# Carbohydrate, protein and fat metabolism during exercise following oral carnitine supplementation in man.

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Running Head: Carnitine supplementation and exercise metabolism.

Abstract:

Twenty non-vegetarian active males were pair-matched and randomly assigned to receive 2 g L-Carnitine L-tartrate (LC)·$d^{-1}$ or placebo for 2 weeks. Subjects exercised for 90 min at 70% $\dot{V}O_{2max}$ following 2 days of a prescribed diet (mean ± *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. Following 2 weeks LC supplementation the plasma ammonia response to exercise tended to be suppressed (0 vs. 2wk at 60 min exercise: 97 ± 26 vs. 80 ± 9; and 90 min exercise: 116 ± 47 vs. 87 ± 25 μmol·$L^{-1}$), with no change in the placebo group. The data indicate that 2 weeks of LC supplementation does not affect fat, carbohydrate and protein contribution to metabolism during prolonged moderate intensity cycling exercise. However, the tendency towards suppressed ammonia accumulation indicates that oral LC supplementation may have the potential to reduce the metabolic stress of exercise or alter ammonia production/removal which warrants further investigation.

Key Words: L-Carnitine L-tartrate, cyclists, fat oxidation, carbohydrate oxidation, protein utilisation.

Introduction:

The two primary, inter-related 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 (Constantin-Teodosiu, Cederblad & Hultman, 1992). We have previously observed enhanced carbohydrate (CHO) oxidation during 60 min cycling exercise in endurance-trained males following supplementation with L-Carnitine (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 utilisation following supplementation in trained athletes may be a result of the short-chain acyl group buffering role of carnitine but this action could also affect amino acid oxidation in skeletal muscle. Furthermore, it may act in the same way in other metabolically active tissues such as the liver, brain, or heart thus influencing whole body substrate utilisation.

To date, studies investigating the effect of LC supplementation on fuel utilisation during exercise have utilised gas analysis techniques, using calculations for CHO and fat oxidation based on a non-protein 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 branched-chain amino acids in skeletal muscle by stimulating the conversion of branched-chain keto acids (BCKA) to carnitine esters (De Palo, Metus, Gatti *et al.*, 1993; Veerkamp,

Van Moerkerk & Wagenmakers, 1985). In doing so, the inhibition of BCKA dehydrogenase, one of the primary regulators of muscular 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 may further enhance the breakdown of BCAA during exercise by buffering the usual accumulation of BCKA's (Hoppel, 2003). Conversely, if LC supplementation were to enhance fatty acid uptake and metabolism during exercise, it may reduce amino acid catabolism. If amino acid oxidation during exercise were to change following LC supplementation, this would question the validity of using the non-protein respiratory quotient to estimate fat and CHO oxidation during exercise. To date, the effect of LC supplementation on amino acid 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 non-vegetarian male athletes actively involved in endurance training were recruited. The subjects' characteristics are shown in Table 1. All subjects were fully informed about the study and underwent pre-participation 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 consistent

92 endurance-based training was being undertaken. No subject was suffering
93 from any metabolic disorder and none was taking any medication or
94 nutritional supplements other than multivitamins/minerals or commercial
95 sports drinks during training. All experimental procedures were approved by
96 the University Ethics of Research Committee, and all subjects were free to
97 withdraw from the study at any time without obligation.
98 The study was undertaken using a double blind, placebo-controlled, pair-
99 matched parallel design. Pair matching was undertaken primarily on the basis
100 of submaximal exercise workload and age. Subjects attended the laboratory on
101 4 occasions over 4 - 5 weeks. The first visit was used to determine $\dot{V}O_{2max}$,
102 power output at 70% $\dot{V}O_{2max}$, and body composition using skinfolds (bicep,
103 tricep, subscapular, supraspinale, abdomen, mid thigh, calf; Norton & Olds,
104 2000). The maximal test was undertaken on an electrically braked cycle
105 ergometer (Lode Excalibur Sport V2.1, Lode BV, The Netherlands) in a
106 laboratory where the temperature was maintained between 20-21$^{o}$C.
107 All subsequent exercise trials, involving 90 minutes of steady state exercise at
108 70% of $VO_{2max}$, were undertaken on the same day of the week and same time
109 of day. In the second visit subjects undertook a familiarisation trial to ensure
110 the correct power output had been selected and to familiarise subjects with all
111 testing procedures. The final two visits were conducted before and after two
112 weeks of LC supplementation.
113
114 *Supplementation*
115 Supplementation consisted of 2 capsules taken twice daily with breakfast and
116 evening meals (*i.e*. 4 capsules$\cdot d^{-1}$ total) for 14 d. The supplement capsule

117 consisted of 746 mg L-Carnitine L-tartrate (L-Carnipure®, Lonza Ltd., Basel,
118 Switzerland), thereby providing 2 g L-Carnitine·$d^{-1}$ (LC). The placebo capsule
119 consisted of a methyl cellulose filler of the same weight as the carnitine (P).
120 Subjects' compliance to the supplementation was assessed by checking for any
121 remaining capsules at the end of 2 weeks and verbal questioning.
122
123 *Dietary and Exercise Controls*
124 Each subject was prescribed their dietary intake for 48 h prior to and 24 h post
125 trial, based on attaining a minimum of 6 g carbohydrate·kg body mass $(BM)^{-1}$·$d^{-1}$
126 and 1.5 g protein·kg $BM^{-1}$·$d^{-1}$ and achieving estimated energy
127 requirements (Burke, 1996). These 2 d diets were designed around their
128 typical dietary intake taken from a 7 d food diary. Compliance was assessed
129 by using a checklist where subjects were asked to note any changes to their
130 prescribed diet. Along with their prescribed diet, subjects were requested to
131 undertake the same exercise in the 48 h prior to each trial to ensure that any
132 differences observed in nitrogen balance could be attributed to a carnitine
133 treatment effect and not to an effect of low glycogen stores (Lemon & Mullin,
134 1980).
135
136 *The Trials*
137 Subjects attended the laboratory for baseline measurements before treatment
138 commenced (0wk), and at the end of the two week supplementation period
139 (2wk). A 24 h urine collection was commenced 24 h prior to the trial
140 commencement time. Each trial was undertaken 2 h following a standardised
141 meal consisting of 1 g·kg $BM^{-1}$ carbohydrate (bread and jam). The last dose of

142 their supplement was taken 3 h before the exercise trial, with a small snack
143 which formed part of their prescribed diet. Upon arrival at the laboratory, a
144 pre-trial urine sample was collected prior to assessing nude BM, and a heart
145 rate monitor supplied. Subjects then rested in a supine position whilst a
146 cannula (20 gauge, SSS Healthcare) was inserted into an antecubital vein.
147 Following 5 min of seated rest a blood sample was drawn without stasis, along
148 with a free flowing capillary sample from a pre-heated hand for analysis of
149 capillary pH, $pCO_2$ and bicarbonate (Radiometer ABL 700, Copenhagen). The
150 cannula was kept patent at all times using a saline flush of 1 mL following
151 sample collections. Subjects then began cycling for 5 min at 50% of their
152 required power output, followed by 85 min at a constant power output
153 equivalent to (mean ± *SD*) 69.7 ± 4.4% $\dot{V}O_{2max}$, at a self-selected pedal
154 cadence. Expired gas was collected over 4 min at 15-min intervals (e.g. 13-17
155 min) from time zero using an online gas analysis system (Sensormedics Vmax
156 29, Holland) calibrated with known gases prior to each test. Heart rate was
157 recorded at 60-second intervals throughout the trial, and a rating of perceived
158 exertion (RPE, 15-point Borg scale; Borg, 1982) recorded every 10 minutes
159 throughout exercise. Venous blood was drawn at rest and 15, 30, 60 and 90
160 min during exercise, and fingerprick capillary samples were taken from a pre-
161 warmed hand at rest, 30, 60 and 90 min of exercise. Water was provided
162 throughout the trial, with encouragement to achieve sufficient fluid intake to
163 prevent a reduction in body mass based on data collected in the familiarisation
164 trials. Subjects were cooled with a fan throughout all trials. Following
165 completion of the 90 min, subjects rested during the removal of the cannula,
166 towel-dried, had final nude BM recorded, and emptied their bladder again for

sampling. For the next 22 h, subjects maintained their prescribed dietary intake and undertook another complete urine collection (thus completing 24 h from the beginning of their trial). If a subject needed to empty his bladder at any point during the 90 min they were 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 subjects.

*Blood and Urine Analysis*

Prior to, and at 15, 30, 60 and 90 min of exercise, duplicate 100 µL aliquots of whole blood were immediately deproteinised in 1 mL ice-cold 0.4 M perchloric acid (PCA), shaken vigorously and kept on ice until centrifugation at 10000 rpm for 3 min. Samples were subsequently frozen at -20$^{o}$C until analysis. Blood lactate and glycerol were measured by fluorimetric procedures (Jenway 6200 fluorimeter, Jenway Ltd, Essex; Boobis & Maughan, 1983; Maughan, 1982). The remaining blood was mixed well in EDTA tubes and duplicate samples were drawn into capillary tubes which were centrifuged at 10000 rpm for microhaematocrit 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 haemoglobin (cyanmethaemoglobin method) were assayed within 3 h of blood draws using standard reagent kits (Hitachi U2001, Hitachi Instruments Ltd, USA). Blood and plasma volume changes were calculated from haematocrit and haemoglobin 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 5000 rpm at 4$^{o}$C for 10 min, with plasma extracted into duplicate tubes and frozen at -60$^{o}$C until analysis. The rest and 90 min samples were used for analysis of noradrenaline and adrenaline analysis by HPLC with electrochemical detection using the methodology outlined by Goldstein *et al.* (1981) and rest, 60 and 90 min samples for plasma carnitine fractions determination by radiometric methods using liquid scintillation as outlined by McGarry & Foster (1985).

Samples for amino acid assessment (BCAA's, alanine and glutamate) were prepared by mixing 80 µL plasma (from EDTA collection tube) with 20 µL 1.375 mM internal standard 1 (L-Methionine) and 10 µL 3.3 M perchloric acid. This mix was immediately vortexed, then centrifuged at 1300 rpm for 10 min. The supernatant was removed for analysis against a known standard by HPLC using fluorescence detection and pre-column derivitisation with 18 o-pthalaldehyde (Hypersel amino acid method, ThermoHypersil-Keystone, Runcorn, UK) according to the method of Heinrikson & Meredith (1984). Also, duplicate 250 µL aliquots of plasma drawn from the lithium heparin tube were immediately frozen at -20$^{o}$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 h urine collections prior to and after each exercise trial. A 5 mL sample of mixed urine was collected and frozen at -60$^{o}$C until analysis, and the total volume of urine excreted over the 24 h period measured to the nearest mL. Urinary carnitine fractions were subsequently analysed (McGarry & Foster,

1985). An additional 5 mL sample was drawn from every urine collection prior to volume measurement (including the immediate pre-exercise, immediate post-exercise, and any intervening collection) and was frozen at -20$^{o}$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 analyser (Foss, Denmark).

Nitrogen balance (assuming stable sweat and faecal losses) was estimated by comparing the difference between 24 h prescribed dietary protein intake (divided by 6.25 to calculate nitrogen intake) and 24 h 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 prior to analysis. Within group differences were assessed using repeated measures analysis of variance (RMANOVA) with time and trial as within subjects factors. Significant main effects were then assessed using 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 RM ANOVA with time as a within-subjects factor and treatment group as a between-subjects factor. Differences between groups were then assessed using independent-samples T-test with Bonferroni correction (SPSS version 11.0.0, SPSS Inc. 2001). Significance was accepted at $p < 0.05$ or Bonferroni adjusted value. All data are expressed as mean ± *SD* unless otherwise specified.

242

243 Results:

244 There was no difference between 0 and 2 wk in the 2 d pre-trial or the 24 h
245 post-trial diets (Table 2). Subjects were in apparent small positive nitrogen
246 balance throughout all exercise trials, with no difference between 0 and 2
247 week trials or between treatment groups (Table 3).
248 Pre-trial training and dietary controls were effective in ensuring that there
249 were no differences between trials or groups for pre-exercise plasma glucose
250 (5.4 ± 1.0 and 5.5 ± 0.9 $mmol \cdot L^{-1}$ for P 0wk and P 2wk, 5.3 ± 0.7 and 5.4 ± 0.6
251 $mmol \cdot L^{-1}$ LC 0wk and LC 2wk, respectively) or body mass (75.0 ± 9.8 and
252 75.3 ± 9.8 kg P 0wk and P 2wk, 75.7 ± 9.3 and 75.9 ± 9.4 kg LC 0wk and LC
253 2wk, respectively). Blood and plasma volume fell by the same degree (6-7%
254 and 10-11%, respectively) in the first 15 min of steady state exercise ($p <$
255 0.01), and did not change further over the duration of exercise in any trial.
256 Further, no differences were found between trials or groups for body mass
257 change (-0.40 ± 0.27 vs. -0.50 ± 41 kg for P 0wk and P 2wk, and -0.50 ± 0.21
258 vs. -0.41 ± 0.37 kg for LC 0wk and LC 2wk, respectively) or fluid intake over
259 exercise (1.34 ± 0.28 vs. 1.34 ± 0.31 L for P 0wk and P 2wk, and 1.19 ± 0.31
260 vs. 1.22 ± 0.33 L for LC 0wk and LC 2wk, respectively); changes in hydration
261 status over the exercise periods were therefore small (0.5%) and the same in
262 each trial. Exercise HR, cadence and RPE did not differ between the 0 and 2
263 wk trials within either group, although cadence was higher in the LC group
264 (88 rpm) than P group (82 rpm, $p < 0.05$). HR and RPE increased over the
265 duration of exercise ($p < 0.01$) whilst cadence fell (~5 rpm).

266

*Haematological and Urinary Data*

No differences were found for pH, $pCO_2$, bicarbonate, glucose or FFA responses to supplementation between P and LC groups (Table 4). There was no significant change over the duration of 90 min steady state exercise for pH, $pCO_2$ and plasma glucose, whereas FFA and glycerol increased progressively throughout exercise in all trials ($p < 0.01$). Mean blood lactate (Table 4) was below 2 $mmol.L^{-1}$ in both groups at all times. There was no difference between groups for blood lactate at 0 weeks, nor between 0 and 2 weeks in LC and P groups. Blood lactate was elevated at 15 and 30 mins in the LC group at 2 wk but did not quite reach statistical significance from 0wk. There was no difference for glycerol between trials in the P group, whereas in the LC group glycerol fell an average of 0.12 $mmol.L^{-1}$ from the 0 to 2 wk trial both at rest and during exercise ($p = 0.07$).

There was no difference in the exercise response of adrenaline (change over exercise, 0 wk P: 4.08 ± 3.10, 2 wk P: 3.32 ± 2.10, 0 wk LC: 3.21 ± 3.40, 2 wk LC: 2.06 ± 1.62 $nmol{\cdot}L^{-1}$) or noradrenaline between trials within either group (0 wk P: 8.76 ± 4.03, 2 wk P: 9.41 ± 3.73, 0 wk LC: 6.64 ± 2.37, 2 wk LC: 6.32 ± 1.66 $nmol{\cdot}L^{-1}$).

There were no between trial changes for 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 h (Table 3) or immediately before to after exercise (pre-exercise: 0.7 ± 0.5 g P 0wk, 0.9 ± 0.7 g P 2wk, 1.1 ± 1.1 g LC 0wk, 1.2 ± 1.0 g LC 2wk; post-exercise: 0.9 ± 0.6 g P 0wk, 0.9 ± 0.6 g P 2wk, 1.1 ± 0.3 g LC 0wk, 1.2 ± 0.4 g LC 2wk). There was no change over the exercise period in blood BCAA or alanine concentrations, but blood urea

292 nitrogen increased progressively from 15 through to 90 min of exercise ($p <$
293 0.01). Resting plasma glutamate was higher following 2 wk LC than 0 wk LC
294 ($p < 0.05$), with no change between 0 and 2 wk P (Table 3). Plasma glutamate
295 concentrations fell over the duration of exercise in all trials ($p < 0.05$).
296 Plasma ammonia increased over the exercise duration in all trials except for
297 2wk LC trial. Analysis revealed that plasma ammonia concentration was
298 suppressed towards the end of exercise at 2 wk in the LC group compared with
299 0wk LC but this did not quite reach statistical significance (Figure 1).
300
301 *Substrate Metabolism*:
302 No significant difference was found in $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ or respiratory
303 exchange ratio (RER) during exercise between 0 and 2 wk within either P
304 (mean RER across the exercise period of 0.80 ± 0.03 and 0.80 ± 0.04 for P
305 0wk and P 2wk, respectively) or LC groups (mean RER across the exercise
306 period of 0.80 ± 0.05 and 0.81 ± 0.04 for LC 0wk and LC 2wk, respectively),
307 and all except $\dot{V}CO_2$ changed across the exercise period, reflecting the
308 expected cardiovascular and ventilatory drift. Due to the absence of
309 differences in nitrogen balance or plasma amino acid concentrations during
310 exercise as a consequence of LC supplementation, CHO and fat utilisation
311 were estimated using the non-protein RER (Peronnet & Massicotte, 1991).
312 There was no 0 to 2 wk trial difference in CHO oxidation between groups
313 (Figure 2) although CHO oxidation was higher at all time points during
314 exercise in the 2wk trial compared with 0wk trial in the LC group. There was a
315 trend towards a between group difference in 0 to 2 wk changes in fat oxidation
316 during the 90 min exercise ($p = 0.07$, Figure 3).

317 Total CHO oxidised (mean ± SD) over the exercise period was estimated to be
318 139 ± 33 and 132 ± 40 g (P group) and 137 ± 36 and 147 ± 32 g (LC group)
319 for 0 and 2 wk trials, respectively. For the same trials, total fat oxidised was
320 estimated to be 100 ± 16 and 105 ± 16 g (P group) and 105 ± 19 and 99 ± 21 g
321 (LC group) for 0 and 2 wk trials, respectively.
322
323 *Carnitine Status*
324 Resting plasma total and acyl-carnitine fractions increased following 2 wk LC
325 supplementation (by 61 ± 42% and 152 ± 105%, $p < 0.01$, respectively) with
326 no change in free carnitine (17 ± 35% change, $p = 0.27$). There was no change
327 in any of these parameters at 2 wk P. Urinary carnitine excretion increased
328 following 2 wk LC supplementation (mean 6.4-fold, 25.2-fold and 1.9-fold
329 increase for total, free and acyl-carnitine over 24 h in urine, $p < 0.01$), with no
330 change in the P group.
331
332 Discussion:
333 By prescribing diets for 2 d prior to and 24 h after exercise and standardising
334 pre-trial exercise, we have attempted to minimise the effect of factors which
335 could influence substrate metabolism during exercise, such as pre-exercise
336 muscle glycogen content (van Hall, Saltin & Wagenmakers, 1999), plasma
337 glucose and FFA concentrations (Coyle, Coggan, Hemmert *et al*., 1985) and
338 hydration status (Shirreffs, Armstrong & Cheuvront, 2004). Therefore, the
339 absence of any differences in blood urea nitrogen, urinary nitrogen excretion,
340 nitrogen balance, plasma BCAA or alanine changes over exercise provides
341 strong evidence that there is no change in protein contribution to metabolism

following 2 weeks of LC supplementation, although this should be confirmed by an isotopic tracer study. The fact that there were no differences in $\dot{V}CO_2$, $pCO_2$, pH and bicarbonate concentration between trials, and that $\dot{V}CO_2$, $pCO_2$, and bicarbonate are 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 present study (Peronnet & Massicotte, 1991).

The results of this study indicate no significant effect of LC supplementation on carbohydrate use during 90 min steady state exercise, but there was a tendency towards a reduction in fat oxidation. In addition, plasma glycerol concentration tended to be lower, and blood lactate higher, following 2 weeks LC supplementation, thereby supporting a tendency towards reduced mobilisation 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 following 2 weeks LC supplementation (Abramowicz & Galloway, 2005). Very few well controlled studies involving 2 weeks LC supplementation have measured expired gas during exercise with which to compare these results. Marconi *et al.* (1985) found no difference in RER during 120 min walking at 65% $\dot{V}O_{2max}$ in competitive walkers following 4 g $LC.d^{-1}$, nor did Vukovich *et al.* (1994) in participants performing 60 minutes of cycling exercise at 70% $\dot{V}O_{2max}$ following 6 g $LC.d^{-1}$ combined with a high fat preload. In contrast, RER was decreased (indicating higher fat oxidation) in competitive runners (Williams, Walker, Nute *et al.*, 1987) and untrained males (Wyss, Ganzit & Rienzi, 1990) after 3 weeks LC supplementation. It is possible that the duration of LC supplementation influences the effects

367 observed on fuel metabolism during exercise and it has been suggested that
368 periods of supplementation 8 wk or longer may be required to observe effects
369 on skeletal muscle metabolism as this is the usual procedure in animal studies
370 (J. Harmeyer, personal communication). Indeed, Arenas *et al.* (1991) observed
371 that carnitine ingestion (1g twice daily over 6 months) prevented a training-
372 induced decrease in muscle free and total carnitine in trained athletes but to
373 date no studies using shorter periods of supplementation have demonstrated
374 any alteration in muscle carnitine content with oral supplementation.

375 Plasma BCAA, alanine, glutamate and blood urea nitrogen concentration
376 responses to exercise were similar to those reported in other exercise trials in
377 humans (De Palo *et al.*, 1993) and with LC supplementation (Angelini,
378 Vergani, Costa *et al.*, 1986; MacLean, Spriet, Hultman *et al.*, 1991). The
379 increased plasma urea nitrogen over the exercise bouts indicate that amino
380 acids were catabolised during exercise in this study (MacLean *et al.*, 1991).
381 The lack of change in urinary nitrogen excretion, which has been used to
382 assess protein contribution to exercise in other studies (Lemon & Mullin,
383 1980), either over the exercise period or over 24 h after exercise indicates a
384 low contribution of protein to exercise (<5% total energy expenditure). This
385 may be due to the fact that our subjects were endurance-trained, and because
386 the prescribed diets ensured they maintained energy balance, sufficient
387 carbohydrate for training needs, and a positive nitrogen balance.
388 The novel finding of a tendency for blunting of ammonia ($NH_3$) accumulation
389 toward the end of prolonged endurance exercise by LC in this study is
390 consistent with the findings that hyperammonemia is present in many cases of
391 carnitine insufficiency (Llansola, Erceg, Hernandez-Viadel *et al*., 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, Inglese, Meyer *et al.*, 1997). However, Oyono-Enguelle *et al.* (1988) found no difference in ammonia accumulation during exercise after 4 wk supplementation with 2 g $LC \cdot d^{-1}$, which may be related to the lower exercise intensity (< 50% $\dot{V}O_{2max}$) and / or shorter duration (60 min) not stimulating the degree of ammonia production noted under our exercise conditions. The mean resting $NH_3$ concentrations in the current study are within the normal range of 20 - 60 μM (Graham, Turcotte, Kiens *et al.*, 1997) and the elevation over exercise is similar to values reported during exercise of similar intensity and duration (Bellinger, Bold, Wilson *et al.*, 2000; MacLean *et al.*, 1991; Terjung & Tullson, 1992). This accumulation of plasma ammonia over exercise correlates with muscle $NH_3$ concentration and efflux (MacLean *et al.*, 1991). The primary sources of increased $NH_3$ are believed to be from deamination of AMP, increased amino acid catabolism, or decreased removal, and $NH_3$ may provide a marker of muscle metabolic stress because its production increases towards the end of endurance exercise and reflects the extent of the reliance of active muscle on amino acid catabolism (Terjung & Tullson, 1992) or reflects low glycogen levels (Sahlin & Broberg, 1990). Thus, $NH_3$ accumulation has been linked with fatigue during exercise (Ogino, Kinugawa, Osaki *et al.*, 2000). In the absence of any change in estimated carbohydrate oxidation or nitrogen balance in the present study it would seem that glycogen depletion and/or increased catabolism of amino acids cannot explain the apparent

417 blunting of ammonia accumulation during prolonged exercise following a
418 period of carnitine ingestion and this effect could therefore be linked to
419 increased removal from the circulation.
420 Another mechanism for an attenuated $NH_3$ accumulation could therefore be
421 through glutamate processing during exercise. Glutamate can accept an $NH_3$
422 group to form glutamine, which is then released from muscle; it can also be
423 transaminated with pyruvate to form alanine, or can be deaminated, producing
424 $NH_3$ (Snow, Carey, Stathis *et al.*, 2000). Since we also observed no change in
425 alanine or BCAA oxidation, it is possible that the lower $NH_3$ reflects an
426 increased glutamine generation due to a more plentiful supply of glutamate
427 precursor prior to exercise, as was observed in this study. Furthermore, plasma
428 $NH_3$ and hypoxanthine concentrations have been shown to be correlated
429 (Ogino *et al.*, 2000), and reduced hypoxanthine has been reported by Volek *et*
430 *al*. (2002) following LC supplementation suggesting that carnitine can reduce
431 metabolic stress. Regardless of the mechanism, lowered $NH_3$ concentrations
432 (especially towards the end of moderate-high intensity endurance exercise)
433 may reflect better maintenance of the ATP:AMP ratio within exercising
434 muscle or other metabolically active tissues and thus appear to be indicative of
435 reduced metabolic stress during exercise. However, if it is assumed that
436 muscle carnitine content did not increase in our subject group, this raises the
437 possibility that the effects we have observed on ammonia accumulation are the
438 result of extramuscular metabolic actions of carnitine in organs such as liver,
439 kidney, heart and brain tissue which may affect ammonia production or
440 removal and therefore deserve further focussed attention.
441

442 Conclusion:

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

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FIGURE LEGENDS

Figure 1. Plasma ammonia changes over exercise following 2 wk P (A) and LC (B) (mean ± *SEM*).

* $p$ = 0.03 (not statistically significant due to Bonferroni correction, p<0.01), mean difference ($\mu mol \cdot L^{-1}$) 17.1, 95% CI (0.52 to 33.73)

† p = 0.09, mean difference ($\mu mol \cdot L^{-1}$) 29.4, 95% CI (-8.13 to 66.93)

Figure 2. Rate of CHO oxidation during 90 min exercise in P (A) and LC (B) (mean ± *SEM*).

Figure 3. Rate of fat oxidation during 90 min exercise in P (A) and LC (B) (mean ± *SEM*).

1 Table 1. Subject characteristics (mean ± *SD*), n = 10 in each group.

2

| CHARACTERISTIC | PLACEBO | LC |
|---|---|---|
| Age (y) | 32 ± 9 | 34 ± 10 |
| Height (cm) | 179 ± 7 | 178 ± 4 |
| Body Mass (kg) | 75.7 ± 10.2 | 76.0 ± 9.5 |
| Sum of Skinfolds (mm) | 62 ± 26 | 62 ± 27 |
| $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) | 4.92 ± 0.46 | 4.96 ± 0.64 |
| Workload ($W \cdot kg^{-1}$) | 3.1 ± 0.6 | 3.0 ± 0.6 |
| Training History (y) | 8.9 ± 5.3 | 9.0 ± 5.9 |
| Current Cycle Training ($h \cdot wk^{-1}$) | 6.5 ± 3.6 | 5.1 ± 2.4 |

3

1 Table 2. Composition of prescribed diets (mean ± SD, n=10 in each group).

| MACRONUTRIENT | 2 DAY PRE-TRIAL | | 24 HR POST TRIAL | |
|---|---|---|---|---|
| | 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 exercise.

| Trial / Time (min) | | Urea $N_2$ ($mg \cdot dL^{-1}$) | Total BCAA ($\mu mol \cdot L^{-1}$) | Plasma alanine ($\mu mol \cdot L^{-1}$) | Plasma glutamate ($\mu mol \cdot L^{-1}$) | $N_2$ excretion (g in 24 h) | $N_2$ balance* (g) |
|---|---|---|---|---|---|---|---|
| P 0wk | 0 | 15.3 ± 2.0 | 418 ± 44 | 355 ± 80 | 63 ± 9 | 15 ± 5 | 4.3 ± 4.3 |
| | 90 | 16.2 ± 1.9 | 415 ± 44 | 393 ± 58 | 50 ± 12[b] | 16 ± 6 | 5.4 ± 5.3 |
| P 2wk | 0 | 15.5 ± 2.3 | 414 ± 82 | 382 ± 71 | 64 ± 18 | 16 ± 5 | 3.7 ± 4.9 |
| | 90 | 16.3 ± 2.2 | 421 ± 72 | 413 ± 107 | 54 ± 14[b] | 19 ± 6 | 2.6 ± 6.7 |
| LC 0wk | 0 | 15.0 ± 2.7 | 405 ± 59 | 386 ± 59 | 55 ± 13 | 17 ± 6 | 2.5 ± 4.7 |
| | 90 | 15.7 ± 2.5 | 375 ± 40 | 412 ± 60 | 45 ± 17[b] | 17 ± 6 | 5.1 ± 5.0 |
| LC 2wk | 0 | 14.5 ± 2.5 | 432 ± 109 | 407 ±85 | 66 ± 26[a] | 15 ± 6 | 4.3 ± 4.5 |
| | 90 | 15.7 ± 2.5 | 445 ± 154 | 457 ± 113 | 52 ± 25[b] | 16 ± 4 | 4.9 ± 5.0 |

* nitrogen balance data refers to 24 h pre and 24 h post exercise, not 0 and 90 min
[a] greater than LC 0 wk resting value, $p < 0.05$
[b] significant change from resting value, $p < 0.05$

Table 4: Blood pH, $pCO_2$ (kPa), plasma bicarbonate (mM, $HCO_3$), plasma FFA (mM), blood glycerol (mM), plasma glucose (mM) and blood lactate (mM) responses to exercise in placebo and carnitine supplemented groups

| Variable / trial | | Rest | 15 min | 30 min | 60 min | 90 min |
|---|---|---|---|---|---|---|
| pH P | 0wk | 7.42 (0.01) | - | 7.38 (0.02) | 7.39 (0.01) | 7.41 (0.02) |
| | 2wk | 7.41 (0.01) | - | 7.38 (0.02) | 7.39 (0.03) | 7.39 (0.02) |
| pH LC | 0wk | 7.41 (0.03) | - | 7.39 (0.02) | 7.40 (0.03) | 7.40 (0.03) |
| | 2wk | 7.42 (0.02) | - | 7.38 (0.03) | 7.39 (0.03) | 7.40 (0.03) |
| $pCO_2$ P | 0wk | 5.47 (0.26) | - | 5.52 (0.28) | 5.46 (0.34) | 5.34 (0.34) |
| | 2wk | 5.48 (0.40) | - | 5.48 (0.44) | 5.39 (0.41) | 5.42 (0.33) |
| $pCO_2$ LC | 0wk | 5.28 (0.35) | - | 5.27 (0.30) | 5.28 (0.39) | 5.20 (0.33) |
| | 2wk | 5.38 (0.34) | - | 5.40 (0.37) | 5.35 (0.40) | 5.24 (0.31) |
| $HCO_3$ P | 0wk | 25.7 (1.0) | - | 23.9 (1.3) | 24.4 (1.1) | 24.7 (1.2) |
| | 2wk | 25.5 (0.8) | - | 23.8 (1.2) | 24.1 (1.6) | 24.1 (1.2) |
| $HCO_3$ LC | 0wk | 25.0 (1.4) | - | 23.5 (1.6) | 24.2 (1.4) | 24.0 (1.0) |
| | 2wk | 25.7 (1.4) | - | 23.5 (1.3) | 23.9 (1.2) | 24.1 (1.3) |
| FFA P | 0wk | 0.28 (0.23) | 0.17 (0.13) | 0.28 (0.26) | 0.42 (0.26) | 0.61 (0.32) |
| | 2wk | 0.26 (0.13) | 0.18 (0.07) | 0.26 (0.14) | 0.42 (0.17) | 0.54 (0.26) |
| FFA LC | 0wk | 0.33 (0.14) | 0.24 (0.09) | 0.37 (0.17) | 0.60 (0.29) | 0.76 (0.32) |
| | 2wk | 0.23 (0.17) | 0.18 (0.09) | 0.32 (0.13) | 0.52 (0.22) | 0.75 (0.34) |
| Glycerol P | 0wk | 0.07 (0.07) | 0.12 (0.12) | 0.16 (0.11) | 0.21 (0.12) | 0.28 (0.11) |
| | 2wk | 0.10 (0.10) | 0.14 (0.11) | 0.15 (0.10) | 0.21 (0.11) | 0.29 (0.10) |
| Glycerol LC | 0wk | 0.21 (0.13) | 0.25 (0.14) | 0.26 (0.15) | 0.34 (0.14) | 0.42 (0.16) |
| | 2wk | 0.12 (0.07) | 0.14 (0.08) | 0.17 (0.08) | 0.21 (0.06) | 0.28 (0.11) |
| Glucose P | 0wk | 5.41 (1.06) | 4.44 (0.59) | 4.45 (0.75) | 4.29 (0.48) | 3.93 (0.38) |
| | 2wk | 5.55 (0.83) | 4.41 (0.70) | 4.57 (0.79) | 4.52 (0.74) | 4.20 (0.72) |
| Glucose LC | 0wk | 5.38 (0.68) | 4.39 (0.46) | 4.56 (0.79) | 4.45 (0.55) | 4.18 (0.51) |
| | 2wk | 5.42 (0.56) | 4.23 (0.76) | 4.39 (0.60) | 4.34 (0.50) | 4.19 (0.46) |
| Lactate P | 0wk | 0.40 (0.33) | 1.25 (0.46) | 1.49 (0.55) | 1.14 (0.39) | 1.25 (0.30) |
| | 2wk | 0.38 (0.24) | 1.52 (0.54) | 1.33 (0.56) | 1.27 (0.69) | 1.53 (0.32) |
| Lactate LC | 0wk | 0.27 (0.20) | 1.49 (0.41) | 1.52 (0.26) | 1.34 (0.58) | 1.36 (0.67) |
| | 2wk | 0.26 (0.23) | 1.80 (0.71) | 1.96 (0.61) | 1.43 (0.69) | 1.25 (0.37) |

Figure 1.

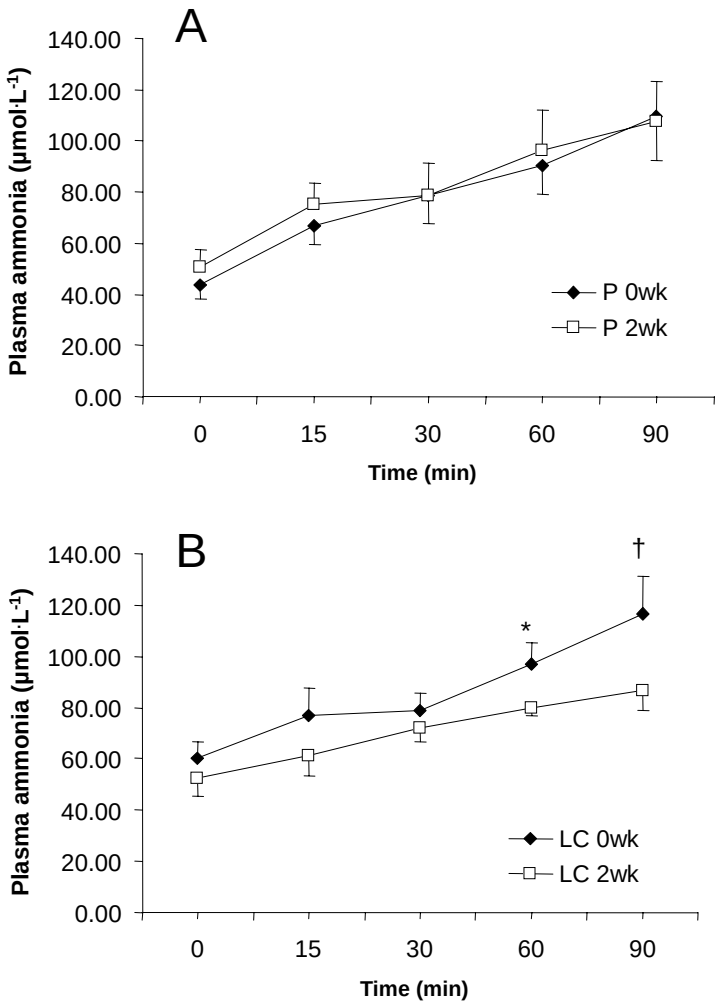

A
Plasma ammonia (µmol·L-1)
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
15
30
60
90
Time (min)
P 0wk
P 2wk
B
Plasma ammonia (µmol·L-1)
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
15
30
60
90
Time (min)
*
†
LC 0wk
LC 2wk

Figure 2.

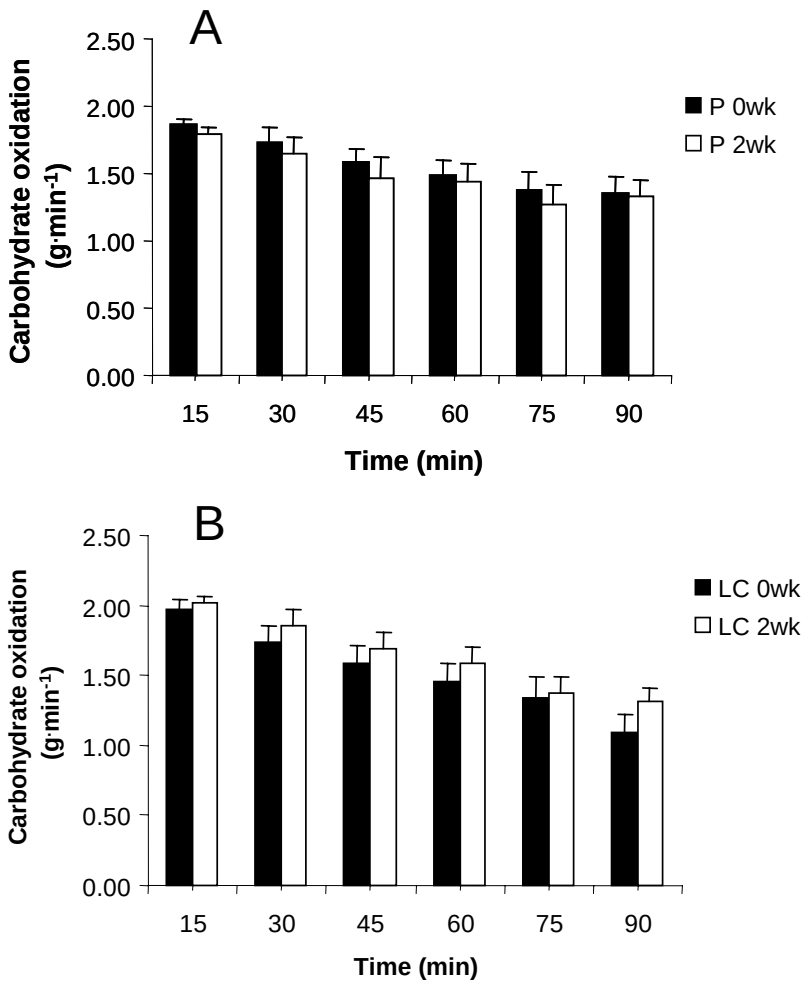
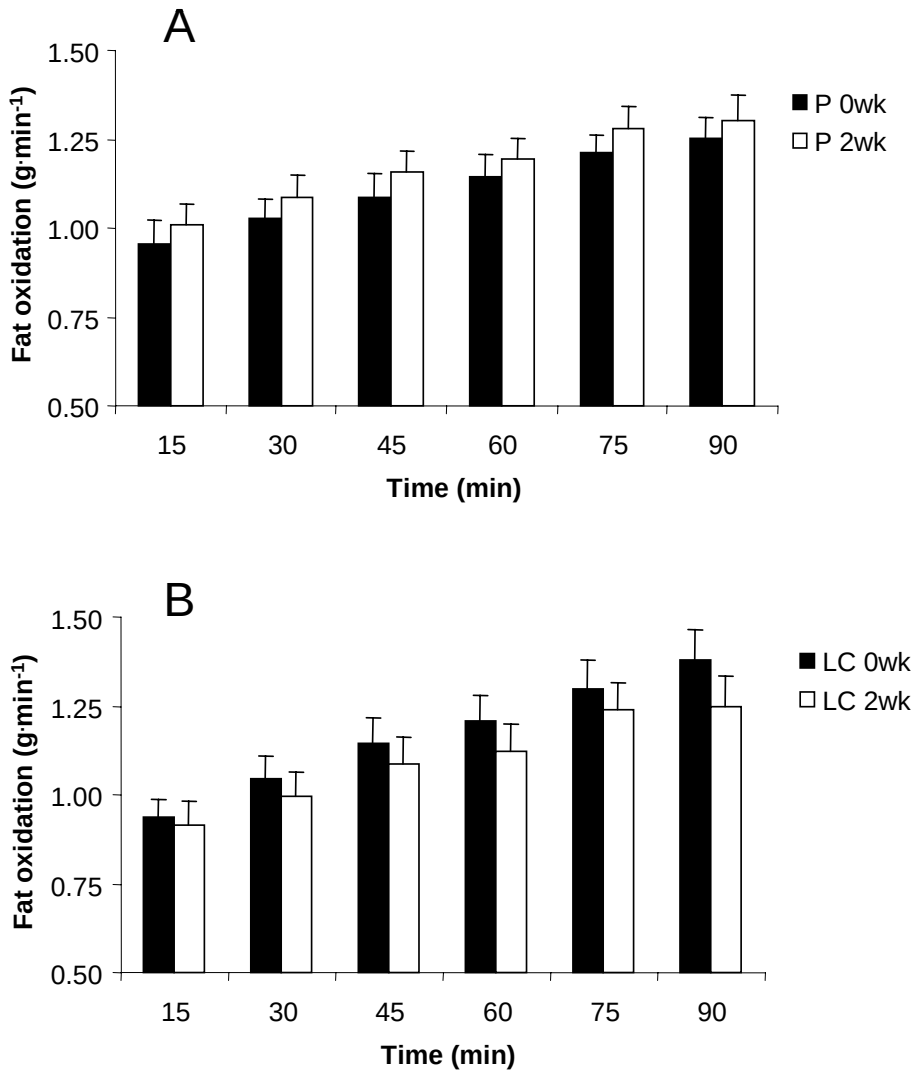

A
Carbohydrate oxidation (g·min-1)
2.50
2.00
1.50
1.00
0.50
0.00
15
30
45
60
75
90
Time (min)
P 0wk
P 2wk
B
Carbohydrate oxidation (g·min-1)
2.50
2.00
1.50
1.00
0.50
0.00
15
30
45
60
75
90
Time (min)
LC 0wk
LC 2wk

Figure 3.


A
1.50
1.25
1.00
0.75
0.50
Fat oxidation (g·min-1)
15
30
45
60
75
90
Time (min)
P 0wk
P 2wk
B
1.50
1.25
1.00
0.75
0.50
Fat oxidation (g·min-1)
15
30
45
60
75
90
Time (min)
LC 0wk
LC 2wk