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ORIGINAL ARTICLE

Effects of oral L-carnitine supplementation on insulin sensitivity indices in response to glucose feeding in lean and overweight/obese males

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Abstract Infusion of carnitine has been observed to increase non-oxidative glucose disposal in several studies, but the effect of oral carnitine on glucose disposal in non-diabetic lean versus overweight/obese humans has not been examined. This study examined the effects of 14 days of L-carnitine L-tartrate oral supplementation (LC) on blood glucose, insulin, NEFA and GLP-1 responses to an oral glucose tolerance test (OGTT). Sixteen male participants were recruited [lean ($n = 8$) and overweight/obese ($n = 8$)]. After completing a submaximal predictive exercise test, participants were asked to attend three experimental sessions. These three visits were conducted in the morning to obtain fasting blood samples and to conduct 2 h OGTTs. The first visit was a familiarisation trial and the final two visits were conducted 2 weeks apart following 14 days of ingestion of placebo (PL, 3 g glucose/day) and then LC (3 g LC/day) ingested as two capsules 3×/day with meals. On each visit, blood was drawn at rest, at intervals during the OGTT for analysis of glucose, insulin, non-esterified fatty acids (NEFA) and total glucagon-like peptide-1 (GLP-1). Data obtained were used for determination of usual insulin sensitivity indices (HOMA-IR, AUC glucose, AUC insulin, 1st phase and 2nd phase β-cell function, estimated insulin sensitivity index and estimated metabolic clearance rate). Data were analysed using RMANOVA and post hoc comparisons where appropriate. There was a significant difference between groups for body mass, % fat and BMI with no significant difference in age and height. Mean (SEM) plasma glucose concentration at 30 min was significantly lower ($p < 0.05$) in the lean group on the LC trial compared with PL [8.71(0.70) PL; 7.32(0.36) LC; mmol/L]. Conversely, plasma glucose concentration was not different at 30 min, but was significantly higher at 90 min ($p < 0.05$) in the overweight/obese group on the LC trial [5.09(0.41) PL; 7.11(0.59) LC; mmol/L]. Estimated first phase and second phase β-cell function both tended to be greater following LC in the lean group only. No effects of LC were observed on NEFA or total GLP-1 response to OGTT. It is concluded that LC supplementation induces changes in blood glucose handling/disposal during an OGTT, which is not influenced by GLP-1. The glucose handling/disposal response to oral LC is different between lean and overweight/obese suggesting that further investigation is required. LC effects on gastric emptying and/or direct 'insulin-like' actions on tissues should be examined in larger samples of overweight/obese and lean participants, respectively.

Keywords Glucose disposal · Muscle glycogen · Exercise

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Introduction

L-Carnitine is a widely used conditionally essential nutrient added to a range of functional food products including weight loss formulations, infant formula milk, and sports bars and drinks. Carnitine is widely recognised as a key factor involved in the regulation and transport of activated fatty acids into mitochondria for oxidation (Brass and Hiatt 1998) and thus many human studies have attempted to enhance skeletal muscle fatty acid oxidation by supplementing with carnitine (Abramowicz and Galloway 2005;

Broad et al. 2005; Decombaz et al. 1993; Hawley et al. 1998a; Hawley et al. 1998b; Vukovich et al. 1994). The large majority of these studies have observed no effect of supplemental carnitine on whole body fat utilisation of healthy active adults during moderate intensity exercise (for review see Galloway and Broad 2005), but there are observations of enhanced carbohydrate oxidation during exercise following oral supplementation in endurance-trained male athletes (Abramowicz and Galloway 2005).

It is known that increasing the total carnitine content of skeletal muscle is difficult in the short term, but is attainable using hyperinsulinaemic euglycaemic clamp methodology to stimulate the sodium-dependent transport of carnitine into skeletal muscle (Stephens et al. 2006b) and through prolonged oral carnitine supplementation combined with carbohydrate (Wall et al. 2010). Infusion of carnitine is associated with an increase in muscle glycogen content (Stephens et al. 2006a) and an elevated plasma carnitine concentration, though infusion has been observed to increase non-oxidative glucose disposal during hyperinsulinaemic euglycaemic clamps in controls and type 2 diabetics (Giancaterini et al. 2000; Mingrone et al. 1999). It is interesting to note that Stephens et al. (2007b) reported an increase in retention of oral carnitine when ingested with carbohydrate and attributed this to an increased muscle carnitine uptake, but these authors do not report muscle carnitine content, muscle glycogen data or data on glucose disposal in response to oral carnitine ingestion. In their review (Stephens et al. 2007a), it is reported that elevation in plasma carnitine likely increases glycogen storage through non-oxidative glucose disposal, which is consistent with the increased glucose disposal effect of elevating plasma carnitine via infusion observed in previous studies. The mechanism for this enhanced glucose disposal effect of carnitine has not been fully investigated, and glucose disposal responses to oral carnitine ingestion and glucose feeding have not been evaluated. Furthermore, investigation of oral carnitine ingestion on glucose disposal could potentially be influenced by differences in the incretin hormone response (components of the entero-insular axis that enhance the insulin response to a meal), and therefore it should be monitored in any oral carnitine supplementation study. To date, no one has investigated the potential role of oral carnitine ingestion on glucose disposal.

Since it is widely recognised that obesity is linked to impaired insulin sensitivity (Kahn et al. 2006), it is important to assess the potential effects of carnitine on insulin sensitivity indices and glucose disposal in lean to overweight/obese adults, as they may respond differently to oral carnitine supplementation. Indeed, there is one report of enhanced whole body fat oxidation at rest in overweight participants following carnitine ingestion (Wutzke and Lorenz 2004), which has not been observed in lean participants. Therefore, the primary aim of the present study was to evaluate whether oral supplementation with carnitine could impact upon insulin sensitivity indices and glucose disposal during an oral glucose tolerance test after an overnight fast in healthy lean and overweight/obese males. The secondary aim was to investigate whether any alteration in glucose disposal or insulin response to oral carnitine supplementation was independent of the incretin hormone response to glucose feeding. We hypothesised that lean active males would have higher insulin sensitivity, thus there would possibly be a greater impact of supplemental oral carnitine on glucose disposal and that this would be independent to the response of the incretin hormone glucagon-like peptide-1 (GLP-1).

Methods

Sixteen male participants were recruited for this study, which had local ethics committee approval. Each participant attended the laboratory on four occasions over a 5- to 6-week period. The first visit was for initial pre-screening, which included medical history and physical activity questionnaires, body fat estimation (bioelectric impedance analysis, Bodystat), body mass and height assessment. They then performed a three-stage submaximal predicted VO_{2peak} test on a cycle ergometer (Lode Excalibur, Lode, Netherlands). The three-stage submaximal test started with 5 min of exercise at 50 W with increases in workload determined by the heart rate response to exercise recorded at the end of minute 4 and 5 of each stage. Participants were recruited as lean ($n = 8$) or overweight/obese ($n = 8$) and their physical characteristics are shown in Table 1. Participants attended the laboratory 1 week later first thing in the morning after an overnight fast to perform a preliminary familiarisation trial. Two days prior to this trial, participants were asked to record their dietary intake and to record any physical activity that they undertook. Participants were told not to exercise heavily during this 2-day period and were then asked to replicate their diet and activity in the 2 days prior to all remaining trials. The familiarisation trial included a standard 75-g, 2-h oral glucose tolerance test (75-g glucose, OGTT). This preliminary trial was included to ensure that all participants were familiarised with the OGTT protocol.

The final two visits were the two main experimental trials and participants attended the laboratory first thing in the morning after an overnight fast (>10 h) and on each visit completed a standard 75-g OGTT trial. The two visits were performed following either a 2-week period of supplementation with placebo (PL, 3 g/day, anhydrous glucose) or a 2-week period of supplementation with L-carnitine L-tartrate (LC, 3 g/day). The supplements were ingested in

Table 1 Physical characteristics of participants in lean and overweight/obese groups

Variable	Lean	Overweight/obese
n	8	8
Age (year)	24.0 ± 7.4 (18.9–29.1)	29.0 ± 9.9 (22.2–35.8)
Height (cm)	180.0 ± 5.8 (176.0–184.0)	178.7 ± 6.7 (174.1–183.3)
Body mass (kg)	76.1 ± 5.6 (72.2–80.0)*	103.3 ± 13.0 (94.3–112.3)*
BMI (kg/m^2)	23.5 ± 1.0 (22.8–24.2)*	32.5 ± 4.9 (29.1–35.9)*
Estimated body fat (%)	12.3 ± 2.9 (9.3–14.3)*	25.9 ± 6.3 (21.5–30.3)*
Fat mass (kg)	9.3 ± 2.2 (7.8–10.8)*	27.3 ± 9.4 (20.8–33.8)*
Lean mass (kg)	66.8 ± 5.7 (62.9–70.7)*	76.0 ± 6.2 (71.7–80.3)*
Fasting glucose (mmol/L)	5.6 ± (5.2–6.0)	5.2 ± (4.7–5.7)
Fasting insulin (μIU/ml)	6.9 ± (5.6–8.2)	15.4 ± (5.8–23.8)
VO_{2peak} ($L\ min^{-1}$)	4.12 ± 0.65 (3.67–4.57)	4.01 ± 0.90 (3.38–4.64)
VO_{2peak} ($mL\ kg\ lean^{-1}\ min^{-1}$)	61.3 ± 6.2 (57.0–65.6)†	52.8 ± 10.9 (45.2–60.4)†

Values are mean ± SD (95% CI)

* Significantly different between lean and overweight/obese groups ($P < 0.05$)

† Tendency towards statistical significance between groups ($P < 0.10$)

gelatin capsules taken as two capsules three times per day, with meals. Treatments were administered in a single blind-ordered fashion with placebo ingested first and with the final dose of supplement ingested with the evening meal on the night before the trial. An ordered design was used, as we have previously shown that after cessation of chronic LC supplementation, morning fasted plasma total and free carnitine can remain elevated by >10% above baseline for up to 3 weeks and only returns to baseline by 4 weeks after the end of the ingestion period (Galloway and Broad 2004). To avoid any potential carryover effects of an elevated fasting plasma carnitine concentration, we administered placebo first, but participants were unaware of this.

OGTT test protocol

After arrival at the laboratory, participants had their body mass measured and then lay supine on a treatment couch whilst a cannula was inserted into an antecubital vein. Once the cannula was in place, participants moved to a seated position, and after a period of seated rest (10 min), an initial resting blood sample was drawn (0 min sample). Participants were then provided with a drink containing 75 g of glucose with 15 ml of a sugar-free lemon flavouring made up to a total volume of 300 ml. The drink was then consumed over a period of 1 min and upon finishing the drink, the OGTT timer was started. Participants remained in a seated position in a comfortable environment (20–22°C) for the whole of the 2-h OGTT period, and blood samples were drawn from the cannula at intervals during the test. After drawing a sample, a small volume of sterile saline (1–2 ml) was used to flush the cannula to ensure patency.

Blood sampling and analysis

Blood samples (7.5 ml) were drawn from the indwelling venous cannula at rest (0 min), and at 15, 30, 45, 60, 90 and 120 min during the OGTT. All blood samples were drawn into a dry syringe during visits 2, 3 and 4, and blood was dispensed into one 2.5-ml KEDTA blood collection tube and one 5-ml lithium heparin blood collection tube. All samples were then mixed and immediately centrifuged at 4,000 rpm for 10 min at 4°C. Plasma was removed and transferred to microcentrifuge tubes and then frozen at −80°C until analysis. Samples were used for analysis of plasma glucose (glucose oxidase method, ABX diagnostics), plasma non-esterified fatty acids (NEFA, enzymatic analysis, Alpha labs NEFA-C kit), plasma insulin (ELISA method, DRG human insulin kit) and total GLP-1 (Assay Technology, DRU, Novo Nordisk, Denmark). The total GLP-1 assay used was a two-sited immuno assay, where two antibodies were applied with one antibody recognising a mid-molecular region epitope (position 15–22) and the other antibody specific for a C-terminus sequence (position 26–33). The assay therefore quantifies GLP-1 and related metabolites with amino acids from approximately position 15 to 33. The inter-rack assay coefficient of variation was 7.5%. Glucose and insulin data were used to calculate the usual insulin sensitivity indices (HOMA-IR, AUC glucose, AUC insulin) with the additional parameters of insulin sensitivity index, β-cell function (1st phase and 2nd phase) and estimated metabolic clearance rate, all calculated using the methods of Stumvoll et al. (2000).

Statistical analysis

All data are expressed as mean (SEM) unless otherwise stated, and 95% confidence intervals are shown where appropriate. Data from the OGTT trials were analysed using a repeated measures analysis of variance (RMANOVA) with trial (placebo or carnitine) and time (0 min–120 min time points) as within-subjects factors, and group (lean or overweight/obese) as a between-subjects factor. Following observation of a main effect, these were followed up with

post hoc analysis using one-way ANOVA and paired samples *t* test where appropriate to determine at which location the differences occurred. Correlation analysis was used to determine whether any associations existed between BMI, estimated % body fat and predicted maximal aerobic capacity (corrected for lean mass) with the magnitude of the change in responses of the key OGTT insulin sensitivity indices between PL and LC trials. Significance was taken as $P < 0.05$.

Results

The physical characteristics of the participants are shown in Table 1. The overweight/obese group had a significantly higher total body mass, BMI and estimated body fat percentage, and also higher fat mass and lean mass than the lean group (all $P < 0.01$). There was no difference in absolute VO_{2peak}, but it tended to be different when expressed per kilogram of lean mass ($P < 0.10$). The overweight/obese group also had a significantly lower baseline calculated insulin sensitivity index and lower estimated metabolic clearance rate compared with the lean group on the placebo (PL) trial, but there was no difference in baseline HOMA-IR, AUC glucose or AUC insulin between groups on the PL trial (Table 2).

Responses to OGTT

There was a significant effect ($P < 0.01$) for plasma glucose response to supplementation with a lower peak plasma glucose during the OGTT at 30 min ($P < 0.05$) on the LC trial in the lean group, and a higher plasma glucose at 90 min ($P < 0.05$) in the overweight.obese group on the LC trial, compared with the PL trial (Fig. 1). Peak plasma glucose on the LC trial occurred at the 15-min sample time in lean and at the 45-min sample time in the overweight/obese group. On the PL trial, peak plasma glucose concentration occurred at the 30-min sample time for both groups.

There was no difference in the plasma insulin response to supplementation and no interactions were observed, but there was a significant time effect ($P < 0.01$) as expected (Fig. 2). There was no between-subjects group effect suggesting that the insulin response to PL and LC trials was similar in both the lean and overweight/obese groups. Total AUC glucose and total AUC insulin were not significantly different between trials for either group (Table 2); however, a group by trial interaction was observed ($P < 0.05$) with a decline in AUC glucose in lean from PL to LC trials [mean (95% CI) change was −39.1 (−80.5 to 2.3) mmol L^{-1}/2 h] and an increase in AUC glucose in overweight/obese from PL to LC trials [mean (95% CI) change was 47.5 (−1.2 to 96.2) mmol L^{-1}/2 h]. There was a

Table 2 Oral glucose tolerance test insulin sensitivity indices of participants in lean and overweight/obese groups on placebo (PL) and L-carnitine (LC)-supplemented trials. Values are mean (95% CI). No statistically significant differences were noted in variables between PL and LC trials within each group

Variable	Trial	Lean	Overweight/obese
HOMA-IR (arbitrary units)	PL	1.7 (1.4–2.0)	3.5 (1.3–5.7)
	LC	2.1 (1.6–2.6)	3.2 (1.7–4.7)
AUC glucose (mM/2 h)	PL	153 (121–186)	265 (98–432)
	LC	114 (97–131)‡	312 (99–526)‡
AUC insulin (pM/2 h)	PL	3,739 (2,480–4,998)	6,117 (3,087–9,147)
	LC	3,414 (2,029–4,799)‡	7,536 (4,256–10,816)‡
1st phase β-cell function (pM)	PL	223 (18–428)	340 (193–487)
	LC	409 (305–513)†	428 (315–541)
2nd phase β-cell function (pM)	PL	93 (55–131)	117 (88–146)
	LC	129 (109–149)†	135 (112–158)
Insulin sensitivity index (μmol kg^{-1} min^{-1} pM^{-1})	PL	0.13 (0.13–0.13)*	0.09 (0.08–0.10)*
	LC	0.13 (0.13–0.13)*	0.09 (0.08–0.10)*
Metabolic clearance rate (mg kg^{-1} min^{-1})	PL	10.9 (10.7–11.1)*	7.9 (6.7–9.1)*
	LC	10.9 (10.7–11.1)*	7.8 (6.5–9.1)*

* Significantly different between lean and overweight/obese groups ($P < 0.01$)

‡ Significant interaction between trial and group ($P < 0.05$)

† Tendency towards statistical significance between PL and LC trials within group ($P < 0.10$)

similar interaction with AUC insulin ($P < 0.05$), with an increase in AUC insulin on LC in the overweight/obese group, but no change in the lean group.

There was a tendency towards a significant difference in the estimated first phase and second phase beta cell function from data obtained during the OGTT trials in the lean group, with a higher first phase and higher second phase beta cell function estimate on the LC trial ($P < 0.10$, Table 2). This was not observed in the overweight/obese group. There was no difference in HOMA-IR, estimated insulin sensitivity index or estimated metabolic clearance rate between PL and LC trials in either group.

There was a significant time effect ($P < 0.01$) with a decline in plasma NEFA occurring throughout the OGTT period in both groups (Fig. 3). Total GLP-1 response during the OGTT was not influenced by carnitine supplementation (no trial effect, Fig. 4), but there was a significant time effect ($P < 0.01$) and group effect ($P < 0.01$) with total GLP-1 significantly higher in the overweight/obese group than in the lean group at all time points during the OGTT. Mean total GLP-1 concentration throughout the protocol was 107.3(11.6) pM on PL and 112.1(15.3) pM on LC for the overweight/obese group, and 74.3(13.7) pM on PL and 69.7(14.3) pM on LC for the lean group.

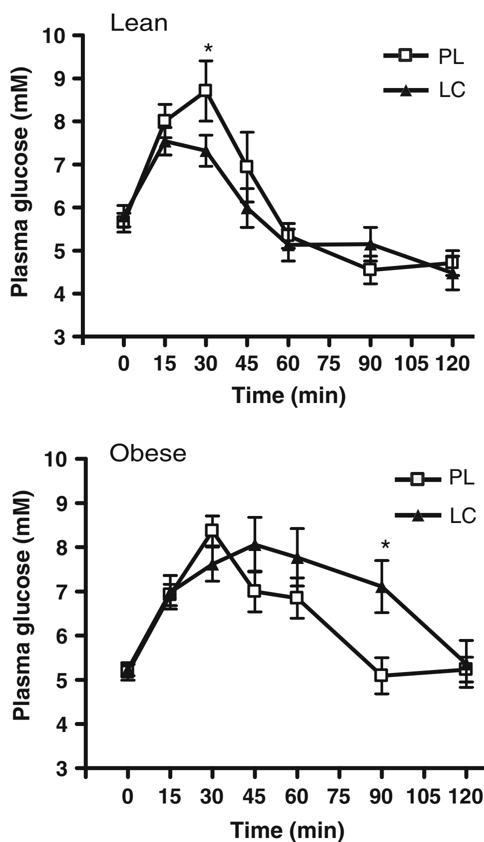

Fig. 1 Plasma glucose response to OGTT on placebo (PL) and L-carnitine (LC) trials in lean (Lean, $n = 8$) and overweight/obese (Obese, $n = 8$) groups. Values are mean (SEM). * indicates significant difference ($P < 0.05$) from PL

Associations between glucose disposal response and BMI, body fat and VO_{2peak}

There were significant associations between the change in AUC glucose with BMI, estimated % body fat and relative VO_{2peak} (expressed relative to lean body mass). A positive correlation (Pearson correlation coefficient, P value) was noted for change in AUC glucose with BMI ($r = 0.58$, $P = 0.02$) and % body fat ($r = 0.65$, $P = 0.01$), with a lower BMI and a lower % body fat associated with a greater change in AUC glucose on LC (i.e. a lower total AUC glucose on the LC trial compared with PL). A significant negative correlation was observed for change in AUC glucose to lean mass adjusted relative VO_{2peak} ($r = -0.65$, $P = 0.01$), with those having a higher relative VO_{2peak} per kilogram lean mass also having a greater change in AUC glucose on LC (again meaning a lower total AUC glucose on the LC trial for more aerobically fit participants).

Discussion

Oral LC supplementation (3 g/day) for 2 weeks appears to have an impact upon glucose homeostasis and insulin release in healthy lean and overweight/obese males during a 2-h OGTT. Despite the lack of a baseline differences in fasting glucose, insulin and HOMA-IR, and the small between-group differences in insulin sensitivity index and estimated metabolic clearance rate at baseline on the PL trial, it is clear that there are some potentially important group differences in blood glucose response to an OGTT following LC supplementation. Furthermore, the association between a greater change in AUC glucose following LC supplementation in those with lower body fat, lower BMI and higher VO_{2peak} relative to lean body mass suggests that fitter and leaner participants had a greater glucose disposal response to LC supplementation in this study. The overweight/obese participants had the opposite response with delayed glucose disposal. Whilst we acknowledge that administering an absolute amount of LC to both groups effectively means that the overweight/obese group obtained less LC per kilogram per day, we wished to standardise to the OGTT protocol in which we administered 75 g of glucose to all participants. Further studies should investigate whether matched dosing per kilogram body mass or per kilogram lean body mass impacts upon the outcomes noted here.

It is interesting that to date no one has investigated the glucose disposal response to oral carnitine supplementation

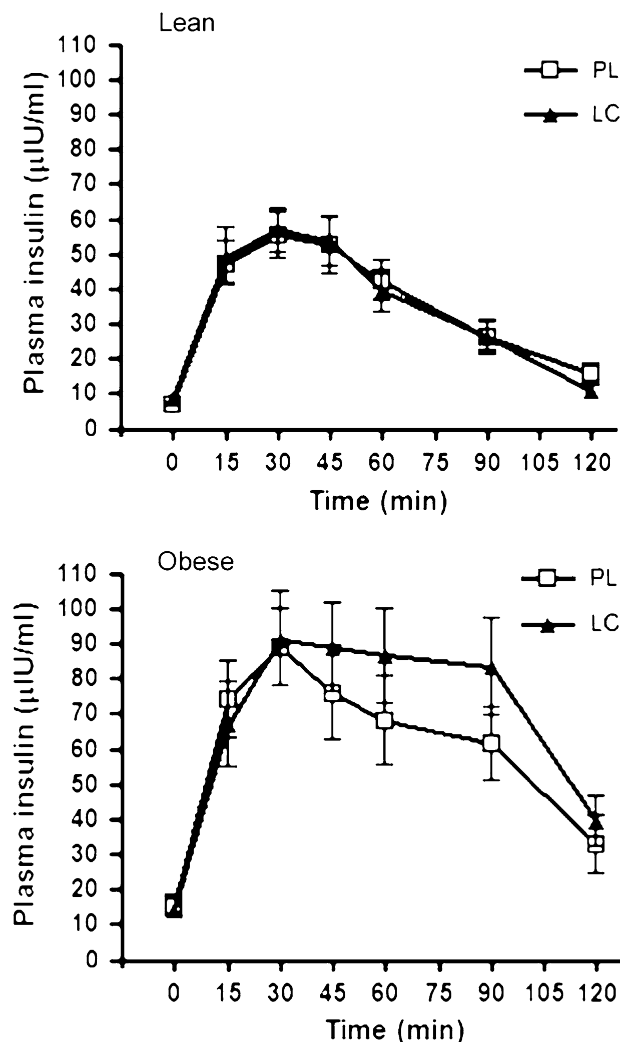

Fig. 2 Plasma insulin response to OGTT on placebo (PL) and L-carnitine (LC) trials in lean (Lean, $n = 8$) and overweight/obese (Obese, $n = 8$) groups. Values are mean (SEM). No differences were observed between trials

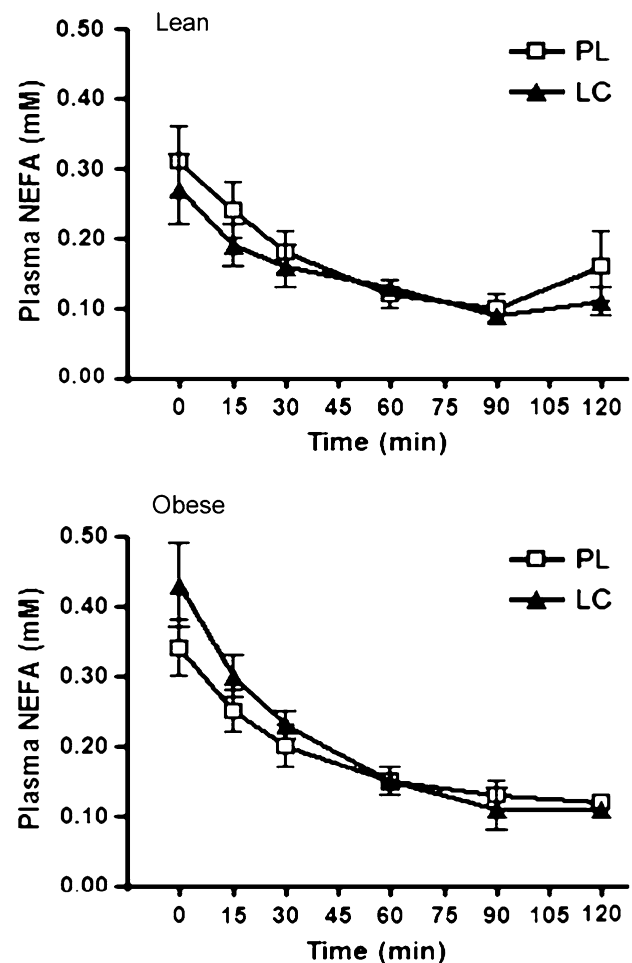

Fig. 3 Plasma NEFA response to OGTT on placebo (PL) and L-carnitine (LC) trials in lean (Lean, $n = 8$) and overweight/obese (Obese, $n = 8$) groups. Values are mean (SEM). No differences were observed between trials

in healthy adults, and these current data indicate a similar pattern of response to that observed following carnitine infusion, at least in the lean adult males studied. The mechanisms behind the potentially beneficial impact of carnitine on glucose disposal have not been fully explored, and the promising studies on glucose disposal in patients with type 2 diabetes in the early 1990s have not led to large-scale clinical studies. Mingrone (2004) published a review on carnitine in type 2 diabetes, in which he concluded that carnitine was effective in improving insulin-mediated glucose disposal. In addition, Rahbar et al. (2005) have observed lowering of fasting glucose following 12 weeks of carnitine supplementation in patients with type 2 diabetes. Power et al. (2007) have subsequently also confirmed these observations in a mouse model and suggest that carnitine could be used as an adjunctive therapy in diabetes. Furthermore, Poorabbas et al. (2007) have observed that plasma carnitine concentration is significantly lower in patients with complications associated with their type 2 diabetes (retinopathy, neuropathy and hyperlipidaemia). However, some confusion exists in literature because other studies have not observed these beneficial actions of carnitine in type 2 diabetes patients (Gonzalez-Ortiz et al. 2008). Therefore, the current data add to the literature by demonstrating that oral carnitine supplementation in healthy lean adults has similar effects to those observed following infusion of carnitine. The data support the notion that this effect is likely independent of changes in insulin or GLP-1 response to glucose feeding and thus points towards a direct effect of carnitine on skeletal muscle glucose uptake. Interestingly, this pattern of response is not observed in the overweight/obese adults studied, and the response in that group suggests that further study should be conducted to examine differences in

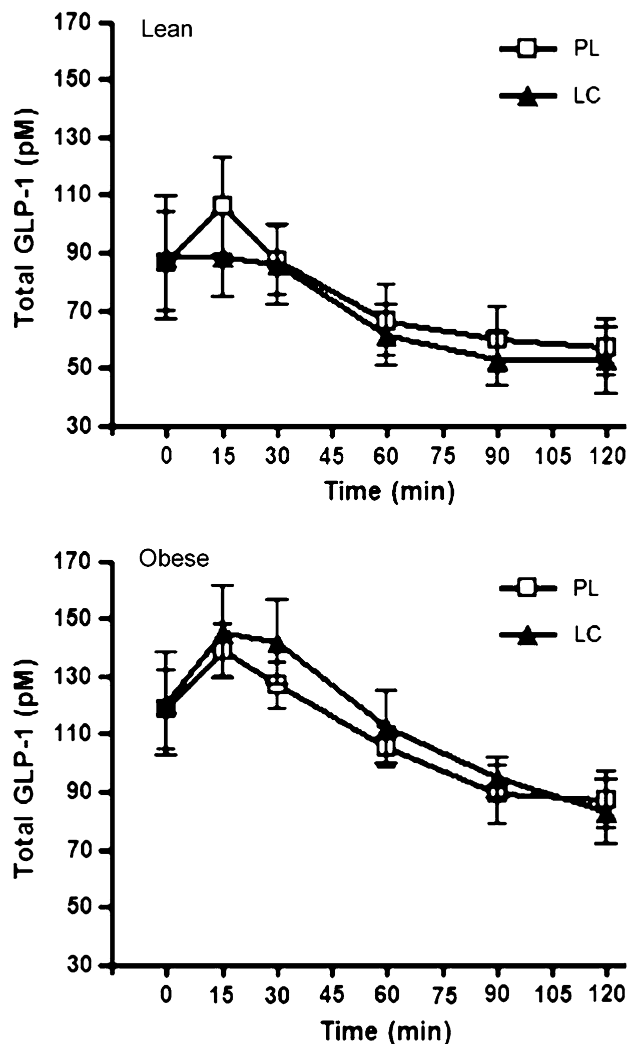

Fig. 4 Plasma total GLP-1 response to OGTT on placebo (PL) and L-carnitine (LC) trials in lean (Lean, $n = 8$) and overweight/obese (Obese, $n = 8$) groups. Values are mean (SEM). No differences were observed between trials

substrate handling/disposal between lean and overweight/obese adults with nutritional supplement interventions.

Responses to OGTT

The blunting of peak glucose concentration to a glucose challenge following LC supplementation despite similar insulin release for lean adult males in the present study indicates that LC could either have a direct 'insulin-like' or sensitising effect on tissues, or could play a metabolic role in buffering acyl groups in β-cell mitochondrial metabolism, which would act to maintain glucose oxidation and thus potentially enhance insulin release for a given glucose concentration (Newsholme et al. 2005). Indeed, the potential for LC to reduce peak plasma glucose through a direct 'insulin-like' action on tissues is supported by previous observations of 'insulin-like' actions on rat cardiac muscle (Rodgers et al. 2001) and the observed increased skeletal muscle glycogen storage following carnitine infusion in humans (Stephens et al. 2006a). This 'insulin-like' action of LC could explain the earlier and lower peak glucose concentration observed in the lean group during the OGTT in the present study. However, this proposed action is not consistent with the observed higher mean glucose concentration at the 90-min sample time on the LC trial in the overweight/obese group. Indeed, the overweight/obese group demonstrated a higher blood glucose concentration at 90 min on the LC trial due to a delayed peak glucose response to feeding.

Therefore, the pattern of response in plasma glucose suggests that supplemental oral carnitine can influence glucose disposal in response to carbohydrate feeding (at least in lean active subjects) and could also partly explain the elevated muscle glycogen data observed following carnitine infusion and hyperinsulinaemia (Stephens et al. 2006a). Furthermore, these enhanced glucose disposal and glycogen storage effects could explain the increased reliance on carbohydrate oxidation in endurance-trained athletes following a 2-week period of carnitine supplementation combined with a high-carbohydrate diet (Abramowicz and Galloway 2005). Further studies with larger sample sizes is required to examine lean versus overweight/obese group differences in response to LC supplementation and to determine whether these could be related to differences in habitual diet composition. Diet was controlled for each trial but not standardised between groups in the present study, and the food records were not detailed enough to warrant full dietary analysis.

The potential effects of LC supplementation on insulin secretion through maintenance of glucose or fatty acid oxidation in the β-cell by buffering of acyl groups or through generation of glutamate for maintenance of ATP/ADP ratio in the β-cell are also attractive hypotheses, as these mechanisms of action have been demonstrated for other amino acids that have been shown to enhance insulin secretion (Newsholme et al. 2005). We do not have any direct evidence from the present study to support an effect on pancreatic glucose or fatty acid metabolism, but carnitine is known to play a key role in buffering acyl group accumulation in skeletal muscle (Roepstorff et al. 2005) and could act in a similar manner in pancreatic cells to assist in maintenance of ATP/ADP ratio and thus provide optimal conditions for promoting insulin release. Furthermore, we have previously shown that LC supplementation over the same time period used in the present study resulted in an increased resting plasma glutamate concentration (Broad et al. 2008), which may support changes to β-cell function. However, without a significant increase in insulin secretion response during the OGTT following

LC supplementation, it seems likely that these acyl group buffering mechanisms or enhanced glutamate supply may not explain the observations we have made. The tendency towards an increase in estimated first and second phase β-cell function suggests a greater early release of insulin in relation to plasma glucose concentration in response to glucose feeding after LC supplementation; however, this could reflect either reduced rate of glucose entry into the circulation or increased rate of glucose removal from the circulation. These two actions could occur through the mechanisms already discussed (direct insulin-like actions) or alternatively through a change in gastric emptying rate or alteration in incretin hormone (GLP-1 or GIP) response to glucose feeding.

Gastric emptying rate has not previously been investigated in human LC supplementation studies, but data on rats do not support an acute carnitine-mediated reduction in gastric emptying rate (Matsuda et al. 1998). However, the response of both glucose and insulin in the overweight/obese group in the present study appears to reflect a change in glucose delivery to the circulation, which would likely be related to an altered gastric emptying or intestinal transport of glucose in the LC trial. Given the effect that prior protein meal ingestion can have on gastric emptying and glucose response to carbohydrate feeding (Ma et al. 2009), it would be interesting to speculate that chronic LC supplementation or acute ingestion of the last dose the evening before the OGTT might be acting to influence glycaemic response to feeding through delayed gastric emptying in overweight/obese participants. From the present data, this could potentially explain the delay in peak glucose concentration on the LC trial in the overweight/obese group, but is not consistent with the response observed in the lean group. Therefore, further work is required to investigate any effects of acute/chronic carnitine feeding on gastric emptying of glucose solutions in overweight/obese adults.

Alternatively, if LC had altered the GLP-1 response to feeding, this could also explain our observations. GLP-1 is one of the main incretin hormones responsible for altering the glucose and insulin response to feeding through an action of ingested nutrients on intestinal L-cell GLP-1 release (Deacon 2005; Holst 2007). GLP-1 can act on the pancreas to stimulate insulin secretion, but can also reduce glucagon secretion and delay gastric emptying, thus having a multifaceted action on glucose homeostasis (Baggio and Drucker 2007). However, the present data clearly demonstrate that LC supplementation does not impact upon total GLP-1 response to glucose feeding. The observed higher total GLP-1 concentration throughout the protocol in overweight/obese and the interaction between trial and group may add further support to the notion that there are differential effects of LC supplementation on responses to glucose feeding in lean versus overweight/obese groups.

However, it must be acknowledged that our total GLP-1 data should be interpreted with some caution as the resting plasma total GLP-1 concentration was high in both trials in both groups of participants. It may be that storage of our plasma samples has led to high fasting GLP-1, or that the baseline samples which were obtained 10 min after venous cannulation reflect a stress-induced response of GLP-1 to the cannulation procedure. Indeed, stress-induced sympathetic activation has been shown to result in enhanced early release of other gut peptides (Zhang et al. 1993) and may be responsible for the high fasted GLP-1 values observed in the present study. This is despite a familiarisation trial involving the cannulation procedure being conducted. We are unaware of any studies specifically examining stress hormone-induced elevation in total GLP-1 in humans, and studies in animal models are at best inconclusive (Kinzig et al. 2003); (Azlina et al. 2005). Storage studies do not suggest an influence on GLP-1 concentration, but do indicate that standardisation of evening meal on the night before trials should be performed (Chandarana et al. 2009). Given that participants in the present study replicated their diet and activity for 2 days prior to each trial, this should not have been an issue in the present work.

Conclusions

We can therefore conclude that supplementation with LC appears to influence glucose homeostasis in response to OGTT. The size and nature of the response is different between lean and overweight/obese adult males and appears to be related to body fat, BMI and VO_{2max} expressed relative to lean body mass. The pattern of response in glucose with LC supplementation in lean participants (earlier timing of peak glucose and lower 30 min glucose concentration) seems to support a mechanism of enhanced glucose disposal through a direct insulin-like action on skeletal muscle. The pattern of change in overweight/obese with LC (delayed timing of peak glucose and higher 90 min glucose concentration) appears to support delayed gastric emptying, but these observations need further evaluation. This differential response to an OGTT in lean and overweight/obese participants following oral LC supplementation could explain some of the conflicting reports on metabolic responses to carnitine supplementation evident in the literature.

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References

Abramowicz WN, Galloway SD (2005) Effects of acute versus chronic L-carnitine L-tartrate supplementation on metabolic responses to steady state exercise in males and females. Int J Sport Nutr Exerc Metab 15:386–400

Azlina MF, Nafeeza MI, Khalid BA (2005) A comparison between tocopherol and tocotrienol effects on gastric parameters in rats exposed to stress. Asia Pac J Clin Nutr 14:358–365

Baggio LL, Drucker DJ (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131–2157

Brass EP, Hiatt WR (1998) The role of carnitine and carnitine supplementation during exercise in man and in individuals with special needs. J Am Coll Nutr 17:207–215

Broad EM, Maughan RJ, Galloway SD (2005) Effects of four weeks L-carnitine L-tartrate ingestion on substrate utilization during prolonged exercise. Int J Sport Nutr Exerc Metab 15:665–679

Broad EM, Maughan RJ, Galloway SD (2008) Carbohydrate, protein, and fat metabolism during exercise after oral carnitine supplementation in humans. Int J Sport Nutr Exerc Metab 18:567–584

Chandarana K, Drew ME, Emmanuel J, Karra E, Gelegen C, Chan P, Cron NJ, Batterham RL (2009) Subject standardization, acclimatization, and sample processing affect gut hormone levels and appetite in humans. Gastroenterology 136:2115–2126

Deacon CF (2005) What do we know about the secretion and degradation of incretin hormones? Regul Pept 128:117–124

Decombaz J, Deriaz O, Acheson K, Gmuender B, Jequier E (1993) Effect of L-carnitine on submaximal exercise metabolism after depletion of muscle glycogen. Med Sci Sports Exerc 25:733–740

Galloway SDR, Broad EM (2004) Effects of L-Carnitine L-tartrate supplementation on substrate metabolism during exercise. Faseb Journal 18:A770–A770

Galloway SDR, Broad EM (2005) Oral L-carnitine supplementation and exercise metabolism. Monatshefte fur Chemie 136:1391–1410

Giancaterini A, De GA, Mingrone G, Gniuli D, Liverani E, Capristo E, Greco AV (2000) Acetyl-L-carnitine infusion increases glucose disposal in type 2 diabetic patients. Metabolism 49:704–708

Gonzalez-Ortiz M, Hernandez-Gonzalez SO, Hernandez-Salazar E, Martinez-Abundis E (2008) Effect of oral L-carnitine administration on insulin sensitivity and lipid profile in type 2 diabetes mellitus patients. Ann Nutr Metab 52:335–338

Hawley JA, Brouns F, Jeukendrup A (1998) Strategies to enhance fat utilisation during exercise. Sports Med 25:241–257

Holst JJ (2007) The physiology of glucagon-like peptide 1. Physiol Rev 87:1409–1439

Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846

Kinzig KP, D'Alessio DA, Herman JP, Sakai RR, Vahl TP, Figueiredo HF, Murphy EK, Seeley RJ (2003) CNS glucagon-like peptide-1 receptors mediate endocrine and anxiety responses to interoceptive and psychogenic stressors. J Neurosci 23:6163–6170

Ma J, Stevens JE, Cukier K, Maddox AF, Wishart JM, Jones KL, Clifton PM, Horowitz M, Rayner CK (2009) Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. Diabetes Care 32:1600–1602

Matsuda K, Yuasa H, Watanabe J (1998) Physiological mechanism-based analysis of dose-dependent gastrointestinal absorption of L-carnitine in rats. Biopharm Drug Dispos 19:465–472

Mingrone G (2004) Carnitine in type 2 diabetes. Ann NY Acad Sci 1033:99–107

Mingrone G, Greco AV, Capristo E, Benedetti G, Giancaterini A, De GA, Gasbarrini G (1999) L-carnitine improves glucose disposal in type 2 diabetic patients. J Am Coll Nutr 18:77–82

Newsholme P, Brennan L, Rubi B, Maechler P (2005) New insights into amino acid metabolism, beta-cell function and diabetes. Clin Sci (Lond) 108:185–194

Poorabbas A, Fallah F, Bagdadchi J, Mahdavi R, Aliasgarzadeh A, Asadi Y, Koushavar H, Vahed JM (2007) Determination of free L-carnitine levels in type II diabetic women with and without complications. Eur J Clin Nutr 61:892–895

Power RA, Hulver MW, Zhang JY, Dubois J, Marchand RM, Ilkayeva O, Muoio DM, Mynatt RL (2007) Carnitine revisited: potential use as adjunctive treatment in diabetes. Diabetologia 50:824–832

Rahbar AR, Shakerhosseini R, Saadat N, Taleban F, Pordal A, Gollestan B (2005) Effect of L-carnitine on plasma glycemic and lipidemic profile in patients with type II diabetes mellitus. Eur J Clin Nutr 59:592–596

Rodgers RL, Christe ME, Tremblay GC, Babson JR, Daniels T (2001) Insulin-like effects of a physiologic concentration of carnitine on cardiac metabolism. Mol Cell Biochem 226:97–105

Roepstorff C, Halberg N, Hillig T, Saha AK, Ruderman NB, Wojtaszewski JF, Richter EA, Kiens B (2005) Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. Am J Physiol Endocrinol Metab 288:E133–E142

Stephens FB, Constantin-Teodosiu D, Laithwaite D, Simpson EJ, Greenhaff PL (2006a) An acute increase in skeletal muscle carnitine content alters fuel metabolism in resting human skeletal muscle. J Clin Endocrinol Metab 91:5013–5018

Stephens FB, Constantin-Teodosiu D, Laithwaite D, Simpson EJ, Greenhaff PL (2006b) Insulin stimulates L-carnitine accumulation in human skeletal muscle. FASEB J 20:377–379

Stephens FB, Constantin-Teodosiu D, Greenhaff PL (2007a) New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. J Physiol 581:431–444

Stephens FB, Evans CE, Constantin-Teodosiu D, Greenhaff PL (2007b) Carbohydrate ingestion augments L-carnitine retention in humans. J Appl Physiol 102:1065–1070

Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van HT, Renn W, Gerich J (2000) Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 23:295–301

Vukovich MD, Costill DL, Fink WJ (1994) Carnitine supplementation: effect on muscle carnitine and glycogen content during exercise. Med Sci Sports Exerc 26:1122–1129

Wall BT, Stephens FB, Constantin-Teodosiu D, Marimuthu K, Macdonald IA, Greenhaff PL (2010) Increasing muscle carnitine content alters muscle fuel metabolism and improves exercise performance in humans. In: 15th Annual Congress of the European College of Sports Science Antalya, Turkey

Wutzke KD, Lorenz H (2004) The effect of L-carnitine on fat oxidation, protein turnover, and body composition in slightly overweight subjects. Metabolism 53:1002–1006

Zhang T, Uchida T, Gomez G, Lluis F, Thompson JC, Greeley GH Jr (1993) Neural regulation of peptide YY secretion. Regul Pept 48:321–328