Carbohydrate, Protein, and Fat Metabolism During Exercise After Oral Carnitine Supplementation in Humans

Elizabeth M. Broad, Ronald J. Maughan, and Stuart D.R. Galloway

Twenty nonvegetarian active males were pair-matched and randomly assigned to receive 2 g of L-carnitine L-tartrate (LC) or placebo per day for 2 wk. Participants exercised for 90 min at 70% VO_{2max} after 2 days of a prescribed diet (M ± SD: 13.6 ± 1.6 MJ, 57% carbohydrate, 15% protein, 26% fat, 2% alcohol) before and after supplementation. Results indicated no change in carbohydrate oxidation, nitrogen excretion, branched-chain amino acid oxidation, or plasma urea during exercise between the beginning and end of supplementation in either group. After 2 wk of LC supplementation the plasma ammonia response to exercise tended to be suppressed (0 vs. 2 wk at 60 min exercise, 97 ± 26 vs. 80 ± 9, and 90 min exercise, 116 ± 47 vs. 87 ± 25 µmol/L), with no change in the placebo group. The data indicate that 2 wk of LC supplementation does not affect fat, carbohydrate, and protein contribution to metabolism during prolonged moderate-intensity cycling exercise. The tendency toward suppressed ammonia accumulation, however, indicates that oral LC supplementation might have the potential to reduce the metabolic stress of exercise or alter ammonia production or removal, which warrants further investigation.

**Keywords:** L-carnitine L-tartrate, cyclists, fat oxidation, carbohydrate oxidation, protein utilization

The two primary, interrelated roles of L-carnitine (LC) in metabolism are to transport long- and medium-chain fatty acids into mitochondria for β-oxidation (Fritz, 1963) and to buffer excess short-chain acyl groups, such as acetyl-CoA, thereby maintaining optimum energy flux within mitochondria (ConstantinTeodosiu, Cederblad, & Hultman, 1992). We have previously observed enhanced carbohydrate (CHO) oxidation during 60 min of cycling exercise in endurance-trained males after supplementation with LC for 2 weeks (Abramowicz & Galloway, 2005), whereas the promoted benefits of carnitine supplementation include increased fat oxidation. We have hypothesized that this shift in substrate utilization after supplementation in trained athletes might be a result of the short-chain

---

Broad and Galloway are with the Dept. of Sports Studies, University of Stirling, FK9 4LA, Scotland UK. Maughan is with the School of Sport and Exercise Sciences, Loughborough University, Leicestershire, LE11 3TU, UK.
acyl-group-buffering role of carnitine, but this action could also affect amino acid oxidation in skeletal muscle. Furthermore, it might act in the same way in other metabolically active tissues such as the liver, brain, or heart, thus influencing whole-body substrate utilization.

To date, studies investigating the effect of LC supplementation on fuel utilization during exercise have used gas-analysis techniques, with calculations for CHO and fat oxidation based on a nonprotein respiratory quotient. Protein is generally believed to contribute 5–10% of the total energy demand in prolonged exercise (Graham & MacLean, 1992), with branched-chain amino acid (BCAA) oxidation making the major contribution. Carnitine has been shown to facilitate the metabolism of BCAAs in skeletal muscle by stimulating the conversion of branched-chain keto acids (BCKAs) to carnitine esters (De Palo et al., 1993; Veerkamp, Van Moerkerk, & Wagenmakers, 1985). In doing so, the inhibition of BCKA dehydrogenase, one of the primary regulators of muscle amino acid metabolism, is removed and free coenzyme-A is released for use in the many energy-producing mitochondrial reactions. It has therefore been suggested that supplementation with carnitine might further enhance the breakdown of BCAAs during exercise by buffering the usual accumulation of BCKAs (Hoppel, 2003). Conversely, if LC supplementation were to enhance fatty-acid uptake and metabolism during exercise, it might reduce amino acid catabolism. If amino acid oxidation during exercise were to change after LC supplementation, this would bring into question the validity of using the nonprotein respiratory quotient to estimate fat and CHO oxidation during exercise. To date, the effect of LC supplementation on amino acids’ contribution to metabolism during exercise in humans has not been investigated.

The aim of this study was to determine whether supplementation with L-carnitine L-tartrate alters the fuel contribution to metabolism in endurance-trained male athletes and specifically to examine any changes in protein contribution to metabolism.

Methods

Twenty nonvegetarian male athletes actively involved in endurance training were recruited. The participants’ characteristics are shown in Table 1. All participants were fully informed about the study and underwent preparticipation screening (medical history and physical activity questionnaires) before written informed consent was obtained. The study was undertaken during the early preparation phase of the cycling and triathlon competitive season to ensure that consistent endurance-based training was being undertaken. No participant was suffering from any metabolic disorder, and none was taking any medication or nutritional supplements other than multivitamins/minerals or commercial sports drinks during training. All experimental procedures were approved by the university ethics of research committee, and all participants were free to withdraw from the study at any time without obligation.

The study was undertaken using a double-blind, placebo-controlled, pair-matched parallel design. Pair matching was undertaken primarily on the basis of submaximal exercise workload and age. Participants came to the laboratory on
four occasions over 4–5 weeks. The first visit was used to determine VO$_{2\text{max}}$, power output at 70% VO$_{2\text{max}}$, and body composition using skinfolds (biceps, triceps, subscapular, supraspinal, abdomen, midthigh, and calf; Norton & Olds, 2000). The maximal test was undertaken on an electrically braked cycle ergometer (Lode Excalibur Sport V2.1, Lode BV, The Netherlands) in a laboratory where the temperature was maintained at 20–21 °C.

All subsequent exercise trials, involving 90 min of steady-state exercise at 70% of VO$_{2\text{max}}$, were undertaken on the same day of the week and at same time of day. On the second visit participants undertook a familiarization trial to ensure that the correct power output had been selected and to familiarize them with all testing procedures. The final two visits were conducted before and after 2 weeks of LC supplementation.

### Supplementation

Supplementation consisted of two capsules taken twice daily with breakfast and evening meals (i.e., four capsules/day total) for 14 days. The supplement capsule consisted of 746 mg L-carnitine L-tartrate (L-Carnipure, Lonza Ltd., Basel, Switzerland), thereby providing 2 g of LC per day. The placebo capsule (P) consisted of a methylcellulose filler of the same weight as the carnitine. Participants’ compliance to the supplementation was assessed by checking for any remaining capsules at the end of 2 weeks and verbal questioning.

### Dietary and Exercise Controls

Each participant was prescribed their dietary intake for 48 hr pre- and 24 hr post-trial, based on attaining a minimum of 6 g carbohydrate · kg body mass (BM)$^{-1}$ · day$^{-1}$ and 1.5 g protein · kg BM$^{-1}$ · day$^{-1}$ and achieving estimated energy requirements (Burke, 1996). These 2-day diets were designed around participants’ typical dietary intake taken from a 7-day food diary. Compliance was assessed by using a checklist on which participants were asked to note any changes to their prescribed diet. Along with their prescribed diet, participants were asked to undertake the same exercise in the 48 hr before each trial to ensure

---

**Table 1 Participant Characteristics ($M \pm SD$), $n = 10$ in Each Group**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32 ± 9</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179 ± 7</td>
<td>178 ± 4</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>75.7 ± 10.2</td>
<td>76.0 ± 9.5</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>62 ± 26</td>
<td>62 ± 27</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (L/min)</td>
<td>4.92 ± 0.46</td>
<td>4.96 ± 0.64</td>
</tr>
<tr>
<td>Workload (W/kg)</td>
<td>3.1 ± 0.6</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Training history (years)</td>
<td>8.9 ± 5.3</td>
<td>9.0 ± 5.9</td>
</tr>
<tr>
<td>Current cycle training (hr/week)</td>
<td>6.5 ± 3.6</td>
<td>5.1 ± 2.4</td>
</tr>
</tbody>
</table>
that any differences observed in nitrogen balance could be attributed to a carni-
tine treatment effect and not to an effect of low glycogen stores (Lemon &
Mullin, 1980).

**The Trials**

Participants came to the laboratory for baseline measurements before treatment
commenced (0 weeks) and at the end of the 2-week supplementation period. A
24-hr urine collection was commenced 24 hr before the trial start time. Each trial
was undertaken 2 hr after a standardized meal consisting of 1 g/kg BM carbo-
hydrate (bread and jam). The last dose of the supplement was taken 3 hr before the
exercise trial, with a small snack that formed part of the prescribed diet. On par-
ticipants’ arrival at the laboratory, we collected a pretrial urine sample before
assessing nude BM and supplied a heart-rate monitor. Participants then rested in a
supine position while a cannula (20 gauge, SSS Healthcare) was inserted into an
antecubital vein. After 5 min of seated rest a blood sample was drawn without
stasis, along with a free-flowing capillary sample from a preheated hand for analy-
sis of capillary pH, pCO₂, and bicarbonate (Radiometer ABL 700, Copenhagen).
The cannula was kept patent at all times using a saline flush of 1 ml after sample
collections. Participants then began cycling for 5 min at 50% of their required
power output, followed by 85 min at a constant power output equivalent to
(M ± SD) 69.7% ± 4.4% VO₂max, at a self-selected pedal cadence. Expired gas was
collected over 4 min at 15-min intervals (e.g., 13–17 min) from time zero using an
online gas-analysis system (Sensormedics Vmax 29, Holland) calibrated with
known gases before each test. Heart rate was recorded at 60-s intervals throughout
the trial, and a rating of perceived exertion (15-point Borg scale; Borg, 1982)
recorded every 10 min throughout exercise. Venous blood was drawn at rest and
15, 30, 60, and 90 min during exercise, and finger-prick capillary samples were
taken from a prewarmed hand at rest and at 30, 60, and 90 min of exercise. Water
was provided throughout the trial, with encouragement to achieve sufficient fluid
intake to prevent a reduction in body mass based on data collected in the familiar-
ization trials. Participants were cooled with a fan throughout all trials. After com-
pleting the 90 min, they rested during the removal of the cannula, towel-dried, had
final nude BM recorded, and emptied their bladder again for sampling. For the
next 22 hr, participants maintained their prescribed dietary intake and undertook
another complete urine collection (thus completing 24 hr from the beginning of
their trial). If a participant needed to empty his bladder at any point during the
90 min he was allowed 2 min to attend to this, with a sample being drawn and the
volume included in calculations of fluid loss over the trial, and this did not alter
the total duration of activity conducted by the participants.

**Blood and Urine Analysis**

Before and at 15, 30, 60, and 90 min of exercise, duplicate 100-µl aliquots of
whole blood were immediately deproteinized in 1 ml of ice-cold 0.4-M perchlo-
ric 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 fluorometric procedures (Jenway 6200
The remaining blood was mixed well in EDTA tubes, and duplicate samples were drawn into capillary tubes that were centrifuged at 10,000 rpm for microhematocrit measurement. A further 1.5-ml portion of the blood sample was centrifuged before duplicate aliquots of plasma were drawn off for glucose and free-fatty-acid (FFA) analysis. Plasma glucose (Sigma Diagnostic), plasma FFA (Wako Chemicals, Germany), and hemoglobin (cyanmethemoglobin method) were assayed within 3 hr of blood draws using standard reagent kits (Hitachi U2001, Hitachi Instruments Ltd., USA). Blood and plasma volume changes were calculated from hematocrit and hemoglobin using standard equations (Dill & Costill, 1974).

Additional blood was collected into lithium heparin tubes at rest and at 60 and 90 min exercise, centrifuged at 5,000 rpm at 4 °C for 10 min, and plasma extracted into duplicate tubes and frozen at –60 °C until analysis. The rest and 90-min samples were used for noradrenaline analysis and adrenaline analysis by high-performance liquid chromatography with electrochemical detection using the methodology outlined by Goldstein, Feuerstein, Izzo, Kopin, and Keiser (1981), and samples at rest and at 60 and 90 min were used to determine plasma carnitine fractions by radiometric methods using liquid scintillation as outlined by McGarry and Foster (1985).

Samples for amino acid assessment (BCAAs, alanine, and glutamate) were prepared by mixing 80 µl of plasma (from the EDTA collection tube) with 20 µl of 1.375-mM internal standard 1 (L-methionine) and 10 µl of 3.3-M perchloric acid. This mix was immediately vortexed, then centrifuged at 1,300 rpm for 10 min. The supernatant was removed for analysis against a known standard by high-performance liquid chromatography using fluorescence detection and precolumn derivatization with 18 o-phthalaldehyde (Hypersel amino acid method, ThermoHypersil-Keystone, Runcorn, UK) according to the method of Heinrikson and Meredith (1984). In addition, duplicate 250-µl aliquots of plasma drawn from the lithium heparin tube were immediately frozen at –20 °C until subsequent analysis for urea nitrogen and ammonia using Sigma Diagnostics kit 171-C for ammonia and 640-B for urea nitrogen (Sigma Diagnostics, St. Louis, MO, USA).

Urinary carnitine excretion was determined in each treatment period by means of 24-hr urine collections before and after each exercise trial. 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-hr period measured to the nearest milliliter. Urinary carnitine fractions were subsequently analyzed (McGarry & Foster, 1985). An additional 5-ml sample was drawn from every urine collection before volume measurement (including the immediate preexercise, immediate postexercise, and any intervening collection) and was frozen at –20 °C until analysis for urinary nitrogen determination via the total Kjeldahl nitrogen in water method (Tecator application sub note ASN 3503) on a Tecator Kjeltec auto 1030 analyzer (Foss, Denmark).

Nitrogen balance (assuming stable sweat and fecal losses) was estimated by comparing the difference between 24-hr prescribed dietary protein intake (divided by 6.25 to calculate nitrogen intake) and 24-hr urinary nitrogen excretion, both before and after each exercise trial (Tarnopolsky, MacDougall, & Atkinson, 1988).
Statistics

All data were checked for normality of distribution and homogeneity of variance before analysis. Within-group differences were assessed using repeated-measures analysis of variance with time and trial as within-participant factors. Significant main effects were then assessed using a paired \( t \) test with Bonferroni correction to determine at which time points the differences lay. Changes between 0 and 2 weeks were compared between groups using repeated-measures analysis of variance with time as a within-participant factor and treatment group as a between-participants factor. Differences between groups were then assessed using an independent-samples \( t \) test with Bonferroni correction (SPSS version 11.0.0, SPSS Inc.). Significance was accepted at \( p < .05 \) or Bonferroni-adjusted value. All data are expressed as \( M \pm SD \) unless otherwise specified.

Results

There was no difference between 0 and 2 weeks in the 2-day pretrial or the 24-hr posttrial diet (Table 2). Participants were in apparent small positive nitrogen balance throughout all exercise trials, with no difference between 0- and 2-week trials or between treatment groups (Table 3).

Pretrial training and dietary controls were effective in ensuring that there were no differences between trials or groups for preexercise plasma glucose (5.4 ± 1.0 and 5.5 ± 0.9 mmol/L for P at 0 weeks and P at 2 weeks, 5.3 ± 0.7 and 5.4 ± 0.6 mmol/L for LC at 0 weeks and LC at 2 weeks, respectively) or BM (75.0 ± 9.8 and 75.3 ± 9.8 kg for P at 0 weeks and P at 2 weeks, 75.7 ± 9.3 and 75.9 ± 9.4 kg LC at 0 weeks and LC at 2 weeks, respectively). Blood and plasma volume fell by the same degree (6–7% and 10–11%, respectively) in the first 15 min of steady-state exercise (\( p < .01 \)) and did not change further over the duration of exercise in any trial. Furthermore, no differences were found between trials or groups for BM change (−0.40 ± 0.27 vs. −0.50 ± 0.41 kg for P at 0 weeks and P at 2 weeks and −0.50 ± 0.21 vs. −0.41 ± 0.37 kg for LC at 0 weeks and LC at 2 weeks, respectively) or fluid intake over exercise (1.34 ± 0.28 vs. 1.34 ± 0.31 L for P at 0 weeks and P at 2 weeks and 1.19 ± 0.31 vs. 1.22 ± 0.33 L for LC at 0 weeks and LC at 2 weeks, respectively); changes in hydration status over the exercise periods were therefore small (0.5%) and the same in each trial. Exercise heart rate, cadence, and rating of perceived exertion did not differ between the 0- and 2-week trials.

| Table 2 | Composition of Prescribed Diets (\( M \pm SD \)), \( n = 10 \) in Each Group |
|---|---|---|---|
| Macronutrient | 2 Days Pretrial | 24 hr Posttrial |
| | Placebo | LC | Placebo | LC |
| Energy (MJ) | 13.5 ± 1.2 | 13.7 ± 1.9 | 14.0 ± 1.9 | 13.2 ± 2.0 |
| CHO (g) | 490 ± 59 | 499 ± 79 | 495 ± 68 | 500 ± 81 |
| Protein (g) | 123 ± 11 | 122 ± 14 | 119 ± 14 | 118 ± 19 |
| Fat (g) | 97 ± 10 | 91 ± 26 | 113 ± 26 | 83 ± 22 |
Table 3  Blood Urea Nitrogen, Plasma Amino Acids, Urinary Nitrogen Excretion, and Nitrogen Balance Before and After 90 min of Exercise in Placebo- (P) and Carnitine-Supplemented (LC) Groups

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time (min)</th>
<th>Urea N₂ (mg/dL)</th>
<th>Total BCAA (µmol/L)</th>
<th>Plasma alanine (µmol/L)</th>
<th>Plasma glutamate (µmol/L)</th>
<th>N₂ excretion (g in 24 hr)</th>
<th>N₂ balancea (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 0 weeks</td>
<td>0</td>
<td>15.3 ± 2.0</td>
<td>418 ± 44</td>
<td>355 ± 80</td>
<td>63 ± 9</td>
<td>15 ± 5</td>
<td>4.3 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>16.2 ± 1.9</td>
<td>415 ± 44</td>
<td>393 ± 58</td>
<td>50 ± 12b</td>
<td>16 ± 6</td>
<td>5.4 ± 5.3</td>
</tr>
<tr>
<td>P 2 weeks</td>
<td>0</td>
<td>15.5 ± 2.3</td>
<td>414 ± 82</td>
<td>382 ± 71</td>
<td>64 ± 18</td>
<td>16 ± 5</td>
<td>3.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>16.3 ± 2.2</td>
<td>421 ± 72</td>
<td>413 ± 107</td>
<td>54 ± 14b</td>
<td>19 ± 6</td>
<td>2.6 ± 6.7</td>
</tr>
<tr>
<td>LC 0 weeks</td>
<td>0</td>
<td>15.0 ± 2.7</td>
<td>405 ± 59</td>
<td>386 ± 59</td>
<td>55 ± 13</td>
<td>17 ± 6</td>
<td>2.5 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15.7 ± 2.5</td>
<td>375 ± 40</td>
<td>412 ± 60</td>
<td>45 ± 17b</td>
<td>17 ± 6</td>
<td>5.1 ± 5.0</td>
</tr>
<tr>
<td>LC 2 weeks</td>
<td>0</td>
<td>14.5 ± 2.5</td>
<td>432 ± 109</td>
<td>407 ± 85</td>
<td>66 ± 26c</td>
<td>15 ± 6</td>
<td>4.3 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15.7 ± 2.5</td>
<td>445 ± 154</td>
<td>457 ± 113</td>
<td>52 ± 25b</td>
<td>16 ± 4</td>
<td>4.9 ± 5.0</td>
</tr>
</tbody>
</table>

Note. BCAA = branched-chain amino acids.

aNitrogen-balance data refer to 24 hr pre- and 24 hr postexercise, not 0 and 90 min. bSignificant change from resting value, p < .05. cGreater than LC 0-weeks resting value, p < .05.
within either group, although cadence was higher in the LC group (88 rpm) than the P group (82 rpm; \( p < .05 \)). Heart rate and rating of perceived exertion increased over the duration of exercise \( (p < .01) \), whereas cadence fell (~5 rpm).

**Hematological and Urinary Data**

No differences were found for pH, pCO\(_2\), bicarbonate, glucose, or FFA responses to supplementation between the P and LC groups (Table 4). There was no significant change over 90 min of steady-state exercise for pH, pCO\(_2\), and plasma glucose, whereas FFA and glycerol increased progressively throughout exercise in all trials \( (p < .01) \). Mean blood lactate (Table 4) was below 2 mmol/L in both groups at all times. There was no difference between groups for blood lactate at 0 weeks, nor between 0 and 2 weeks. Blood lactate was elevated at 15 and 30 min in the LC group at 2 weeks but did not quite reach statistical significance from 0 weeks. There was no difference for glycerol between trials in the P group, whereas in the LC group glycerol fell an average of 0.12 mmol/L from the 0- to 2-weeks trial both at rest and during exercise \( (p = .07) \).

There was no difference in the exercise response of adrenaline (change over exercise, 0 weeks P, 4.08 ± 3.10; 2 weeks P, 3.32 ± 2.10; 0 weeks LC, 3.21 ± 3.40; and 2 weeks LC, 2.06 ± 1.62 nmol/L) or noradrenaline (0 weeks P, 8.76 ± 4.03; 2 weeks P, 9.41 ± 3.73; 0 weeks LC, 6.64 ± 2.37; and 2 weeks LC, 6.32 ± 1.66 nmol/L) between trials within either group.

There were no between-trials changes in blood concentrations of urea nitrogen, total BCAA, or alanine in either group (Table 3). There was also no difference in urinary nitrogen excretion either over 24 hr (Table 3) or from immediately before to after exercise (preexercise: 0.7 ± 0.5 g P 0 weeks, 0.9 ± 0.7 g P 2 weeks, 1.1 ± 1.1 g LC 0 weeks, and 1.2 ± 1.0 g LC 2 weeks; postexercise: 0.9 ± 0.6 g P 0 weeks, 0.9 ± 0.6 g P 2 weeks, 1.1 ± 0.3 g LC 0 weeks, and 1.2 ± 0.4 g LC 2 weeks). There was no change over the exercise period in blood BCAA or alanine concentrations, but blood urea nitrogen increased progressively from 15 through to 90 min of exercise \( (p < .01) \). Resting plasma glutamate was higher in LC after 2 weeks than at 0 weeks \( (p < .05) \), with no change between 0 and 2 weeks in P (Table 3). Plasma glutamate concentrations fell over the duration of exercise in all trials \( (p < .05) \).

Plasma ammonia increased over the exercise duration in all trials except for the 2-weeks LC trial. Analysis revealed that plasma ammonia concentration was suppressed toward the end of exercise at 2 weeks in the LC group compared with 0 weeks LC, but this did not quite reach statistical significance (Figure 1).

**Substrate Metabolism**

No significant difference was found in VO\(_2\), VCO\(_2\), VE, or respiratory-exchange ratio (RER) during exercise between 0 and 2 weeks within either the P (mean RER across the exercise period of 0.80 ± 0.03 and 0.80 ± 0.04 for P at 0 weeks and P at 2 weeks, respectively) or the LC group (mean RER across the exercise period of 0.80 ± 0.05 and 0.81 ± 0.04 for LC at 0 weeks and LC at 2 weeks, respectively), and all except VCO\(_2\) changed across the exercise period, reflecting the expected cardiovascular and ventilatory drift. Because of the absence of differences in
Table 4 Responses of Blood pH, pCO₂ (kPa), Plasma Bicarbonate (mM, HCO₃), Plasma Free Fatty Acids (FFA; mM), Blood Glycerol (mM), Plasma Glucose (mM), and Blood Lactate (mM) to Exercise in Placebo- (P) and Carnitine-Supplemented (LC) Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Rest</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH P</td>
<td>0 weeks</td>
<td>7.42 (0.01)</td>
<td>—</td>
<td>7.38 (0.02)</td>
<td>7.39 (0.01)</td>
<td>7.41 (0.02)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>7.41 (0.01)</td>
<td>—</td>
<td>7.38 (0.02)</td>
<td>7.39 (0.03)</td>
<td>7.39 (0.02)</td>
</tr>
<tr>
<td>pH LC</td>
<td>0 weeks</td>
<td>7.41 (0.03)</td>
<td>—</td>
<td>7.39 (0.02)</td>
<td>7.40 (0.03)</td>
<td>7.40 (0.03)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>7.42 (0.02)</td>
<td>—</td>
<td>7.38 (0.03)</td>
<td>7.39 (0.03)</td>
<td>7.40 (0.03)</td>
</tr>
<tr>
<td>pCO₂ P</td>
<td>0 weeks</td>
<td>5.47 (0.26)</td>
<td>—</td>
<td>5.52 (0.28)</td>
<td>5.46 (0.34)</td>
<td>5.34 (0.34)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>5.48 (0.40)</td>
<td>—</td>
<td>5.48 (0.44)</td>
<td>5.39 (0.41)</td>
<td>5.42 (0.33)</td>
</tr>
<tr>
<td>pCO₂ LC</td>
<td>0 weeks</td>
<td>5.28 (0.35)</td>
<td>—</td>
<td>5.27 (0.30)</td>
<td>5.28 (0.39)</td>
<td>5.20 (0.33)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>5.38 (0.34)</td>
<td>—</td>
<td>5.40 (0.37)</td>
<td>5.35 (0.40)</td>
<td>5.24 (0.31)</td>
</tr>
<tr>
<td>HCO₃ P</td>
<td>0 weeks</td>
<td>25.7 (1.0)</td>
<td>—</td>
<td>23.9 (1.3)</td>
<td>24.4 (1.1)</td>
<td>24.7 (1.2)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>25.5 (0.8)</td>
<td>—</td>
<td>23.8 (1.2)</td>
<td>24.1 (1.6)</td>
<td>24.1 (1.2)</td>
</tr>
<tr>
<td>HCO₃ LC</td>
<td>0 weeks</td>
<td>25.0 (1.4)</td>
<td>—</td>
<td>23.5 (1.6)</td>
<td>24.2 (1.4)</td>
<td>24.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>25.7 (1.4)</td>
<td>—</td>
<td>23.5 (1.3)</td>
<td>23.9 (1.2)</td>
<td>24.1 (1.3)</td>
</tr>
<tr>
<td>FFA P</td>
<td>0 weeks</td>
<td>0.28 (0.23)</td>
<td>0.17 (0.13)</td>
<td>0.28 (0.26)</td>
<td>0.42 (0.26)</td>
<td>0.61 (0.32)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.26 (0.13)</td>
<td>0.18 (0.07)</td>
<td>0.26 (0.14)</td>
<td>0.42 (0.17)</td>
<td>0.54 (0.26)</td>
</tr>
<tr>
<td>FFA LC</td>
<td>0 weeks</td>
<td>0.33 (0.14)</td>
<td>0.24 (0.09)</td>
<td>0.37 (0.17)</td>
<td>0.60 (0.29)</td>
<td>0.76 (0.32)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.23 (0.17)</td>
<td>0.18 (0.09)</td>
<td>0.32 (0.13)</td>
<td>0.52 (0.22)</td>
<td>0.75 (0.34)</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Rest</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol P</td>
<td>0 weeks</td>
<td>0.07 (0.07)</td>
<td>0.12 (0.12)</td>
<td>0.16 (0.11)</td>
<td>0.21 (0.12)</td>
<td>0.28 (0.11)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.10 (0.10)</td>
<td>0.14 (0.11)</td>
<td>0.15 (0.10)</td>
<td>0.21 (0.11)</td>
<td>0.29 (0.10)</td>
</tr>
<tr>
<td>Glycerol LC</td>
<td>0 weeks</td>
<td>0.21 (0.13)</td>
<td>0.25 (0.14)</td>
<td>0.26 (0.15)</td>
<td>0.34 (0.14)</td>
<td>0.42 (0.16)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.12 (0.07)</td>
<td>0.14 (0.08)</td>
<td>0.17 (0.08)</td>
<td>0.21 (0.06)</td>
<td>0.28 (0.11)</td>
</tr>
<tr>
<td>Glucose P</td>
<td>0 weeks</td>
<td>5.41 (1.06)</td>
<td>4.44 (0.59)</td>
<td>4.45 (0.75)</td>
<td>4.29 (0.48)</td>
<td>3.93 (0.38)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>5.55 (0.83)</td>
<td>4.41 (0.70)</td>
<td>4.57 (0.79)</td>
<td>4.52 (0.74)</td>
<td>4.20 (0.72)</td>
</tr>
<tr>
<td>Glucose LC</td>
<td>0 weeks</td>
<td>5.38 (0.68)</td>
<td>4.39 (0.46)</td>
<td>4.56 (0.79)</td>
<td>4.45 (0.55)</td>
<td>4.18 (0.51)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>5.42 (0.56)</td>
<td>4.23 (0.76)</td>
<td>4.39 (0.60)</td>
<td>4.34 (0.50)</td>
<td>4.19 (0.46)</td>
</tr>
<tr>
<td>Lactate P</td>
<td>0 weeks</td>
<td>0.40 (0.33)</td>
<td>1.25 (0.46)</td>
<td>1.49 (0.55)</td>
<td>1.14 (0.39)</td>
<td>1.25 (0.30)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.38 (0.24)</td>
<td>1.52 (0.54)</td>
<td>1.33 (0.56)</td>
<td>1.27 (0.69)</td>
<td>1.53 (0.32)</td>
</tr>
<tr>
<td>Lactate LC</td>
<td>0 weeks</td>
<td>0.27 (0.20)</td>
<td>1.49 (0.41)</td>
<td>1.52 (0.26)</td>
<td>1.34 (0.58)</td>
<td>1.36 (0.67)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>0.26 (0.23)</td>
<td>1.80 (0.71)</td>
<td>1.96 (0.61)</td>
<td>1.43 (0.69)</td>
<td>1.25 (0.37)</td>
</tr>
</tbody>
</table>
nitrogen balance or plasma amino acid concentrations during exercise as a consequence of LC supplementation, CHO and fat utilization were estimated using the nonprotein RER (Peronnet & Massicotte, 1991). There was no 0- to 2-weeks trial difference in CHO oxidation between groups (Figure 2), although CHO oxidation was higher at all time points during exercise in the 2-weeks trial than in the 0-weeks trial in the LC group. There was a trend toward a between-groups difference in 0- to 2-weeks trial changes in fat oxidation during the 90 min of exercise ($p = .07$; Figure 3).

Figure 1 — Plasma ammonia changes over exercise after 2 weeks of supplementation with (a) placebo (P) and (b) L-carnitine L-tartrate (LC; $M \pm SEM$). *$p = .03$ (not statistically significant because of Bonferroni correction, $p < .01$), mean difference 17.1 µmol/L, 95% CI 0.52–33.73. †$p = .09$, mean difference 29.4 µmol/L, 95% CI –8.13 to 66.93.
Total CHO oxidized over the exercise period was estimated to be 139 ± 33 and 132 ± 40 g in the P group and 137 ± 36 and 147 ± 32 g in the LC group for 0- and 2-weeks trials, respectively. For the same trials, total fat oxidized was estimated to be 100 ± 16 and 105 ± 16 g in the P group and 105 ± 19 and 99 ± 21 g in the LC group for 0- and 2-weeks trials, respectively.

Carnitine Status

Resting plasma total and acyl-carnitine fractions increased after 2 weeks of LC supplementation (by 61% ± 42% and 152% ± 105%, respectively, \( p < .01 \)), with no change in free carnitine (17% ± 35% change, \( p = .27 \)). There was no change in any of these parameters at 2 weeks in P. Urinary carnitine excretion increased...
after 2 weeks of LC supplementation (mean 6.4-fold, 25.2-fold, and 1.9-fold increase for total, free, and acyl carnitine over 24 hr in urine, $p < .01$), with no change in the P group.

**Discussion**

By prescribing diets for 2 days before and 24 hr after exercise and standardizing pretrial exercise, we attempted to minimize the effect of factors that could influence substrate metabolism during exercise, such as preexercise muscle glycogen content (van Hall, Saltin, & Wagenmakers, 1999), plasma glucose and FFA concentrations (Coyle, Coggan, Hemmert, Lowe, & Walters, 1985), and hydration status (Shirreffs, Armstrong, & Cheuvront, 2004). Therefore, the absence of any differences in blood urea nitrogen, urinary nitrogen excretion, nitrogen balance, plasma BCAA, or alanine changes over exercise provides strong evidence that
there is no change in protein contribution to metabolism after 2 weeks of LC supplementation, although this should be confirmed by an isotopic tracer study. The fact that there were no differences in $\text{VCO}_2$, $\text{pCO}_2$, pH, and bicarbonate concentration between trials and that $\text{VCO}_2$, $\text{pCO}_2$, and bicarbonate were stable across the exercise period supports our assumption that the RER adequately reflects the respiratory quotient and relative fat:CHO oxidation during steady-state exercise in the current study (Peronnet & Massicotte, 1991).

The results of this study indicate no significant effect of LC supplementation on CHO use during 90 min of steady-state exercise, but there was a tendency toward a reduction in fat oxidation. In addition, plasma glycerol concentration tended to be lower, and blood lactate higher, after 2 weeks of LC supplementation, thereby supporting a tendency toward reduced mobilization and/or oxidation of fatty acids. This is contrary to the promoted benefits of LC supplementation but supports the trend shown by other studies in our laboratory after 2 weeks of LC supplementation (Abramowicz & Galloway, 2005). There have been very few well-controlled studies involving 2 weeks of LC supplementation that have measured expired gas during exercise with which to compare these results. Marconi, Sassi, Carpinelli, and Cerretelli (1985) found no difference in RER during 120 min of walking at 65% $\text{VO}_2\text{max}$ in competitive walkers after 4 g LC/day, nor did Vukovich, Costill, and Fink (1994) in participants performing 60 min of cycling exercise at 70% $\text{VO}_2\text{max}$ after 6 g LC/day combined with a high fat preload. In contrast, RER was decreased (indicating higher fat oxidation) in competitive runners (Williams, Walker, Nute, Jackson, & Brooks, 1987) and untrained males (Wyss, Ganzit, & Rienzi, 1990) after 3 weeks of LC supplementation. It is possible that the duration of LC supplementation influences the effects observed on fuel metabolism during exercise, and it has been suggested that periods of supplementation of 8 weeks or longer might be required to observe effects on skeletal-muscle metabolism because this is the usual procedure in animal studies (J. Harmeyer, personal communication). Indeed, Arenas et al. (1991) observed that carnitine ingestion (1 g twice daily over 6 months) prevented a training-induced decrease in muscle free and total carnitine in trained athletes, but to date no studies using shorter periods of supplementation have demonstrated any alteration in muscle carnitine content with oral supplementation.

Responses of plasma BCAA, alanine, glutamate, and blood urea nitrogen concentration to exercise were similar to those reported in other exercise trials in humans (De Palo et al., 1993) and with LC supplementation (Angelini et al., 1986; MacLean, Spriet, Hultman, & Graham, 1991). The increased plasma urea nitrogen over the exercise bouts indicates that amino acids were catabolized during exercise in this study (MacLean et al.). The lack of change in urinary nitrogen excretion, which has been used to assess protein contribution to exercise in other studies (Lemon & Mullin, 1980), either over the exercise period or over 24 hr after exercise, indicates a low contribution of protein to exercise (<5% of total energy expenditure). This might be because our participants were endurance trained and because the prescribed diets ensured that they maintained energy balance, sufficient CHO for training needs, and a positive nitrogen balance.

The novel finding of a tendency for blunting of ammonia ($\text{NH}_3$) accumulation toward the end of prolonged endurance exercise by LC in this study is consistent with the findings that hyperammonemia is present in many cases of carnitine
insufficiency (Llansola, Erceg, Hernandez-Viadel, Felipo, 2002). LC provision has also previously been shown to reduce blood and brain ammonia and increase glutamate concentrations, preventing the acute toxic effects of hyperammonemia in mice (Grisolia, O’Connor, & Costell, 1984) and in epileptic children undergoing valproate therapy (Gidal et al., 1997). Oyono-Enguelle et al. (1988), however, found no difference in ammonia accumulation during exercise after 4 weeks of supplementation with 2 g of LC per day, which might be related to the lower exercise intensity (<50% VO2max) and/or shorter duration (60 min) not stimulating the degree of ammonia production noted under our exercise conditions. The mean resting NH3 concentrations in the current study are within the normal range of 20–60 µM (Graham, Turcotte, Kiens, & Richter, 1997), and the elevation over exercise is similar to values reported during exercise of similar intensity and duration (Bellinger, Bold, Wilson, Noakes, & Myburgh, 2000; MacLean et al., 1991; Terjung & Tullson, 1992). This accumulation of plasma ammonia over exercise correlates with muscle NH3 concentration and efflux (MacLean et al.). The primary sources of increased NH3 are believed to be from deamination of AMP, increased amino acid catabolism, or decreased removal, and NH3 might provide a marker of muscle metabolic stress because its production increases toward the end of endurance exercise and reflects the extent of the reliance of active muscle on amino acid catabolism (Terjung & Tullson) or reflects low glycogen levels (Sahlin & Broberg, 1990). Thus, NH3 accumulation has been linked with fatigue during exercise (Ogino et al., 2000). In the absence of any change in estimated CHO oxidation or nitrogen balance in the current study it would seem that glycogen depletion or increased catabolism of amino acids cannot explain the apparent blunting of ammonia accumulation during prolonged exercise after a period of carnitine ingestion, and this effect could therefore be linked to increased removal from the circulation.

Another mechanism for an attenuated NH3 accumulation could therefore be through glutamate processing during exercise. Glutamate can accept an NH3 group to form glutamine, which is then released from muscle; it can also be transaminated with pyruvate to form alanine or can be deaminated, producing NH3 (Snow, Carey, Stathis, Febbraio, & Hargreaves, 2000). Because we also observed no change in alanine or BCAA oxidation, it is possible that the lower NH3 reflects an increased glutamine generation resulting from a more plentiful supply of glutamate precursor before exercise, as was observed in this study. Furthermore, plasma NH3 and hypoxanthine concentrations have been shown to be correlated (Ogino et al., 2000), and reduced hypoxanthine has been reported by Volek et al. (2002) after LC supplementation, suggesting that carnitine can reduce metabolic stress. Regardless of the mechanism, lowered NH3 concentrations (especially toward the end of moderate- to high-intensity endurance exercise) might reflect better maintenance of the ATP:AMP ratio in exercising muscle or other metabolically active tissues and thus appear to be indicative of reduced metabolic stress during exercise. If it is assumed, however, that muscle carnitine content did not increase in our participant group, this raises the possibility that the effects we observed on ammonia accumulation are the result of extramuscular metabolic actions of carnitine in organs such as liver, kidney, heart, and brain tissue that might affect ammonia production or removal and therefore deserve further focused attention.
Conclusion

This study indicates that LC supplementation does not appear to alter the proportional contribution of protein, CHO, or fat to energy metabolism during prolonged exercise in this sample of well-trained endurance athletes. LC supplementation appears to blunt the accumulation of ammonia, which might reflect reduced metabolic stress in the exercising muscle or increased ammonia removal from the circulation, and this warrants further investigation.

Acknowledgments

The authors would like to thank Lonza Ltd., Basel, for their support of this research, and Prof. Johein Harmeyer, Germany, for the analysis of carnitine fractions and his advice.

References


