Carbohydrate ingestion augments L-carnitine retention in humans

Francis B. Stephens, Claire E. Evans, Dumitru Constantin-Teodosiu, and Paul L. Greenhaff

Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences,
Queen’s Medical Centre, University of Nottingham, Nottingham, United Kingdom

Published: 11 September 2006; accepted in final form 27 November 2006

Carbohydrate ingestion augments L-carnitine retention in humans. J Appl Physiol 102: 1065–1070, 2007. First published November 30, 2006; doi:10.1152/japplphysiol.01011.2006.—Maintaining hyperinsulinemia (~150 mU/l) during steady-state hypercarnitinemia (~550 μmol/l) increases skeletal muscle total carnitine (TC) content by ~15% within 5 h. The present study aimed to investigate whether an increase in whole body carnitine retention can be achieved through L-carnitine feeding in conjunction with a dietary-induced elevation in circulating insulin. On two randomized visits (study A), eight men ingested 3 g/day L-carnitine followed by 4 × 500-ml solutions, each containing flavored water (Con) or 94 g simple sugars (glucose syrup; CHO). In addition, 14 men ingested 3 g/day L-carnitine followed by 2 × 500 ml of either Con or CHO for 2 wk (study B). Carbohydrate ingestion in study A resulted in a fourfold greater serum insulin area under the curve when compared with Con (P < 0.001) and in a lower plasma TC concentration throughout the CHO visit (P < 0.05). Twenty-four-hour urinary TC excretion in the CHO visit was lower than in the Con visit in study A (155.0 ± 10.7 vs. 212.1 ± 17.2 mg; P < 0.05). In study B, daily urinary TC excretion increased after 3 days (65.9 ± 18.0 to 281.0 ± 35.0 mg; P < 0.001) and remained elevated throughout the Con trial. During the CHO trial, daily urinary TC excretion increased from a similar basal value of 53.8 ± 9.2 to 166.8 ± 17.3 mg after 3 days (P < 0.01), which was less than during the Con trial (P < 0.01), and it remained lower over the course of the study (P < 0.001). The difference in plasma TC concentration in study A and 24-h urinary TC excretion in both studies suggests that insulin augmented the retention of carnitine in the CHO trials.

NONVEGETARIAN HUMANS OBTAIN ~1 mg/kg of carnitine (L-3-hydroxy-4-N,N,N-trimethylaminobutyric acid) per day from dietary sources such as meat and dairy products and up to 0.4 mg·kg⁻¹·day⁻¹ by endogenous synthesis from trimethyllysine (26), to maintain a total body content of ~300 mg/kg (5, 24). More than 95% of the body’s total carnitine store exists within skeletal muscle tissue as either free or acyl carnitine (5, 24) where, as a substrate for the carnitine acyltransferase enzymes, it plays essential roles in the translocation of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation (11, 12) and as a buffer of excess acetyl group production during intense exercise (1, 8, 9, 17). Previous research has demonstrated that a 65% decrease in skeletal muscle free carnitine content during intense exercise at a workload >70% maximal oxygen uptake (VO₂max) was paralleled by a 30% decrease in the rate of fat oxidation (33). It was, therefore, hypothesized that at higher exercise workloads free carnitine availability might be rate limiting to fat oxidation in skeletal muscle.

To fully elucidate whether free carnitine availability is indeed rate limiting to fat oxidation in healthy humans (i.e., not carnitine deficient) during exercise, the ability to increase skeletal muscle carnitine content before exercise is essential. However, following ~20 yr of research on this topic (for review see Ref. 6), it remains doubtful whether oral or intravenous L-carnitine administration per se can measurably increase skeletal muscle carnitine content and, as a result, modify energy metabolism in healthy human subjects. For example, 2–12 wk of daily L-carnitine feeding (2–4 g/day) had no effect on skeletal muscle carnitine content or indexes of muscle energy metabolism (2, 34, 35) or indeed on net uptake of carnitine across the leg (30). Furthermore, intravenous infusion of L-carnitine in healthy human volunteers did not increase skeletal muscle carnitine content in the fasted state (4, 30) or change substrate oxidation during exercise (4, 21). It is known that carnitine is transported into skeletal muscle via a saturable, Na⁺-dependent, high-affinity, active transport process (23) against a large concentration gradient (>100-fold; Refs. 30, 35). The protein responsible for this process is the novel organic cation transporter 2 (OCTN2), with an in vitro Km for carnitine of 4.3 μmol/l (32). It is clear from these transport characteristics that it is unlikely that plasma carnitine availability per se, even in the nonsupplemented state, will be rate limiting to muscle carnitine transport and storage (fasted plasma carnitine concentration in nonvegetarians is ~50 μmol/l; Ref. 30), perhaps explaining why the aforementioned studies failed to increase skeletal muscle carnitine content. Based on this evidence, alternative strategies to stimulate skeletal muscle carnitine accumulation in humans should be investigated.

Recent research has demonstrated that maintaining hyperinsulinemia (~150 mU/l) in the presence of a supraphysiological plasma carnitine concentration of ~550 μmol/l for 5 h increased skeletal muscle total carnitine content by ~15% in healthy human volunteers (30, 31). This finding was in concordance with the hypothesis that insulin would augment Na⁺-dependent skeletal muscle carnitine transport via OCTN2, secondary to its action of increasing sarcolemmal Na⁺-K⁺-ATPase pump activity and, therefore, intracellular Na⁺ flux. Furthermore, the increase in skeletal muscle carnitine content was associated with a large reduction in glycolytic flux and carbohydrate oxidation at rest [decreased muscle pyruvate dehydrogenase complex (PDC) activity and lactate content, and increased muscle glycogen accumulation], despite conditions of identical carbohydrate administration, suggesting a
carnitine-mediated increase in fat oxidation (31). However, any strategy that involves intravenous infusion of L-carnitine and prolonged periods of elevated circulating insulin concentration is clearly not practical in the everyday setting. It would, therefore, be pertinent to investigate whether physiologically significant increases in skeletal muscle carnitine content can be achieved through the use of L-carnitine feeding in conjunction with a dietary-induced elevation in circulating insulin.

In this respect, the aim of the present study was to determine whether insulin, released as a consequence of carbohydrate ingestion, could increase the retention of carnitine in humans over the course of 1 day or 2 wk of L-carnitine feeding. Given that the increase in muscle carnitine content following a single dose, or 2 wk, of L-carnitine feeding in the presence of elevated circulating insulin is likely to be small (18) due to the poor bioavailability of orally administered L-carnitine (16), muscle carnitine accumulation was estimated indirectly from measurements of plasma and urinary carnitine concentration.

MATERIALS AND METHODS

Subjects

Twenty-two healthy, untrained, nonvegetarian men participated in the present studies (study A and study B), which were approved by the University of Nottingham Medical School Ethics Committee in accordance with the Declaration of Helsinki. Before taking part in the studies, all subjects underwent routine medical screening and completed a general health questionnaire. All gave their written consent to take part in the studies and were aware that they were free to withdraw from the experiment at any point.

Study Protocol

**Study A.** Eight subjects (age 21.9 ± 0.6 yr, body mass 76.8 ± 1.4 kg, and body mass index 23.3 ± 0.6 kg/m²) reported to the laboratory following an overnight fast on two occasions (control and experimental), separated by a 2 wk “wash-out” period. On arrival, subjects voided their bladder and were asked to rest in a semisupine position on a bed, where they remained for the duration of the visit, while a cannula was inserted retrogradely into a superficial vein on the dorsal surface of their nondominant hand for subsequent blood collection. The cannula was kept patent by means of a saline drip, and the hand was then placed in a hand-warming unit (air temperature 55°C) for the duration of the visit to arterialize the venous drainage of the hand (13). Subjects then ingested 4.5 g L-carnitine L-tartrate (3 g L-carnitine; Lonza Group, Basel, Switzerland) dissolved in 200 ml of water (time 0), which also signified the start of a 24-h period of urine collection. Thereafter, subjects ingested a 500-ml solution containing either 4.5 g L-carnitine L-tartrate (3 g L-carnitine; Con) or 94 g of simple sugars (CHO; GlaxoSmithKline, Brentford, UK) on four occasions (total carbohydrate 376 g) throughout the visit (time 0, 1, 4, 7, 10, and 14 h after L-carnitine ingestion). Subjects consumed either flavored sugar-free water (Con; n = 7; age 20.4 ± 0.2 yr, body mass 73.5 ± 3.5 kg, and body mass index 22.0 ± 0.6 kg/m²) or 94 g carbohydrate (CHO; n = 7; age 20.9 ± 0.3 yr, body mass 71.9 ± 2.7 kg, and body mass index 22.3 ± 0.7 kg/m²) of the same composition used in study A (total carbohydrate 188 g). In addition to baseline (day 0), 24-h urine collections were obtained on days 3, 7, 10, and 14.

During the 14-day trial, subjects were allowed to eat and drink a habitual diet at liberty, but they abstained from alcoholic beverages. The subjects were given training in keeping a food intake record and provided with an instruction booklet with an example of a 1-day food intake record. Subjects were asked to provide a food intake record for days 0, 3, 7, 10, and 14 of the study. Food diaries of the subjects’ habitual diet were analyzed by using Microdiet software (version 1.2, Downlee Systems Limited, Chapel-en-le-Frith, UK).

**Sample Collection and Analysis**

In study A, 5 ml of arterialized venous blood was obtained every 20 min for 7 h. Two milliliters of this blood were collected into lithium-heparin containers, and, after centrifugation, the plasma was removed and immediately frozen in liquid nitrogen. These samples were then stored at −80°C and analyzed at a later date for total carnitine concentration using a radioenzymatic assay described by Cederblad et al. (7). The remaining blood was allowed to clot, and after centrifugation the serum was stored frozen at −80°C. Insulin concentration was measured in these samples at a later date using a radioimmunoassay kit (Coat-a-Count Insulin, Euro-DPC, Caernarvon, UK). In both studies, the volume of each 24-h urine collection was recorded, and a 5-ml aliquot was removed and stored at −80°C to be analyzed at a later date for total carnitine (TC) concentration using the same radioenzymatic method as for plasma (7).

**Statistical Analysis**

A two-way repeated-measures ANOVA (time and treatment effects; GraphPad Prism version 4, GraphPad Software, San Diego, CA) was performed to detect differences in plasma carnitine and serum insulin concentration in study A, and daily urinary carnitine excretion in study B. When a significant main effect was detected, data were further analyzed with Student’s t-tests using the Bonferroni correction to locate differences. Student’s t-tests were also used to analyze all other data. The total areas under curve (AUC) for serum insulin concentration × time and urinary carnitine excretion × time were calculated using the least squares method (KaleidaGraph version 3.51, Synergy Software, Reading, PA). The association between plasma carnitine and serum insulin was investigated using Pearson correlation analysis. Statistical significance was declared at P < 0.05, and all values in text and Figs. 1, 2, 4, and 5 represent means ± SE.

**RESULTS**

**Subjects**

The age, mass, and body mass index of the subjects in study B were similar between the Con and CHO groups (20.4 ± 0.2 vs. 20.9 ± 0.3 yr, 73.5 ± 3.5 vs. 71.9 ± 2.7 kg, and 22.0 ± 0.6 vs. 22.3 ± 0.7 kg/m², respectively).

**Serum Insulin**

Basal serum insulin concentration during the Con and CHO visit in study A was similar (12.4 ± 1.5 and 11.4 ± 0.9 mU/L, respectively; Fig. 1). There was no change in serum insulin concentration from basal, which remained at ∼10 mU/L throughout Con. However, the consumption of each carbohydrate solution during CHO (indicated by the arrows in Fig. 1) was accompanied soon after by an increase in serum insulin...
concentration (peak serum insulin concentration was 68.2 ± 12.0, 73.6 ± 22.3, 68.1 ± 29.0, and 46.4 ± 10.2 mU/l at time = 1.7, 3.3, 5, and 6.3 h, respectively). The AUC (serum insulin concentration × time) was fourfold greater on the CHO visit compared with Con (4,387 ± 390 and 17,960 ± 2,898 mU·l⁻¹·min⁻¹ for Con and CHO, respectively; P < 0.001).

Plasma Carnitine

Basal plasma TC concentration was similar between Con and CHO visits in study A (44.5 ± 5.5 and 44.9 ± 4.2 μmol/l, respectively; Fig. 2). Following the ingestion of the L-carnitine, there was a steady increase in plasma TC concentration in Con, reaching a peak of 69.9 ± 6.6 μmol/l after 3 h, which was significantly greater than basal (P < 0.001; Fig 2). Plasma TC then gradually declined toward basal over the remainder of the visit. During the CHO visit, peak plasma TC concentration (63.9 ± 9.7 μmol/l), which was also significantly greater than basal (P < 0.001; Fig 2), occurred slightly earlier than 3 h (160 min). Furthermore, after the first carbohydrate bolus delivery (indicated by the arrows in Fig. 2), plasma TC concentration was significantly lower throughout the CHO visit compared with Con (treatment effect, P < 0.05). Plasma TC concentration during CHO did not deviate from Con for the initial 60 min. The AUC for plasma TC concentration × curve was negatively correlated with the AUC for serum insulin concentration × time during the CHO visit (r = 0.80, P < 0.05; Fig. 3).

Urinary Carnitine

Following L-carnitine ingestion in study A, the mean 24-h urinary TC excretion [concentration (mg/l) × urinary volume (l)] was 212.1 ± 17.2 and 155.0 ± 10.7 for Con and CHO, respectively (Fig. 4). The urinary excretion of TC in the CHO then gradually declined toward basal over the remainder of the visit.
AUGMENTATION OF L-CARNITINE RETENTION

Fig. 5. Daily urinary TC excretion throughout 14 days of L-carnitine feeding (3 g/day) in combination with 2 x 500 ml/day solutions of flavored water (Con) or 94 g of simple sugars (CHO). Values are means ± SE for 7 subjects. **P < 0.01, significant decrease in urinary TC excretion compared with Con.

visit was significantly lower than that of Con visit (P < 0.05).

Daily urinary TC excretion during study B is illustrated in Fig. 5. Daily urinary TC excretion increased from a basal value of 65.9 ± 18.0 to 281.0 ± 35.0 mg after 3 days (P < 0.001) and remained close to this value on days 7, 10, and 14. During the CHO trial, daily urinary TC excretion increased from a similar basal value of 53.8 ± 9.2 to 166.8 ± 17.3 mg after 3 days (P < 0.01), which was significantly less than during the Con trial (P < 0.01). Furthermore, there was a significant treatment effect between the Con and CHO trials over the course of the study (P < 0.001), which is represented by a lower AUC for urinary TC excretion × time in the CHO trial (3.525 ± 178 vs. 2.626 ± 281 mg/day for Con and CHO, respectively; P < 0.05).

Energy Intake

The food diaries in study B did not show any difference in the average daily energy intake between the Con and CHO trials (2,219.7 ± 321.1 vs. 2,406.7 ± 326.9 kcal/day). However, as might be expected, macronutrient composition analysis of the food diaries demonstrated that the contribution from carbohydrate to daily energy intake was greater in the CHO group compared with Con (59.7 ± 8.1 vs. 48.0 ± 7.1%; P < 0.01), whereas the contribution from protein and fat was reduced [10.9 ± 1.5 vs. 15.0 ± 2.3% (P < 0.01) and 29.3 ± 4.0 vs. 37.1 ± 5.7% (P < 0.05), respectively].

DISCUSSION

The principal finding from the present studies was that plasma TC concentration and urinary TC excretion were significantly reduced when L-carnitine ingestion was accompanied by carbohydrate feeding. Furthermore, the reduction in plasma TC concentration from Con after 60 min on the CHO trial in study A corresponded with the time of the first bolus of CHO ingestion. This leads us to conclude that, collectively, our observations demonstrate that carbohydrate feeding augments whole body carnitine retention in humans.

Following the ingestion of L-carnitine in the Con visit in study A, there was an increase in plasma TC concentration to a peak of 69.9 ± 6.6 μmol/l after 3 h (Fig. 2), which is in agreement with previous studies (16, 27). Plasma TC then declined over the remaining 4 h toward its preingestion concentration. However, when L-carnitine feeding was accompanied by CHO ingestion, peak plasma TC concentration occurred earlier than 3 h and plasma TC concentration was lower than the corresponding Con trial over the final 6 h of the experimental visit (i.e., after the first CHO drink). Importantly, the 24-h urinary TC excretion was ~30% less after the CHO visit compared with the Con visit (Fig. 4). We suggest, therefore, that the lowering of plasma TC concentration occurring immediately following CHO ingestion, and the lower urinary TC excretion during the CHO visit, collectively indicate that an increase in whole body carnitine retention occurred when L-carnitine feeding was accompanied by CHO ingestion. Given that skeletal muscle is the major site of carnitine storage within the body, and that maintaining hypercarnitinemia for 5 h in the presence of hyperinsulinemia increases skeletal muscle TC accumulation (30, 31), it is not unreasonable to suggest that this greater retention occurred mainly in this tissue. These findings are in concordance with our hypothesis that insulin, released as a consequence of carbohydrate ingestion, can augment Na⁺-dependent skeletal muscle carnitine uptake by increasing Na⁺-K⁺-ATPase pump activity in humans (30, 31).

Indeed, the AUC for plasma TC concentration × time was negatively correlated with the AUC for serum insulin concentration × curve during the CHO visit (Fig. 3).

It could be suggested that the present observations would also have occurred had carnitine gut absorption been negatively influenced by carbohydrate ingestion, resulting in less carnitine appearing in the circulation and urine. However, the peak in plasma TC concentration was slightly earlier during the CHO visit compared with the Con visit (Fig. 3.2), an observation that is not consistent with impaired or slower intestinal absorption (25). Indeed, the Kₚ of intestinal carnitine transport is between 300 and 550 μmol/l (14, 15, 27). Following the 3-g dose of L-carnitine in this study, jejunal carnitine concentration is likely to increase to ~19,000 μmol/l (19), suggesting that intestinal carnitine absorption would have been saturated and maximal during both visits. In addition, it is likely that carnitine absorption in the small intestine occurs via the carnitine transporter OCTN2 (10), and there are no apparent reports to date indicating that carbohydrate inhibits carnitine absorption or the transporter protein. Importantly, the fact that plasma TC concentration was negatively correlated with serum insulin concentration in study A would suggest that insulin concentration per se, rather than carbohydrate ingestion, was responsible for the effect on plasma TC concentration.

A study by Harper et al. (16) demonstrated 24-h urinary TC excretions of ~160 mg (8% of the total bolus) and 240 mg (4%) following the ingestion of 2 and 6 g of L-carnitine, respectively. This observation is in good agreement with the 24-h urinary TC excretion of study A (7%; Fig. 4). A urinary excretion of 7% would imply whole body carnitine retention of 93% or 2.8 g. However, pharmacokinetic analysis demonstrates that L-carnitine has a poor bioavailability and that <20% of a given dose (2–6 g) is absorbed from the gut into the circulation (16, 25–27), suggesting that, at most, 560 mg of carnitine were retained on the Con visit of study A. Skeletal muscle comprises 40% of total body mass, corresponding to an average of 30.7 kg for the subjects in this study, and the
concentration of carnitine in skeletal muscle is 700 mg/kg wet weight (9, 30). Therefore, if all of the absorbed carnitine were to be transported into skeletal muscle, it would increase skeletal muscle carnitine concentration by 3%. Following the CHO visit of study A, 24-h urinary TC excretion corresponded to 5% of the total dose and was $\sim 60$ mg (0.8 mg/kg) less than that in the Con visit, which equates to $>50\%$ of normal daily carnitine intake (26). Assuming equal amounts of carnitine were absorbed across the gut during both visits of study A and that all absorbed carnitine was either taken up into skeletal muscle tissue or excreted in the urine, it can be calculated that L-carnitine feeding in conjunction with CHO ingestion would have increased skeletal muscle TC concentration by a further 0.1% (i.e., 60 mg) compared with L-carnitine ingestion alone.

In very good agreement with this observation, urinary TC excretion was on average 70 mg/day lower in the CHO group over the 14 days of study B (Fig. 5). Consequently, if maintaining a daily L-carnitine feeding regime with CHO has an additive effect on muscle carnitine content, L-carnitine feeding for 100 days could increase muscle carnitine content by an additional 10%, which we believe could have a significant metabolic impact in contracting skeletal muscle. Indeed, previous research by our laboratory demonstrated that muscle total carnitine content was not reduced 24 h after a 15% increase (31), suggesting that a daily increase in muscle carnitine content can be maintained.

Furthermore, release of carnitine from skeletal muscle is a slow process (22), and skeletal muscle carnitine turnover time (190 h) is much greater than in other tissues (24). Taken together with the maintained effect on whole body TC retention observed in study B, these findings would suggest that daily L-carnitine and carbohydrate administration could well have an additive effect on skeletal muscle TC accumulation. Importantly, if L-carnitine supplementation is to be used as a tool to modify skeletal muscle energy metabolism, the findings in study B also suggest that, at most, only two 500-mL CHO drinks ($2 \times 94$ g CHO) are required to achieve the effect on L-carnitine retention. To provide solid evidence that measurable increases in muscle carnitine content will indeed be achieved when the L-carnitine supplementation procedure is prolonged (e.g., 100 days), a daily L-carnitine and CHO feeding study involving muscle biopsies is required.

One of the metabolic roles of carnitine in skeletal muscle is to regulate the ratio of mitochondrial acetyl-CoA to CoASH by buffering excess acetyl-CoA production from pyruvate (1, 8, 9, 17). However, this acetylation of the free carnitine pool could result in its depletion, and previous research has demonstrated that the decrease in the rate of long-chain fatty acid oxidation, observed at an exercise intensity above 70% V̇O₂ max using a [U-13C]palmitate tracer, was paralleled by a 43% decrease in skeletal muscle carnitine content (3). Given that carnitine is essential for the translocation of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation (11, 12), this observation would suggest that, during high-intensity exercise, free carnitine availability might be rate limiting to fat oxidation in skeletal muscle. Indeed, it was hypothesized that muscle free carnitine availability becomes limiting to carnitine palmitoyltransferase I at a concentration of $\sim 6$ mmol/kg dry muscle or $\sim 1.8$ mM intracellular water (30, 33). Thus, assuming the average 70 mg/day retention in the present studies resided within skeletal muscle and that daily L-carnitine/carbohydrate feeding for 100 days would have an additive effect, then muscle carnitine content would increase by $\sim 2$ mmol/kg dry muscle ($\sim 0.6$ mM intracellular water), which could alleviate the decline in fat oxidation rates routinely observed at exercise intensities above 70% V̇O₂ max, which could be of major relevance to exercise performance due to the sparing of muscle glycogen. In line with this theory, increasing skeletal muscle carnitine availability has been reported to delay fatigue development by 25% in rat soleus muscle strips in vitro (3). In addition, our laboratory’s most recent research (31) has demonstrated that a 15% increase in skeletal muscle carnitine content, achieved during hyperinsulinemia, resulted in a 30% decrease in muscle PDC activity and 40% decrease in muscle lactate content compared with control (euglycemic hyperinsulinemia). Furthermore, following an overnight fast, muscle glycogen and long-chain acyl-CoA content was 30 and 40% greater than control, respectively, despite carbohydrate administration over the previous 24 h being exactly the same.

In conclusion, this is the first study to demonstrate that the whole body retention of orally supplemented L-carnitine can be augmented if accompanied by carbohydrate ingestion. Furthermore, this retention is likely to reside in skeletal muscle, because insulin is known to stimulate muscle total carnitine accumulation. These findings could have a significant effect on the integration of fat and carbohydrate oxidation in contracting skeletal muscle.

ACKNOWLEDGMENTS

We thank Michelle Hazell for technical assistance during the study.

GRANTS

This work was supported by Lonza Ltd. (Basel, Switzerland).

REFERENCES


