difference between TT performance at the beginning of each supplementation period. Time trial time at P_{sw} was significantly faster than P_{0} (mean difference 1:34 min:s, 95% CI 0:33 to 2:35 min:s change, P < 0.01), whereas there was no significant change over the LCLT period (LCLT_{sw} 0:27 min:s, 95% CI -0:43 to 1:37 min:s change over LCLT_{0}, respectively).

Discussion

The main finding from this study was that 4 wk LCLT supplementation had no effect on substrate use during 90 min steady-state cycling, nor on subsequent time trial performance. There was also no difference in response to supplementation across the range of training status of subjects used in this study. Plasma carnitine data demonstrated an elevation in plasma total, free, and acyl-carnitine at rest following LCLT supplementation which was not observed in the placebo period. The plasma carnitine concentrations in the present study are similar to previously reported values (10, 17, 19, 25, 37) and baseline values (P_{0} and LCLT_{0}) are within the normal range for human subjects (total carnitine 30 to 90 \mu mol/L; 31). Over the exercise period, plasma free carnitine did not change, while plasma acyl-carnitine increased significantly, resulting in a small, yet significant, elevation in total carnitine concentration, as has also been reported in studies of similar exercise intensity (9, 19, 37). This increased plasma acyl:free carnitine ratio could be considered reflective of a change in muscle carnitine fractions over moderate-high intensity exercise (2, 33, 37). Urinary carnitine excretion was increased five-fold over baseline during LCLT supplementation, representing 18% of the ingested dose, which is similar to reports from other studies (29).
Table 3  Blood Parameters at Baseline and at the End of 4 Wk Supplementation

<table>
<thead>
<tr>
<th>Measure</th>
<th>Trial</th>
<th>Placebo</th>
<th></th>
<th>LCLT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-exercise</td>
<td>90 min</td>
<td></td>
<td>Pre-exercise</td>
</tr>
<tr>
<td>Noradrenaline (nmol/L)</td>
<td>Baseline</td>
<td>2.3 ± 0.9</td>
<td>8.6 ± 2.6*</td>
<td>2.8 ± 1.7</td>
<td>9.2 ± 5.4*</td>
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<tr>
<td></td>
<td>4 wk</td>
<td>2.6 ± 1.1</td>
<td>9.3 ± 4.4*</td>
<td>2.2 ± 0.7</td>
<td>9.3 ± 5.7</td>
</tr>
<tr>
<td>Adrenaline (nmol/L)</td>
<td>Baseline</td>
<td>0.16 ± 0.11</td>
<td>1.88 ± 1.12*</td>
<td>0.13 ± 0.05</td>
<td>1.79 ± 1.40*</td>
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<td></td>
<td>4 wk</td>
<td>0.15 ± 0.11</td>
<td>1.59 ± 1.75*</td>
<td>0.15 ± 0.17</td>
<td>1.84 ± 1.51*</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>Baseline</td>
<td>0.36 ± 0.11</td>
<td>0.61 ± 0.21*</td>
<td>0.35 ± 0.16</td>
<td>0.55 ± 0.15*</td>
</tr>
<tr>
<td></td>
<td>4 wk</td>
<td>0.34 ± 0.07</td>
<td>0.51 ± 0.15*</td>
<td>0.36 ± 0.07</td>
<td>0.55 ± 0.14*</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>Baseline</td>
<td>5.24 ± 0.62</td>
<td>4.88 ± 0.54*</td>
<td>5.32 ± 0.69</td>
<td>4.84 ± 0.84*</td>
</tr>
<tr>
<td></td>
<td>4 wk</td>
<td>5.53 ± 0.71</td>
<td>4.92 ± 0.55*</td>
<td>5.39 ± 0.66</td>
<td>4.78 ± 0.58*</td>
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<tr>
<td>Glycerol (mmol/L)</td>
<td>Baseline</td>
<td>0.09 ± 0.04</td>
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<td>0.25 ± 0.16*</td>
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<td></td>
<td>4 wk</td>
<td>0.07 ± 0.05</td>
<td>0.20 ± 0.06*</td>
<td>0.09 ± 0.04</td>
<td>0.25 ± 0.07*</td>
</tr>
<tr>
<td>pH</td>
<td>Baseline</td>
<td>7.42 ± 0.01</td>
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<td>7.42 ± 0.01</td>
<td>7.43 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>4 wk</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.02</td>
</tr>
</tbody>
</table>

Note. Values are means ± standard deviation; LCLT, L-Carnitine L-tartrate; FFA, free fatty acids; *significantly different to pre-exercise value (P < 0.01).
Figure 3.—Plasma total carnitine (panel A), free carnitine (panel B), and acyl-carnitine (panel C) over 90 min SS exercise for P and LCLT trials (n = 15): a LCLT_{aw} significantly higher at all time points (P < 0.01) from all other trials for total and free carnitine; b LCLT_{aw} significantly higher than LCLT_{o} at all time points (P < 0.05); c 60 and 90 min exercise significantly higher than rest for total carnitine (P < 0.05) and acyl-carnitine (P < 0.01), except 90 min LCLT_{aw}; d P_{0} higher than LCLT_{o} (P < 0.05) at all time points; e P_{0} higher than LCLT_{o} at rest and 60 min (P < 0.05); f P_{0} higher than LCLT_{o} at 60 and 90 min (P < 0.05).
One of our primary aims was to expand on previous work which had determined an increase in CHO oxidation following 2 wk LCLT supplementation (14) to determine whether supplementing for longer than 2 wk had a greater affect on substrate oxidation. Despite using similar subjects, the same dosage of LCLT, and the same 2 d pre-exercise dietary and exercise controls, at 4 wk LCLT supplementation expired gas data during SS exercise was the same as pre-supplementation values and there were no differences in any hematological variables. Greig et al. (17) noted a lower HR response to exercise following 2 wk of 2 g LC/d compared to placebo, whereas there were no differences following 4 wk supplementation, and another study revealed no influence of LC supplementation during a 90 min cycle at 62.3% $\text{VO}_2\text{max}$ following 4 wk supplementation (41). In contrast, Gorostiaga et al. (16) reported a lower RER during 45 min cycling at 66% $\text{VO}_2\text{max}$ following 2 g LC/d for 4 wk in subjects of similar aerobic capacity to those in the present study (mean $\text{VO}_2\text{max}$ 62.4 mL·kg$^{-1}$·min$^{-1}$). They observed no increase in plasma total carnitine following supplementation, however, a response which differs from all other studies of oral LC supplementation. These authors acknowledged that the changes they observed might have been due to alterations of diet or exercise routine which were not recorded or controlled (16). The combination of data following 2 or 4 wk supplementation indicates that there could be a progressive adaptation to supplemental LCLT. Renal carnitine clearance changes rapidly in response to changes in plasma carnitine concentration (31), and there are large variations in the turnover of body carnitine pools. For example, liver and kidney carnitine pools turn over in around 12 h compared to 191 h for muscle carnitine (30). Hence, over short term LCLT supplementation there could be a temporary mismatch in relative carnitine status that stabilizes by 4 wk of supplementation. While Arenas and colleagues (1)

![Figure 4](image_url)
observed a 12% increase in total muscle carnitine after 2 g LC/d for 4 wk, others have found no change despite an even higher dose (37), and it has been suggested that increasing muscle carnitine stores is likely to be a slow process due to the high concentration gradient existing from muscle to plasma (37, 38). Although muscle biopsies were not taken in the present study, there is no evidence to suggest that 4 wk oral LCLT supplementation affects fatty acid or CHO utilization, or lactate accumulation, by exercising muscle in humans, as other studies have also reported (13, 24, 25, 41).

One aspect of the present study which differs from most other L-Carnitine and exercise studies is the training status of our subjects. All subjects in this study had a VO_{2\text{max}} greater than 50.8 mL·kg⁻¹·min⁻¹, and are therefore generally more exercise trained than individuals used in other carnitine supplementation studies. Training itself induces changes in muscle metabolism that enable humans to use more fat and less CHO for the same intensity of exercise than when untrained (18). In addition, exercise intensities above 50 to 65% VO_{2\text{max}} when muscle glycogen stores are normal coincide with maximal rates of lipid oxidation (10, 32, 38). Hence, our choice of subjects and exercise intensity (65% VO_{2\text{max}}) could have limited how much further fatty acid uptake and oxidation could be influenced by supplementing with LCLT. In comparison, those well-controlled studies which have found either small or significant decreases in RER or increases in fat oxidation during exercise have tended to use lower exercise intensities or less well-trained subjects than in the current study (10, 25, 40).

Although we observed no negative impact of LCLT on TT performance in the present study, the TT times failed to decrease over the LCLT supplementation period as they did in the placebo period. The difference in TT time between P₀ and LCLT₀ was on average 0.9%, within the 1.1% coefficient of variation previously reported for a 20 km TT (26), thereby indicating that consistency of performance was achieved prior to commencement of treatments. An explanation for the lack of improvement in TT performance following LCLT supplementation when this was observed during placebo supplementation is unclear from the data in the current study.

While plasma carnitine concentrations were within the normal range for both P₀ and LCLT₀, the former were significantly higher than the latter. This might be due to an insufficient “washout” period in subjects who underwent LCLT supplementation before the P treatment. Although not significantly different, the CHO oxidation during exercise at P₀ was lower, and fat oxidation higher, than during the other trials, which could possibly be due to the differences in plasma carnitine concentrations. Pre-exercise guidelines regarding 2-d pre-trial food intake collected from subjects showed no difference between total CHO or energy intake between P₀ and LCLT₀, nor was there any difference in resting plasma glucose, FFA, or glycerol concentrations between trials. Accordingly, it is not likely that differences in subject preparation were responsible for the higher plasma carnitine at P₀. In the present work, a minimum 2 wk washout period was employed, with only two subjects undertaking a longer washout period. Other studies of LC supplementation incorporating crossover designs have used durations of 7 d or less (17, 34, 36, 40) to 3 to 5 wk (7, 10, 16, 24). Only Oyono-Enguelle et al. (25) have reported plasma carnitine for a period following cessation of 4 wk LC supplementation, and they found plasma carnitine had returned to baseline after 6 to 8 wk of washout. No
plasma carnitine measures were undertaken prior to 6 wk, however, and the results of the current study raise the question as to whether more information is required on ideal washout periods following LCLT supplementation.

**Conclusion**

The data from the present study indicate that 4 wk of supplementation with 3 g LCLT/d in a group of subjects whose training status ranged from moderately- to well-trained does not affect exercise metabolism during 90 min steady-state exercise. This 4 wk supplementation period also did not benefit subsequent TT performance. Further research is required, with particular consideration of ideal washout durations following prolonged LCLT supplementation and inclusion of muscle biopsies to determine whether LCLT supplementation has any effect on muscle carnitine stores.

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**References**


