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, NG7 2UH, United Kingdom.

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**Address for correspondence:**
Francis B. Stephens
E Floor, School of Biomedical Sciences,
University of Nottingham Medical School,
Queen’s Medical Centre,
Nottingham, NG7 2UH, UK

**E-mail:** francis.stephens@nottingham.ac.uk

**Telephone:** +44 (0)115 8230157

**Fax:** +44 (0)115 9709259
ABSTRACT

Maintaining hyperinsulinaemia (~150mU·L⁻¹) during steady-state hypercarnitinaemia (~550µmol·L⁻¹) increases skeletal muscle total carnitine (TC) content by ~15% within 5h. 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), 8 men ingested 3g·d⁻¹ L-carnitine followed by 4 x 500mL solutions, each containing flavored water (CON) or 94g simple sugars (Glucose syrup; CHO). In addition, 14 men ingested 3g·d⁻¹ L-carnitine followed by 2 x 500mL of either CON or CHO for 2 weeks (Study B). Carbohydrate ingestion in Study A resulted in a 4-fold greater serum insulin AUC when compared with CON (P<0.001), and a lower plasma TC concentration throughout the CHO visit (P<0.05). Twenty-four h urinary TC excretion in the CHO visit was lower than the CON visit in Study A (155.0 ± 10.7 vs. 212.1 ± 17.2mg, respectively; P<0.05). In Study B, daily urinary TC excretion increased after 3 d (65.9 ± 18.0 to 281.0 ± 35.0mg; 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 ± 17.3mg after 3d (P<0.01), which was less than during the CON trial (P<0.01) and remained lower over the course of the study (P<0.001). The difference in plasma TC concentration in Study A and 24h urinary TC excretion in both studies suggests insulin augmented the retention of carnitine in the CHO trials.

Keywords: Novel organic cation transporter 2; Insulin; Skeletal muscle
INTRODUCTION

Non-vegetarian humans obtain approximately 1 mg·kg\(^{-1}\) 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\(^{-1}·d^{-1}\) by endogenous synthesis from trimethyllysine (26), in order to maintain a total body content of around 300 mg·kg\(^{-1}\) (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 greater than 70% maximal oxygen uptake (\(V_O_2_{max}\)), 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 prior to exercise is essential. However, following more than 20 years of research on this topic (for review see 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 to 12 weeks of daily L-carnitine feeding (2-4 g·d\(^{-1}\)) had no effect on skeletal muscle carnitine content or indices of muscle
energy metabolism (2, 34, 35), or indeed 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; 30, 35). The protein responsible for this process is the novel organic cation transporter 2 (OCTN2), with an in vitro $K_m$ for carnitine of 4.3 $\mu$mol·L$^{-1}$ (32). It is clear from these transport characteristics that it is unlikely that plasma carnitine availability per se, even in the non-supplemented state, will be rate limiting to muscle carnitine transport and storage (fasted plasma carnitine concentration in non-vegetarians is approximately 50 $\mu$mol·L$^{-1}$; 30), perhaps explaining why the afore mentioned 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$^{-1}$) in the presence of a supraphysiological plasma carnitine concentration of around 550 $\mu$mol·L$^{-1}$ for 5 h, increased skeletal muscle total carnitine content by approximately 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 by 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 every day 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 weeks of L-carnitine feeding. Given the increase in muscle carnitine content following a single dose, or 2 weeks, 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, non-vegetarian men participated in the present studies (A and 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·m⁻²) reported to the laboratory following an overnight fast on two occasions (control and experimental), separated by a 2 week “wash out” period. On arrival, subjects voided their bladder and were asked to rest in a semi-supine position on a bed, where they remained for the duration of the visit, while a cannula was inserted retrogradely in to a superficial vein on the dorsal surface of their non-dominant 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 (t = 0), which also signified the start of a 24 h period of urine collection. Thereafter, subjects ingested a 500 mL solution
containing either flavored sugar free water (CON) or 94 g of simple sugars (CHO; Original Lucozade, GlaxoSmithKline, Brentford, UK) on four occasions (total carbohydrate 376 g) throughout the visit (t = 1, 2.5, 4, and 5.5 h). Arterialized-venous blood samples (5 mL) were collected at time 0 and at 20 min intervals thereafter for 7 h. Subjects were then allowed to leave the laboratory. Urine was collected in 5 L bottles, containing 5 mL of 10% thymol / isopropanol preservative (21), and returned to the laboratory the following morning, 24 h after the ingestion of the L-carnitine. For the 24 h prior to their visit and during the urine collection period, subjects were allowed to eat and drink at liberty but abstained from carnitine containing foods and alcoholic beverages.

Study B

In Study B, following a baseline 24 h urine collection, 14 subjects were randomized into 2 groups (control and experimental). Each subject ingested 4.5 g L-carnitine L-tartrate (containing 3 g L-carnitine) dissolved in 200 mL of water every morning for 14 d. In addition, on two occasions each day (1 and 4 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·m⁻²) 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·m⁻²) 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 day 3, 7, 10, and 14.

During the 14 d trial, subjects were allowed to eat and drink a habitual diet at liberty, but abstain 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, UK).

Sample collection and analysis

In study A, 5 mL of arterialized venous blood was obtained every 20 min for 7 h. Two mL of this blood was 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, Wales). 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 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 Inc., USA) 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. Students’s t tests were also used to analyse all other data. The total area
under serum insulin concentration x time and urinary carnitine excretion x time curves were calculated using the least squares method (KaleidaGraph version 3.51, Synergy Software, USA). 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 Figures represent mean ± standard error of the mean (SEM).
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·m⁻², 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⁻¹, respectively; Figure 1). There was no change in serum insulin concentration from basal, which remained at ~10 mU·L⁻¹ 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⁻¹ at t = 1.7, 3.3, 5, and 6.3 h, respectively). The area under the curve (serum insulin concentration x time) was four-fold greater on the CHO visit compared with CON (4387 ± 390 and 17960 ± 2898 mU·L⁻¹·min⁻¹ for CON and CHO, respectively; P < 0.001).

Plasma Carnitine
Basal plasma total carnitine (TC) concentration was similar between CON and CHO visits in Study A (44.5 ± 5.5 and 44.9 ± 4.2 μmol·L⁻¹, respectively; Figure 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⁻¹ after 3 h, which was significantly greater than basal (P < 0.001; Fig 2.). Plasma TC then gradually declined
towards basal over the remainder of the visit. During the CHO visit, peak plasma TC concentration (63.9 ± 9.7 µmol·L⁻¹), 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 when compared to CON (treatment effect P < 0.05). Plasma TC concentration during CHO did not deviate from CON for the initial 60 min. The area under the plasma TC concentration x time curve was negatively correlated with the area under the serum insulin concentration x time curve during the CHO visit (r = 0.80, P < 0.05; Figure 3).

**Urinary carnitine**

Following L-carnitine ingestion in Study A, the mean 24 h urinary TC excretion (concentration (mg·L⁻¹) x urinary volume (L)) was 212.1 ± 17.2 and 155.0 ± 10.7 for CON and CHO, respectively (Figure 4). The urinary excretion of TC in the CHO visit was significantly lower than that of CON visit (P < 0.05).

Daily urinary TC excretion during Study B is illustrated in Figure 5. Daily urinary TC excretion increased from a basal value of 65.9 ± 18.0 to 281.0 ± 35.0 mg after 3 d (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 d (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 area under the urinary TC excretion x time curve in the CHO trial (3525 ± 178 vs. 2626 ± 281 mg·d 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 (2219.7 ± 321.1 vs. 2406.7 ± 326.9 kcal·d⁻¹, respectively). 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 to CON (59.7 ± 8.1 vs. 48.0 ± 7.1 %, respectively; $P < 0.01$), 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⁻¹ after 3 h (Fig. 2), which is in agreement with previous studies (16, 27). Plasma TC then declined over the remaining 4 h towards its pre-ingestion 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 approximately 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 hyperinsulinaemia 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 area under the plasma TC concentration x time curve was negatively correlated with the area under the serum insulin concentration x time curve during the CARN 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 to the CON visit (Fig. 3.2), an observation which is not consistent with impaired or slower intestinal absorption (25). Indeed, the \(K_m\) of intestinal carnitine transport is between 300 and 550 \(\mu\)mol\cdotL\(^{-1}\) (14, 15, 27). Following the 3 g dose of L-carnitine in this study, jejunal carnitine concentration is likely to increase to approximately 19,000 \(\mu\)mol\cdotL\(^{-1}\) (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 approximately 160 mg (8% of the total bolus) and 240 mg (4%) following the ingestion of 2 g 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 less than 20% of a given dose (2-6 g) is absorbed from the gut into the circulation (16, 25, 26, 27) suggesting that, at most, 560 mg of carnitine was 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⁻¹ 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 approximately 60 mg (0.8 mg·kg⁻¹) less than the CON visit, which equates to greater than 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·d⁻¹ 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 ourselves 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 x 94g CHO) are required to achieve the effect on L-carnitine retention. In order 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 mitochondrial acetyl-CoA/CoASH ratio 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% \( VO_{2\text{max}} \) using a [U-\(^{13}\text{C}\)]palmitate tracer, was paralleled by a 43% decrease in skeletal muscle free carnitine content (33). Given that carnitine is essential for the translocation of long chain fatty acids into the mitochondrial matrix for subsequent \( \beta-\)}
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 1 (CPT1) at a concentration of about 6 mmol·(kg dm)⁻¹ or approximately 1.8 mM intracellular water (30, 33). Thus, assuming the average 70 mg·d⁻¹ 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 around 2 mmol·(kg dm)⁻¹ (~0.6 mM intracellular water), which could alleviate the decline in fat oxidation rates routinely observed at exercise intensities above 70% $V_{O_2,max}$, 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 most recent research (31) has demonstrated that a 15% increase in skeletal muscle carnitine content, achieved during hyperinsulinaemia, resulted in a 30% decrease in muscle PDC activity and 40% decrease in muscle lactate content compared to control (euglycaemic hyperinsulinaemia). 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, as 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.
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REFERENCES


FIGURES AND LEGENDS

Figure 1.
Serum insulin concentration during Study A after the ingestion of 3 g of L-carnitine in 200 ml of water followed by 500 ml solutions of flavored water (CON; open circles) or 94 g of simple sugars (CHO; closed circles) at t = 60, 150, 240, and 330 (indicated by the arrows). The area under the serum insulin-time curve during the CHO visit was significantly higher ($P < 0.001$) than during the CON visit. Values are means ± SEM (n = 8).

Figure 2.
Plasma TC concentration during Study A after the ingestion of 3 g of L-carnitine in 200 ml of water followed by 500 ml solutions of flavored water (CON; open circles) or 94 g of simple sugars (CHO; closed circles) at t = 60, 150, 240, and 330. No significant differences ($P > 0.05$) were found in plasma TC concentration between the CON and CHO visits. ††† $P < 0.001$, CON and CHO significantly higher than basal, respectively. Values are means ± SEM (n = 8).

Figure 3.
Relationship between serum insulin concentration and plasma carnitine concentration during the CHO visit in study A. * $P < 0.05$, significant negative correlation. Values represent individual subjects (n = 8).
Figure 4.

Twenty-four h urinary TC excretion during Study A after the ingestion of 3 g of L-carnitine in 200 ml of water followed by 4 x 500 ml solutions of flavored water (CON) or 94 g of simple sugars (CHO). * P < 0.05, significant decrease in urinary TC excretion. Values are means ± SEM (n = 8).

Figure 5.

Daily urinary TC excretion throughout 14 d of L-carnitine feeding (3 g·d⁻¹) in combination with 2 x 500 ml·d⁻¹ solutions of flavored water (CON; open circles) or 94 g of simple sugars (CHO; closed circles). ** P < 0.01, significant decrease in urinary TC excretion compared to CON. Values are means ± SEM (n = 7). †† † P < 0.001, †† P < 0.01, significantly greater than basal during CON and CARN, respectively.
$r^2 = 0.6495, \ P < 0.05$

Plasma total carnitine AUC (mmolL$^{-1}$min$^{-1}$)

Serum insulin AUC (U·L$^{-1}$·min$^{-1}$)