Protein ingestion acutely inhibits insulin-stimulated muscle carnitine uptake in healthy young men

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Abbreviations: AUC, area under the curve; ATP, adenosine triphosphate; CHO, carbohydrate; CHO-PRO, carbohydrate-whey protein; CON, control; CPT1, carnitine palmitoyltransferase 1; FC, free carnitine; LCMSMS, liquid chromatography tandem mass-spectrometry; MPE, molar percent excess; NCB, net carnitine balance; OCTN2, novel

organic cation transporter 2; PDC, pyruvate dehydrogenase complex; R_a, rate of appearance; R_d, rate of disappearance; SPE, solid phase extraction; TC, total carnitine; TTR, tracer-to-tracee ratio.

Abstract

Background: Increasing skeletal muscle carnitine content represents an appealing intervention in conditions of perturbed lipid metabolism such as obesity and type 2 diabetes, but requires chronic L-carnitine feeding on a daily basis in a high-carbohydrate beverage.

Objective: We investigated whether whey protein ingestion could reduce the carbohydrate load required to stimulate insulin-mediated muscle carnitine accretion.

Design: Seven healthy males (24 ± 5 years, 23 ± 3 $kg \cdot m^{-2}$) ingested 80g carbohydrate (CHO), 40g carbohydrate + 40g protein (CHO-PRO), or flavoured water (CON) beverages 60 min following ingestion of 4.5g L-carnitine tartrate (3g L-carnitine; 0.1% $^2H_3$-L-carnitine). Serum insulin concentration, net forearm carnitine balance (NCB; calculated from arterialised-venous and venous plasma carnitine concentrations and brachial artery plasma flow) and carnitine disappearance and appearance rates (R_d and R_a), were determined at baseline and 20 min intervals for 180 min.

Results: Serum insulin and plasma flow area under curve (AUC) were similarly elevated above CON (0.04 ± 0.1 and -0.5 ± 0.2) by CHO (4.5 ± 0.8; $P<0.01$ and 0.5 ± 0.6; $P<0.05$) and CHO-PRO (3.8 ± 0.6 $U \cdot L^{-1} \cdot min$; $P<0.01$ and 0.4 ± 0.6 $L \cdot min$; $P=0.05$). Plasma carnitine AUC was greater following CHO-PRO (3.5 ± 0.5) than CON (2.1 ± 0.2; $P<0.05$) and CHO (1.9 ± 0.3 $mmol \cdot L^{-1} \cdot min$; $P<0.01$). Peak NCB in CHO (135 ± 60; 120 min) was greater than CON (-26 ± 40) and CHO-PRO (-113 ± 107 $nmol \cdot min^{-1}$), as was R_d AUC in CHO (35.7 ± 25.2) compared to CON (19.7 ± 15.5; $P=0.07$) and CHO-PRO (14.8 ± 9.6 $umol \cdot min$; $P<0.05$). R_a was no different between trials.

Conclusions: Following L-carnitine ingestion, insulin-mediated stimulation of a positive forearm carnitine balance with CHO feeding alone was acutely blunted by an isocaloric CHO-PRO beverage, suggesting that ingestion of whey protein in combination with CHO may inhibit chronic muscle carnitine accumulation.

Introduction

There is growing interest in the role of skeletal muscle carnitine and its associated enzymes in the perturbation of muscle lipid metabolism and aetiology of obesity and type 2 diabetes. For example, incomplete or insufficient β-oxidation of fatty acids may precede the accumulation of deleterious lipid intermediates and has been implicated in the development of skeletal muscle insulin resistance (1, 2). In line with the proposal that skeletal muscle carnitine availability is limiting to CPT1 flux and fatty acid oxidation (3), elevating muscle carnitine content via acute L-carnitine infusion (4) or chronic feeding (5) promotes physiologic and gene expression adaptations that are consistent with enhanced fat oxidation at rest and during moderate-intensity exercise. Conversely during high-intensity exercise, when mitochondrial acetyl-group provision is limited (at least partially) by the activation kinetics of the pyruvate dehydrogenase complex (PDC) enzyme (6), increasing skeletal muscle carnitine availability and acetylation improves the matching of glycolytic, PDC and mitochondrial substrate fluxes (7). The latter observation has implications for the enhancement of mitochondrial ATP delivery, such as in disease states where skeletal muscle perfusion may be impaired (8, 9).

Increasing the availability of carnitine within skeletal muscle is, therefore, a useful research tool to investigate the importance of these processes in humans *in vivo*, as well as to manipulate muscle metabolism for health benefits. However, muscle carnitine accretion, facilitated by the Na^+-dependent transporter OCTN2 (10), occurs against a 100-fold concentration gradient and hence L-carnitine feeding (11, 12) or intravenous infusion (13, 14) alone has no impact upon muscle carnitine content. Simultaneous elevation of plasma carnitine and serum insulin concentrations has proven effective in stimulating muscle carnitine uptake in healthy subjects (4, 14) and as such, a 20% increase in muscle carnitine can be achieved through twice-daily feeding of L-carnitine in a beverage containing 80 g of

carbohydrate over a 12-24 week period (5, 7). However, such a large carbohydrate load *per se* (160 g/day) will likely affect metabolism and alter body composition (5) and so investigation into alternative oral insulinogenic formulations that can stimulate muscle carnitine accumulation using lower carbohydrate loads is warranted. For example, whey protein has previously been fed with carbohydrate to promote insulin-mediated muscle creatine retention (15) and, unlike carbohydrate, prolonged protein supplementation is less likely to influence body fat content (16). Therefore, the aim of the present study was to acutely assess forearm net carnitine balance (NCB) and uptake following oral L-carnitine ingestion in combination with 80 g of carbohydrate alone versus a protein-carbohydrate blend that would produce a similar serum insulin response.

Subjects and Methods

Volunteers

Seven healthy, non-vegetarian males (age 24.2 ± 5.0 yrs, BMI 23.3 ± 3.1 $kg \cdot m^{-2}$) gave written informed consent and attended a routine medical screening prior to starting the study, which was approved by the University of Nottingham Medical School Ethics Committee.

Experimental Protocol

Subjects completed three single-blind, randomised (randomization.com) visits (**Figure 1**) following a 10 hour fast. Upon arrival at the University of Nottingham David Greenfield Physiology Unit, volunteers voided their bladder and laid semi-supine with one hand heated to ~55°C in an air-warming unit. This method has been shown to arterialise venous blood to within 3% of the oxygen saturation values of arterial blood (17). A cannula was placed retrograde into a dorsal vein of the heated hand for arterialised-venous blood sampling and into a deep-lying antecubital vein of the contralateral arm to sample venous blood draining the forearm muscle bed (18, 19). At t =0 min, subjects ingested 4.5 g L-carnitine tartrate (Lonza, Switzerland) with 30 mg [methyl-$^{2}H_{3}$]-L-carnitine (Cambridge Isotopes, MA, USA) in 200 ml water. At t=60 minutes, subjects were given a 500 ml beverage of either 80 g carbohydrate (CHO; Vitargo orange, Swecarb, Sweden), 40 g carbohydrate mixed with 40 g pure whey protein isolate (CHO-PRO; PRO-10.com, UK) or flavoured water (CON). The type (high molecular weight) and amount of carbohydrate used was employed previously to increase muscle carnitine content (5, 7). CHO-PRO was designed to be isocaloric and elicit a similar serum insulin response to CHO. All drinks were identically coloured and flavoured.

Sampling and analysis

Blood was sampled at 10 minute intervals for the determination of arterialised-venous serum insulin (Coat-A-Count Insulin; Seimens Healthcare, USA), and plasma acylcarnitine as well as both arterialised- and deep-venous whole blood glucose (Yellow Springs Instruments, Ohio) plasma free carnitine (20) concentrations and $^{2}H_{3}$-carnitine enrichment. For the latter, plasma was purified by strong cation exchange SPE (30 mg Oasis MCX 33 µm, 80Å; Waters), dried to residue and resuspended in 0.1% formic acid for analysis by LCMSMS. Chromatography was performed in isocratic mode using 5% acetonitrile, 0.1% formic acid in water (C18 Brownlee; 2.1 x 300 mm, 5 µm). The peak area ratio of carnitine (m/z 162 → 60) to $^{2}H_{3}$-carnitine (m/z 165 → 63) was subsequently determined in positive electrospray ionisation mode (Quattro Ultima triple quad, Micromass Ltd, UK). Brachial artery blood flow of the non-heated arm was determined by ultrasound imaging (Aplio SSA-770A, Toshiba Medical Systems) with a 12 MHz transducer synchronised to a three-lead ECG. Luminal diameter was imaged 10 cm proximal to the antecubital fossa and measured using online video callipers. Mean blood velocity was determined at the same anatomical location by integration of the pulsed-wave Doppler signal (21). Blood flow measurements were made by a single, experienced operator and values were converted to plasma flow using individual haematocrit fractional concentrations. Urine was collected from t=0 until t=180 minutes for the determination of urinary TC excretion (20).

Calculations

Plasma flow, F, was calculated as Blood Flow x (1 – haematocrit fraction). NBC was calculated by the Fick principle: $NBC=F\cdot[C_a-C_v']$ where C_a is the arterialised-venous and C_v' is the deep venous free carnitine concentration adjusted for non-steady state conditions (22, 23). Fractional carnitine and glucose extraction were calculated to provide a flow-independent marker of forearm balance: $Extraction =[C_a-C_v']/C_a\cdot100$. The rate of carnitine

disappearance (R_d) across the forearm was calculated from the steady-state $^2H_3$-carnitine molar percent excess (MPE) using arterialised-venous plasma as the precursor pool (24): $R_d=[MPE_a \cdot C_a - MPE_v \cdot C_v] \cdot F/MPE_a$ where MPE is the tracer-to-tracee ratio (TTR) expressed as a percentage enrichment: MPE=TTR/(1+TTR)*100. The rate of carnitine appearance, R_a, was also calculated: $R_a=R_d$-NCB. Consistent with small molecule pharmacokinetics, the calculations of R_d and R_a assume rapid carnitine equilibration between plasma and interstitial fluid compartments. All values were averaged over 20 min. Area under the [variable] x time curve (AUC) above baseline was integrated over 0-180 minutes.

Statistics

Based on our previous carnitine feeding and infusion studies (25, 26), this study was powered at 80% to detect a 10% difference in NCB in 7 subjects with an alpha level of 0.05. Time dependent variables (serum insulin and plasma free concentrations, plasma flow, NCB, carnitine extraction, MPE, R_d and R_a) were analysed using two-way repeated measures analysis of variance (ANOVA) with Bonferoni-corrected paired t-test to isolate main effects post-hoc. Urinary carnitine excretion and AUC were compared using one-way ANOVA with Tukey post-hoc. All statistical analyses were performed with GraphPad Prism 6 (GraphPad Software). Data presented are mean ± standard error of the mean (SE) for seven subjects.

Results

Serum Insulin and glucose extraction

Serum insulin concentration was similar between CON, CHO and CHO-PRO at 0 min (7 ± 2, 5 ± 1 and 6 ± 1 $mU \cdot L^{-1}$, respectively) and did not change during CON (**Figure 2A**). Insulin concentration increased rapidly after CHO and CHO-PRO ingestion, peaking at 100 min (64 ± 10 and 72 ± 10 $mU \cdot L^{-1}$, respectively), and remained elevated above CON thereafter. AUC in CHO (4.5 ± 0.8) and CHO-PRO (3.8 ± 0.6) was 102- and 85-fold greater, respectively, than CON (0.04 ± 0.1 $U \cdot L^{-1} \cdot min$; $P<0.01$), but no different between CHO and CHO-PRO. An interaction effect was observed ($P<0.001$) such that glucose extraction during CHO and CHO-PRO was higher than CON from 100-180 minutes and also greater in CHO than CHO-PRO at 100 and 140 minutes (**Figure 2B**).

Plasma free, acyl and urinary total carnitine

Baseline plasma arterialised-venous free carnitine (FC) concentration was no different (42 ± 3, 44 ± 2 and 42 ± 3 $\mu mol \cdot L^{-1}$) for CON, CHO and CHO-PRO, respectively and increased equivalently over the first hour after L-carnitine ingestion. Following the ingestion of the treatment drink, FC increased sharply in CHO-PRO and remained elevated above CHO and CON, which continued to rise steadily, for the remainder of the visit ($P<0.001$; **Figure 3A**). FC AUC during CHO-PRO (3.5 ± 0.5) was 67% and 84% greater than CON (2.1 ± 0.2; $P<0.05$) and CHO (1.9 ± 0.3 $mmol \cdot L^{-1} \cdot min$; $P<0.01$), respectively. Plasma acylcarnitine was unchanged in CON and CHO ($P>0.05$ versus baseline) but increased in CHO-PRO such that it was higher in CHO-PRO than CON between 80-120 minutes ($P<0.001$) and higher than CHO between 80-180 minutes ($P<0.05$). Urinary TC excretion was similar during CON (46 ± 14 mg) and CHO (45 ± 34) but was 98 and 106% greater in CHO-PRO (92 ± 18 mg) than CON and CHO, respectively (both $P<0.05$).

153 *Plasma flow*

154 Plasma flow was similar at baseline for CON, CHO and CHO-PRO (40 ± 7, 47 ± 10 and 44 ±
155 9 ml·min^{-1}, respectively) and did not change over the first hour. A main effect of drink
156 (P<0.05) was observed such that plasma flow was greater in CHO and CHO-PRO than CON
157 (**Figure 3B**). Plasma flow AUC above baseline (0-60 min) was greater in CHO (0.5 ± 0.6;
158 P<0.05) and tended to be greater in CHO-PRO (0.4 ± 0.6; P=0.05) than CON (-0.5 ± 0.2
159 L·min).
160
161 *Net forearm carnitine balance and extraction*
162 Net carnitine balance (NCB) across the forearm is shown in **Figure 3C** and was unchanged
163 over the one hour following L-carnitine ingestion. Following the treatment drink, NCB
164 increased in CHO only (interaction effect; P<0.05), peaking at 120 min (135 ± 60) above
165 CON (-26 ± 40; P<0.05) and CHO-PRO (-113 ± 107 nmol·min^{-1}; P<0.05). This resulted in a
166 greater AUC in CHO (4.1± 3.1) than CON (-8.6 ± 3.0) and CHO-PRO (-14.6 ± 6.4
167 µmol·min; P<0.05). Carnitine extraction was also increased (P<0.05) in CHO compared to
168 CHO-PRO at t=100, 120 and 180 minutes and tended to be increased (P=0.09) above CON at
169 t=120 minutes.
170
171 *Plasma [$^2H_3$]-carnitine enrichment, rate of disappearance and appearance*
172 Plasma [$^2H_3$]-carnitine enrichment reached a steady state after t=80 min in all trials, with a
173 similar MPE attained in CON and CHO (~0.14%), but a slightly higher MPE of ~0.2% in
174 CHO-PRO (**Figure 4A**). Plasma carnitine R_d was unchanged throughout in CON and CHO-
175 PRO but increased in CHO (**Figure 4B)**, resulting in a 1.8 and 2.4-fold greater AUC above
176 zero in CHO (35.7 ± 25.2) than CON (19.7 ± 15.5; P=0.07) and CHO-PRO (14.8 ± 9.6

177 μmol·min; P<0.05), respectively. Carnitine R_a was similar and no different from zero in

178 CON, CHO and CHO-PRO (**Figure 4C**).

Discussion

These novel data provide the most direct measurement to date of acute muscle carnitine uptake *in vivo* in humans and demonstrate that the ingestion of a carbohydrate beverage is able to promote a positive net carnitine balance across the forearm, indicative of accelerated muscle carnitine accretion. Moreover, replacement of some of this carbohydrate with whey protein prohibited any increase in net carnitine balance, despite inducing a similar serum insulin and plasma flow response. Thus it would appear that the mechanism by which insulin stimulates muscle carnitine transport is antagonised by a large bolus of whey protein.

Studies from this lab have previously shown that elevating plasma carnitine concentration via intravenous infusion alone has no impact on muscle carnitine content (14), whilst acute (27) or chronic (11) oral dosing of L-carnitine does not affect net leg carnitine balance or muscle carnitine content, respectively. A recent study of porcine arteriovenous carnitine fluxes confirmed that net muscle carnitine uptake/efflux is negligible under normal conditions, with systemic concentrations of carnitine and acylcarnitines largely governed by gut absorption, hepatic release and renal filtration (28). The latter is consistent with the preservation of total muscle carnitine under conditions of increased metabolic flux, such as during hyperinsulinemic-euglycemic insulin clamp (4), or exercise (7). These data refute the speculated role of skeletal muscle acetylcarnitine influx/efflux in metabolic health (29, 30) and question the validity of the physiological inferences that can be made from *in-vitro* studies of carnitine metabolism (30, 31). In agreement with previous *in-vivo* data and consistent with the high concentration gradient between plasma and tissues, carnitine ingestion in the current study had no detectable impact on NCB during CON. In contrast, CHO facilitated a positive NCB across the forearm, further validating the ingestion of L-carnitine in a carbohydrate beverage as a means to augment muscle carnitine content (7). The majority of the measured plasma carnitine extraction across the forearm likely occurred into

skeletal muscle (32) and thus it is possible to estimate whole-body rates of insulin-stimulated muscle carnitine accretion. Insulin was elevated from 80 to 180 minutes during CHO, over which period the NCB AUC was 7.9 µmols greater than CON. Assuming an average forearm muscle mass of 0.6 kg (18, 33) and whole-body muscle mass of 30 kg, this equates to a whole-body muscle carnitine uptake of 390 µmols above CON. This aligns well with the 370 µmols (60 mg) of carnitine retention predicted from differences in urinary carnitine excretion in a previous study (26). Extended to a chronic feeding scenario, this would equate to a daily increase in muscle carnitine content of 13 µmol·kg ww^{-1}, which would augment muscle total carnitine content stores (~5 mmol·kg ww^{-1}) by 22% over 12 weeks. Again, this extrapolation is in good agreement with the 21% increase in muscle carnitine content reported by (5) and provides indirect validation for our values of net carnitine balance.

Limb/organ balance models normally preclude definitive conclusions on whether substrate uptake, efflux, or a combination of the two has occurred (28). Here, the use of $^{2}H_{3}$-carnitine tracer enables a more direct interrogation of muscle carnitine uptake. The average rate of forearm carnitine disappearance throughout the CON trial (~9 µmol·kg·hr^{-1}) is very similar to estimated rates of basal muscle carnitine uptake (11.6 µmol·kg·hr^{-1}) from compartmental modelling of intravenously-administered [^{3}H]-carnitine kinetics (34). Peak R_d during CHO was numerically 15-fold higher than the equivalent time-point in CON and tended to be greater when compared over the entire treatment period (AUC). It could be argued that increases in NCB and R_d during CHO were related to greater plasma flow. However, carnitine fractional extraction, which does not depend on flow, was also elevated following CHO but not CON, whilst plasma flow was similarly elevated above CON in CHO-PRO (where extraction was unchanged). Moreover, peak carnitine R_d during CHO coincided with peak serum insulin concentration, suggesting that the positive NCB during CHO is more likely attributable to an up-regulation of muscle carnitine transport, rather than plasma flow.

Serum insulin responses were similar between CHO and CHO-PRO and importantly, were identical over the period when forearm carnitine balance was elevated in CHO. It was thus surprising that NCB was not similarly increased during CHO-PRO. This apparent inhibition of insulin-stimulated muscle carnitine uptake is supported by the finding that R_d following CHO-PRO was supressed relative to CHO and further reflected by the augmented plasma carnitine compartment and 2-fold greater urinary carnitine excretion in CHO-PRO compared to CHO. As forearm glucose extraction was greater in CHO than CHO-PRO, it might be speculated that the absence of a positive NBC in CHO-PRO was related to differential forearm glucose metabolism. However, the difference between CHO and CHO-PRO glucose extraction relative to CON was small, and thus insufficient to explain the contrasting NCB. Considering the obligate role of the organic novel cation transporter in muscle carnitine accumulation (10, 35), it seems more plausible that the inhibition of muscle carnitine transport following CHO-PRO be related to OCTN2 activity. Known physiological inhibitors of OCTN2 include acylcarnitines (36) which have been shown to accumulate in response to increased amino acid availability as by-products of excessive amino acid oxidation or incomplete β-oxidation (37). Indeed, plasma acylcarnitine concentrations during CHO-PRO were elevated throughout the period of hyperinsulinemia and thus, could perhaps be responsible for the inhibition of muscle carnitine uptake. In line with this, plasma acylcarnitines are characteristically elevated in the insulin resistant state (38), a scenario in which muscle carnitine accumulation is purportedly compromised. Alternatively, high rates of insulin-responsive, sodium-dependent amino acid flux could restrict cationic muscle carnitine transport following CHO-PRO (39, 40), although this cannot be inferred from the current data. Why the uptake of carnitine by skeletal muscle would be inhibited by protein, given the predominant dietary source of carnitine is meat (41), is somewhat perplexing, though it should be noted that the large bolus of protein ingested in this study is excessive

compared to the protein content of a normal mixed meal. Nevertheless, a slow postprandial transport of carnitine into muscle, together with a negligible rate of appearance of carnitine from muscle, is entirely consistent with the stability and slow turnover of the muscle carnitine pool (34).

It has previously been suggested that amino acids could inhibit L-carnitine intestinal absorption (42). However, the greater urinary and plasma carnitine in CHO-PRO during the current study may provide evidence to the contrary. When compared to CHO, the elevated plasma and urinary carnitine in CHO-PRO can reasonably be accounted for by the estimated difference in muscle carnitine uptake. When comparing CON and CHO-PRO trials however, during which NCB was similarly negligible, the differences in plasma and urinary carnitine would suggest that carnitine absorption was not comparable across all trials. In particular, assuming negligible intracellular release of carnitine during CON and CHO-PRO (R_a was not different from zero), the greater plasma MPE during CHO-PRO likely reflects a greater absorption of exogenous carnitine into the endogenous carnitine pool. Based on previous studies (43-45), it was expected that a 3 g dose of L-carnitine would saturate intestinal active carnitine transport and thus facilitate equivalent carnitine absorption across all trials. However, and in contrast to the suggestion that amino acids may inhibit intestinal carnitine absorption, these findings infer that protein ingestion may increase co-ingested carnitine absorption. This reconciles with the predominant dietary sources of carnitine and, given the blunting of forearm NCB in CHO-PRO, also implies that the mechanism and regulation of intestinal carnitine absorption is perhaps different from that of skeletal muscle.

In Conclusion, the novel use of an acute arteriovenous forearm balance model with $^{2}H_3$-L-carnitine tracer methodology affirms the absence of appreciable muscle carnitine uptake (or efflux) following L-carnitine ingestion alone and confirms the efficacy of a carbohydrate beverage in promoting muscle carnitine accretion. Conversely, a carbohydrate-protein blend

279 entirely blunted this stimulation of muscle carnitine uptake, despite comparable serum insulin
280 concentration, plasma flow responses and apparent increased intestinal carnitine absorption.

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CES, PLG and FBS designed research; CES and AVN conducted research and analysed data; CES wrote the paper; PLG and FBS critically revised the manuscript; FBS had primary responsibility for the final content. All authors read and approved the final manuscript. The authors have no conflict of interest to declare.

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Figures and legends

Figure 1. Experimental protocol for study visits.

Figure 2. Arterialised-venous serum insulin (A) and whole blood glucose extraction (B) following ingestion of 4.5 g L-carnitine-tartrate (t=0 min) and a 500 ml drink (arrow) containing flavoured water (CON; circles), 80 g carbohydrate (CHO; squares) or 40 g carbohydrate + 40 g whey protein (CHO-PRO; triangles) at t=60 min. Values are mean ± SE, n=7 and were compared using two-way (drink x time) ANOVA with Tukey or Bonferoni tests used for post-hoc subgroup comparisons following the observation of a significant main or interaction effect, respectively. A significant interaction effect was found for serum inuslin ($P<0.001$) and glucose extraction ($P<0.001$) with post-hoc subgroup comparisons denoted as follows: *** $P<0.001$ CHO vs CON; † $P<0.05$, †† $P<0.01$, ††† $P<0.001$ CHO-PRO vs CON; ‡ $P<0.05$, ‡‡ $P<0.01$ CHO vs CHO-PRO.

Figure 3. Plasma arterialised-venous free carnitine concentration (A), brachial artery plasma flow (B) and forearm net carnitine balance (C) following ingestion of 4.5 g L-carnitine-tartrate (t=0 min) and a 500 ml drink (arrow) containing flavoured water (CON; circles), 80 g carbohydrate (CHO; squares) or 40 g carbohydrate + 40 g whey protein (CHO-PRO; triangles) at t=60 min. Values are mean ± SE, n=7 and were compared using two-way (drink x time) ANOVA with Tukey or Bonferoni tests used for post-hoc subgroup comparisons following the observation of a significant main or interaction effect, respectively. Plasma flow was greater in both CHO and CHO-PRO vs CON (main effect of drink; $P<0.05$). A interaction effect ($P<0.05$) was found for plasma free carnitine and net carnitine balance with post-hoc subgroup comparisons denoted as follows: * $P<0.05$ CHO vs CON; ††† $P<0.001$ CHO-PRO vs CON; ‡ $P<0.05$, ‡‡ $P<0.01$, ‡‡‡ $P<0.001$ CHO vs CHO-PRO.

Figure 4. Arterialised-venous plasma [$^{3}H_{2}$]-carnitine enrichment (molar percent excess; A), rate of carnitine disappearance (B) and carnitine appearance (C) following ingestion of 4.5 g

L-carnitine-tartrate (t=0 min) and a 500 ml drink (arrow) containing flavoured water (CON; circles), 80 g carbohydrate (CHO; squares) or 40 g carbohydrate + 40 g whey protein (CHO-PRO; triangles) at t=60 min. Values are mean ± SE, n=7 and were compared using two-way (drink x time) ANOVA with Tukey or Bonferoni tests used for post-hoc subgroup comparisons following the observation of a significant main or interaction effect, respectively. An interaction effect (P<0.001) was found for plasma [$^{3}H_{2}$]-carnitine enrichment with post-hoc subgroup comparisons denoted as follows: †† P<0.01, ‡‡‡ P<0.001 CHO-PRO vs CON; ‡ P<0.05, ‡‡ P<0.01, ‡‡‡ P<0.001 CHO vs CHO-PRO.