

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

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19 Running Head: Carnitine supplementation and exercise metabolism.

20 Abstract:

21 Twenty non-vegetarian active males were pair-matched and randomly
22 assigned to receive 2 g L-Carnitine L-tartrate (LC)d⁻¹ or placebo for 2 weeks.
23 Subjects exercised for 90 min at 70% $\dot{V} O_{2\max}$ following 2 days of a prescribed
24 diet (mean \pm SD: 13.6 \pm 1.6 MJ, 57% carbohydrate, 15% protein, 26% fat, 2%
25 alcohol) before and after supplementation. Results indicated no change in
26 carbohydrate oxidation, nitrogen excretion, branched-chain amino acid
27 oxidation, or plasma urea during exercise between the beginning and end of
28 supplementation in either group. Following 2 weeks LC supplementation the
29 plasma ammonia response to exercise tended to be suppressed (0 vs. 2wk at 60
30 min exercise: 97 \pm 26 vs. 80 \pm 9; and 90 min exercise: 116 \pm 47 vs. 87 \pm 25
31 $\mu\text{mol}\cdot\text{L}^{-1}$), with no change in the placebo group. The data indicate that 2 weeks
32 of LC supplementation does not affect fat, carbohydrate and protein
33 contribution to metabolism during prolonged moderate intensity cycling
34 exercise. However, the tendency towards suppressed ammonia accumulation
35 indicates that oral LC supplementation may have the potential to reduce the
36 metabolic stress of exercise or alter ammonia production/removal which
37 warrants further investigation.

38

39

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

42 Introduction:

43 The two primary, inter-related roles of L-carnitine (LC) in metabolism are to
44 transport long- and medium-chain fatty acids into mitochondria for β -
45 oxidation (Fritz, 1963), and to buffer excess short-chain acyl groups, such as
46 acetyl-CoA, thereby maintaining optimum energy flux within mitochondria
47 (Constantin-Teodosiu, Cederblad & Hultman, 1992). We have previously
48 observed enhanced carbohydrate (CHO) oxidation during 60 min cycling
49 exercise in endurance-trained males following supplementation with L-
50 Carnitine (LC) for 2 weeks (Abramowicz & Galloway, 2005), whereas the
51 promoted benefits of carnitine supplementation include increased fat
52 oxidation. We have hypothesized that this shift in substrate utilisation
53 following supplementation in trained athletes may be a result of the short-
54 chain acyl group buffering role of carnitine but this action could also affect
55 amino acid oxidation in skeletal muscle. Furthermore, it may act in the same
56 way in other metabolically active tissues such as the liver, brain, or heart thus
57 influencing whole body substrate utilisation.

58 To date, studies investigating the effect of LC supplementation on fuel
59 utilisation during exercise have utilised gas analysis techniques, using
60 calculations for CHO and fat oxidation based on a non-protein respiratory
61 quotient. Protein is generally believed to contribute 5 - 10% of the total energy
62 demand in prolonged exercise (Graham & MacLean, 1992), with branched-
63 chain amino acid (BCAA) oxidation making the major contribution. Carnitine
64 has been shown to facilitate the metabolism of branched-chain amino acids in
65 skeletal muscle by stimulating the conversion of branched-chain keto acids
66 (BCKA) to carnitine esters (De Palo, Metus, Gatti *et al.*, 1993; Veerkamp,

67 Van Moerkerk & Wagenmakers, 1985). In doing so, the inhibition of BCKA
68 dehydrogenase, one of the primary regulators of muscular amino acid
69 metabolism, is removed and free coenzyme-A is released for use in the many
70 energy-producing mitochondrial reactions. It has therefore been suggested that
71 supplementation with carnitine may further enhance the breakdown of BCAA
72 during exercise by buffering the usual accumulation of BCKA's (Hoppel,
73 2003). Conversely, if LC supplementation were to enhance fatty acid uptake
74 and metabolism during exercise, it may reduce amino acid catabolism. If
75 amino acid oxidation during exercise were to change following LC
76 supplementation, this would question the validity of using the non-protein
77 respiratory quotient to estimate fat and CHO oxidation during exercise. To
78 date, the effect of LC supplementation on amino acid contribution to
79 metabolism during exercise in humans has not been investigated.
80 The aim of this study was to determine whether supplementation with L-
81 Carnitine L-tartrate alters the fuel contribution to metabolism in endurance-
82 trained male athletes and specifically to examine any changes in protein
83 contribution to metabolism.

84

85 Methods:

86 Twenty non-vegetarian male athletes actively involved in endurance training
87 were recruited. The subjects' characteristics are shown in Table 1. All subjects
88 were fully informed about the study and underwent pre-participation screening
89 (medical history and physical activity questionnaires) before written informed
90 consent was obtained. The study was undertaken during the early preparation
91 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°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·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·
126 d⁻¹ 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, pCO₂ 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 \pm SD) $69.7 \pm 4.4\% \dot{V} O_{2\max}$, 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

167 sampling. For the next 22 h, subjects maintained their prescribed dietary
168 intake and undertook another complete urine collection (thus completing 24 h
169 from the beginning of their trial). If a subject needed to empty his bladder at
170 any point during the 90 min they were allowed 2 min to attend to this, with a
171 sample being drawn and the volume included in calculations of fluid loss over
172 the trial, and this did not alter the total duration of activity conducted by the
173 subjects.

174

175 *Blood and Urine Analysis*

176 Prior to, and at 15, 30, 60 and 90 min of exercise, duplicate 100 μ L aliquots of
177 whole blood were immediately deproteinised in 1 mL ice-cold 0.4 M
178 perchloric acid (PCA), shaken vigorously and kept on ice until centrifugation
179 at 10000 rpm for 3 min. Samples were subsequently frozen at -20°C until
180 analysis. Blood lactate and glycerol were measured by fluorimetric procedures
181 (Jenway 6200 fluorimeter, Jenway Ltd, Essex; Boobis & Maughan, 1983;
182 Maughan, 1982). The remaining blood was mixed well in EDTA tubes and
183 duplicate samples were drawn into capillary tubes which were centrifuged at
184 10000 rpm for microhaematocrit measurement. A further 1.5 mL portion of
185 the blood sample was centrifuged before duplicate aliquots of plasma were
186 drawn off for glucose and free fatty acid (FFA) analysis. Plasma glucose
187 (Sigma Diagnostic), plasma FFA (Wako Chemicals, Germany) and
188 haemoglobin (cyanmethaemoglobin method) were assayed within 3 h of blood
189 draws using standard reagent kits (Hitachi U2001, Hitachi Instruments Ltd,
190 USA). Blood and plasma volume changes were calculated from haematocrit
191 and haemoglobin using standard equations (Dill & Costill, 1974).

192 Additional blood was collected into lithium heparin tubes at rest and at 60 and
193 90 min exercise, centrifuged at 5000 rpm at 4°C for 10 min, with plasma
194 extracted into duplicate tubes and frozen at -60°C until analysis. The rest and
195 90 min samples were used for analysis of noradrenaline and adrenaline
196 analysis by HPLC with electrochemical detection using the methodology
197 outlined by Goldstein *et al.* (1981) and rest, 60 and 90 min samples for plasma
198 carnitine fractions determination by radiometric methods using liquid
199 scintillation as outlined by McGarry & Foster (1985).

200 Samples for amino acid assessment (BCAA's, alanine and glutamate) were
201 prepared by mixing 80 µL plasma (from EDTA collection tube) with 20 µL
202 1.375 mM internal standard 1 (L-Methionine) and 10 µL 3.3 M perchloric
203 acid. This mix was immediately vortexed, then centrifuged at 1300 rpm for 10
204 min. The supernatant was removed for analysis against a known standard by
205 HPLC using fluorescence detection and pre-column derivitisation with 18 o-
206 phthalaldehyde (Hypersel amino acid method, ThermoHypersil-Keystone,
207 Runcorn, UK) according to the method of Heinrikson & Meredith (1984).
208 Also, duplicate 250 µL aliquots of plasma drawn from the lithium heparin tube
209 were immediately frozen at -20°C until subsequent analysis for urea nitrogen
210 and ammonia using Sigma Diagnostics kit 171-C for ammonia and 640-B for
211 urea nitrogen (Sigma Diagnostics, St Louis MO, USA).

212 Urinary carnitine excretion was determined in each treatment period by means
213 of 24 h urine collections prior to and after each exercise trial. A 5 mL sample
214 of mixed urine was collected and frozen at -60°C until analysis, and the total
215 volume of urine excreted over the 24 h period measured to the nearest mL.

216 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°C until analysis for urinary nitrogen determination via the total Kjeldahl nitrogen in water method (Tecator application sub note ASN 3503) on a Tecator Kjeltex 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).

228

229 *Statistics*

230 All data were checked for normality of distribution and homogeneity of
231 variance prior to analysis. Within group differences were assessed using
232 repeated measures analysis of variance (RMANOVA) with time and trial as
233 within subjects factors. Significant main effects were then assessed using
234 paired T-test with Bonferroni correction to determine at which time points the
235 differences lay. Changes between 0 and 2 weeks were compared between
236 groups using RM ANOVA with time as a within-subjects factor and treatment
237 group as a between-subjects factor. Differences between groups were then
238 assessed using independent-samples T-test with Bonferroni correction (SPSS
239 version 11.0.0, SPSS Inc. 2001). Significance was accepted at $p < 0.05$ or
240 Bonferroni adjusted value. All data are expressed as mean \pm SD unless
241 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·L⁻¹ for P 0wk and P 2wk, 5.3 ± 0.7 and 5.4 ± 0.6 251 mmol·L⁻¹ LC 0wk and LC 2wk, respectively) or body mass (75.0 ± 9.8 and252 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 ± 0.41 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 over259 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 the265 duration of exercise ($p < 0.01$) whilst cadence fell (~ 5 rpm).

266

267 *Haematological and Urinary Data*

268 No differences were found for pH, pCO₂, bicarbonate, glucose or FFA
 269 responses to supplementation between P and LC groups (Table 4). There was
 270 no significant change over the duration of 90 min steady state exercise for pH,
 271 pCO₂ and plasma glucose, whereas FFA and glycerol increased progressively
 272 throughout exercise in all trials ($p < 0.01$). Mean blood lactate (Table 4) was
 273 below 2 mmol.L⁻¹ in both groups at all times. There was no difference
 274 between groups for blood lactate at 0 weeks, nor between 0 and 2 weeks in LC
 275 and P groups. Blood lactate was elevated at 15 and 30 mins in the LC group at
 276 2 wk but did not quite reach statistical significance from 0wk. There was no
 277 difference for glycerol between trials in the P group, whereas in the LC group
 278 glycerol fell an average of 0.12 mmol.L⁻¹ from the 0 to 2 wk trial both at rest
 279 and during exercise ($p = 0.07$).

280 There was no difference in the exercise response of adrenaline (change over
 281 exercise, 0 wk P: 4.08 ± 3.10 , 2 wk P: 3.32 ± 2.10 , 0 wk LC: 3.21 ± 3.40 , 2 wk
 282 LC: 2.06 ± 1.62 nmol.L⁻¹) or noradrenaline between trials within either group
 283 (0 wk P: 8.76 ± 4.03 , 2 wk P: 9.41 ± 3.73 , 0 wk LC: 6.64 ± 2.37 , 2 wk LC:
 284 6.32 ± 1.66 nmol.L⁻¹).

285 There were no between trial changes for blood concentrations of urea nitrogen,
 286 total BCAA or alanine in either group (Table 3). There was also no difference
 287 in urinary nitrogen excretion either over 24 h (Table 3) or immediately before
 288 to after exercise (pre-exercise: 0.7 ± 0.5 g P 0wk, 0.9 ± 0.7 g P 2wk, 1.1 ± 1.1
 289 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
 290 2wk, 1.1 ± 0.3 g LC 0wk, 1.2 ± 0.4 g LC 2wk). There was no change over the
 291 exercise period in blood BCAA or alanine concentrations, but blood urea

nitrogen increased progressively from 15 through to 90 min of exercise ($p < 0.01$). Resting plasma glutamate was higher following 2 wk LC than 0 wk LC ($p < 0.05$), with no change between 0 and 2 wk P (Table 3). Plasma glutamate concentrations fell over the duration of exercise in all trials ($p < 0.05$). Plasma ammonia increased over the exercise duration in all trials except for 2wk LC trial. Analysis revealed that plasma ammonia concentration was suppressed towards the end of exercise at 2 wk in the LC group compared with 0wk LC but this did not quite reach statistical significance (Figure 1).

300

301 *Substrate Metabolism:*

No significant difference was found in $\dot{V} O_2$, $\dot{V} CO_2$, $\dot{V} E$ or respiratory exchange ratio (RER) during exercise between 0 and 2 wk within either P (mean RER across the exercise period of 0.80 ± 0.03 and 0.80 ± 0.04 for P 0wk and P 2wk, respectively) or LC groups (mean RER across the exercise period of 0.80 ± 0.05 and 0.81 ± 0.04 for LC 0wk and LC 2wk, respectively), and all except $\dot{V} CO_2$ changed across the exercise period, reflecting the expected cardiovascular and ventilatory drift. Due to the absence of differences in nitrogen balance or plasma amino acid concentrations during exercise as a consequence of LC supplementation, CHO and fat utilisation were estimated using the non-protein RER (Peronnet & Massicotte, 1991). There was no 0 to 2 wk trial difference in CHO oxidation between groups (Figure 2) although CHO oxidation was higher at all time points during exercise in the 2wk trial compared with 0wk trial in the LC group. There was a trend towards a between group difference in 0 to 2 wk changes in fat oxidation during the 90 min exercise ($p = 0.07$, Figure 3).

317 Total CHO oxidised (mean \pm 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 $61 \pm 42\%$ and $152 \pm 105\%$, $p < 0.01$, respectively) with
326 no change in free carnitine ($17 \pm 35\%$ 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

342 following 2 weeks of LC supplementation, although this should be confirmed
343 by an isotopic tracer study. The fact that there were no differences in $\dot{V} \text{CO}_2$,
344 pCO_2 , pH and bicarbonate concentration between trials, and that $\dot{V} \text{CO}_2$,
345 pCO_2 , and bicarbonate are stable across the exercise period supports our
346 assumption that the RER adequately reflects the respiratory quotient, and
347 relative fat:CHO oxidation during steady state exercise in the present study
348 (Peronnet & Massicotte, 1991).

349 The results of this study indicate no significant effect of LC supplementation
350 on carbohydrate use during 90 min steady state exercise, but there was a
351 tendency towards a reduction in fat oxidation. In addition, plasma glycerol
352 concentration tended to be lower, and blood lactate higher, following 2 weeks
353 LC supplementation, thereby supporting a tendency towards reduced
354 mobilisation and/or oxidation of fatty acids. This is contrary to the promoted
355 benefits of LC supplementation, but supports the trend shown by other studies
356 in our laboratory following 2 weeks LC supplementation (Abramowicz &
357 Galloway, 2005). Very few well controlled studies involving 2 weeks LC
358 supplementation have measured expired gas during exercise with which to
359 compare these results. Marconi *et al.* (1985) found no difference in RER
360 during 120 min walking at 65% $\dot{V} \text{O}_{2\text{max}}$ in competitive walkers following 4 g
361 $\text{LC} \cdot \text{d}^{-1}$, nor did Vukovich *et al.* (1994) in participants performing 60 minutes
362 of cycling exercise at 70% $\dot{V} \text{O}_{2\text{max}}$ following 6 g $\text{LC} \cdot \text{d}^{-1}$ combined with a high
363 fat preload. In contrast, RER was decreased (indicating higher fat oxidation) in
364 competitive runners (Williams, Walker, Nute *et al.*, 1987) and untrained males
365 (Wyss, Ganzit & Rienzi, 1990) after 3 weeks LC supplementation. It is
366 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 (NH₃) 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 $\text{LC}\cdot\text{d}^{-1}$, which may be related to the lower exercise intensity ($< 50\% \dot{V} \text{O}_{2\text{max}}$) 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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1 FIGURE LEGENDS

2

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

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

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

8

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

11

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

14

15

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

2

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

3

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

MACRONUTRIENT	2 DAY PRE-TRIAL		24 HR POST TRIAL	
	PLACEBO	LC	PLACEBO	LC
Energy (MJ)	13.5 \pm 1.2	13.7 \pm 1.9	14.0 \pm 1.9	13.2 \pm 2.0
CHO (g)	490 \pm 59	499 \pm 79	495 \pm 68	500 \pm 81
Protein (g)	123 \pm 11	122 \pm 14	119 \pm 14	118 \pm 19
Fat (g)	97 \pm 10	91 \pm 26	113 \pm 26	83 \pm 22

1 Table 3. Blood urea nitrogen, plasma amino acids, urinary nitrogen excretion and
 2 nitrogen balance before and after 90 min exercise.

3

Trial / Time (min)		Urea N ₂ (mg·dL ⁻¹)	Total BCAA (μmol·L ⁻¹)	Plasma alanine (μmol·L ⁻¹)	Plasma glutamate (μmol·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

4

* nitrogen balance data refers to 24 h pre and 24 h post exercise, not 0 and 90 min

5

^a greater than LC 0 wk resting value, $p < 0.05$

^b significant change from resting value, $p < 0.05$

6

- 1 Table 4: Blood pH, pCO₂ (kPa), plasma bicarbonate (mM, HCO₃), plasma FFA
 2 (mM), blood glycerol (mM), plasma glucose (mM) and blood lactate (mM) responses
 3 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)

1 Figure 1.

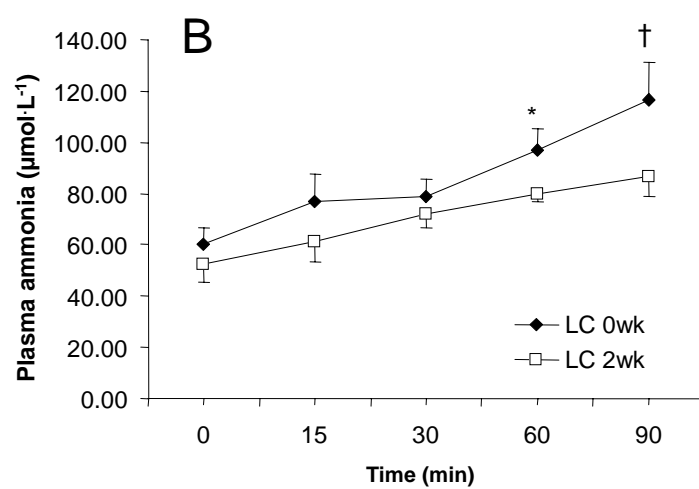
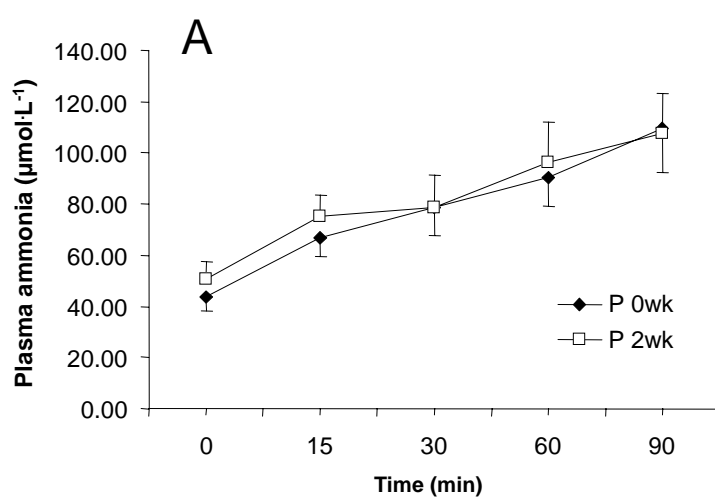
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1 Figure 2.

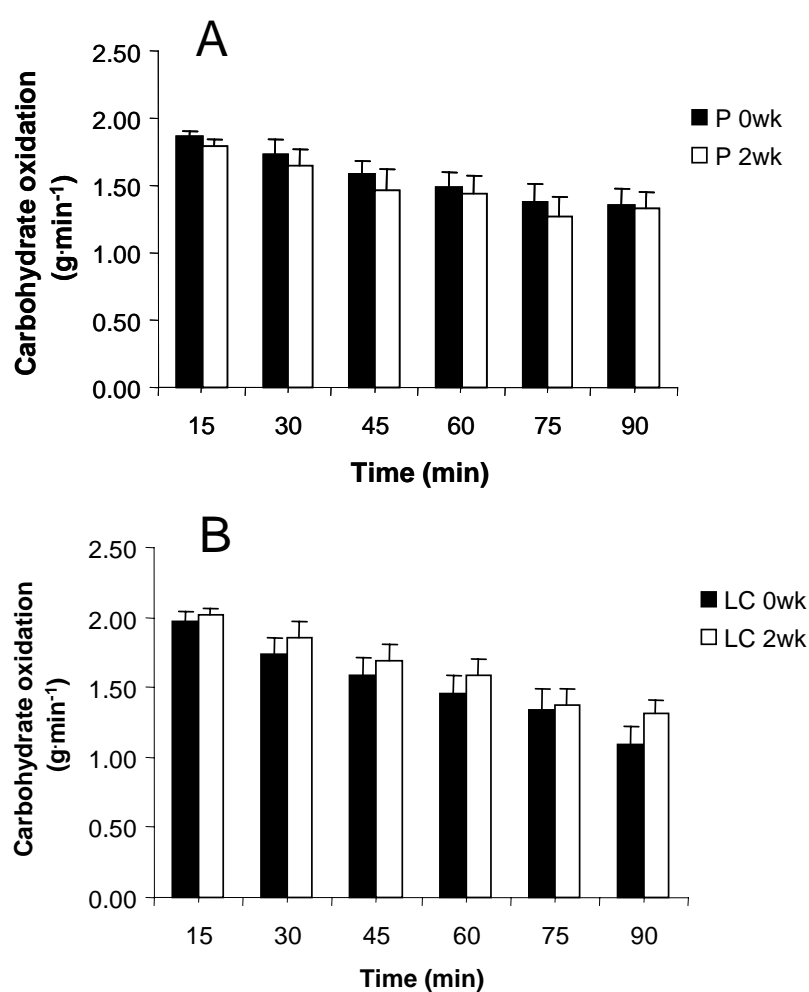
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1 Figure 3.

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