A threshold exists for the stimulatory effect of insulin on plasma L-carnitine clearance in humans

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More than 95% of the body’s total carnitine (TC) (1-3-hydroxy-4-N,N,N-trimethylaminobutyric acid) store exists within skeletal muscle tissue as either free or acyl carnitine (6), where, as a substrate for carnitine palmitoyltransferase I (CPT I), it plays an essential role in the translocation of long-chain fatty acyl groups into the mitochondrial matrix for subsequent β-oxidation (14, 15). Another metabolic role of carnitine in skeletal muscle is to regulate the mitochondrial acetyl-CoA/CoASH ratio, in a reaction catalyzed by carnitine acetyltransferase to form acetylcarnitine, by buffering excess acetyl-CoA production when its rate of formation from pyruvate oxidation outweighs the demands of the TCA cycle, i.e., during high-intensity exercise (1, 9, 11, 18). However, this acetylation depletes the skeletal muscle free carnitine pool, and it has been hypothesized that the resulting reduction in the availability of free carnitine to CPT I might be limiting to fat oxidation in human skeletal muscle during exercise. For example, previous research (31) has demonstrated that a 30% decrease in the rate of fat oxidation during high-intensity exercise >70% V02max, measured using a [U-13C]palmitate tracer, was paralleled by a 65% decrease in skeletal muscle free carnitine content. In addition, decreased fat oxidation rates during moderate-intensity exercise have been reported when free carnitine availability was reduced by 46% as a result of a dietary-induced increase in glycolytic flux (25). In line with this hypothesis, it has been proposed that increasing skeletal muscle TC content via exogenous L-carnitine administration might alleviate the decline in fat oxidation rates routinely observed during these conditions of high glycolytic flux (28). Furthermore, it is possible that such an increase in muscle carnitine content could positively impact upon fat oxidation rates during exercise in metabolic diseases such as obesity and type 2 diabetes. Both conditions are associated with an impaired ability of skeletal muscle to oxidize fatty acids during exercise, occurring perhaps as a result of high glycolytic flux (2, 3, 20) and, consequently, reduced muscle free carnitine availability limiting long-chain fatty acyl group translocation.

The majority of the pertinent studies in humans to date, however, have failed to increase skeletal muscle carnitine content via oral or intravenous L-carnitine administration per se (7, 28, 32). For example, neither feeding 2 g/day L-carnitine for 3 mo (32) nor intravenously infusing L-carnitine for 5 h in the fasted state (28) had an effect on muscle TC content. The most likely reason for these findings is the fact that the Km for carnitine of the sarcolemmal carnitine transporter OCTN2 in vitro is 4.3 μmol/l (30), which would suggest that, in the basal state, where plasma TC concentration is ~50 μmol/l (28, 32), skeletal muscle carnitine uptake is saturated. Thus simply increasing plasma L-carnitine availability will not increase skeletal muscle carnitine accumulation, and therefore, investigation into alternative strategies to increase muscle carnitine content is warranted.

Recent research by ourselves (28, 29) has demonstrated that maintaining hyperinsulinemia (~160 μmol/l) in the presence of a supraphysiological plasma carnitine concentration of ~550 μmol/l for 5 h increased skeletal muscle TC content by ~15% in healthy human volunteers. 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 sarcloemmal Na\(^+\)-K\(^+\) ATPase pump activity and, therefore, intracellular Na\(^+\) flux. Furthermore, the increase in skeletal muscle carnitine content was associated with a large reduction in glycolytic flux and carbohydrate oxidation [decreased muscle pyruvate dehydrogenase complex (PDC) activity and lactate content and increased muscle glycerogen accumulation] despite conditions of identical carbohydrate administration, suggesting a carnitine-mediated increase in fat oxidation (29). However, the serum insulin concentration used to achieve this increase in muscle carnitine content, and its associated metabolic effects, was close to the upper physiological limit and would be difficult to achieve by dietary means alone. If L-carnitine is to be used as a tool to modify muscle pyruvate dehydrogenase complex activity and, therefore, intracellular Na\(^+\)\(^+\)-K\(^+\) ATPase pump activity, it would be pertinent to identify the relationship between serum insulin concentration and muscle carnitine accumulation in humans.

When L-carnitine is administered intravenously (15 mg/kg bolus followed by 10 mg·kg\(^{-1}\)·h\(^{-1}\)), an increase in skeletal muscle TC content occurs during hyperinsulinemia and is paralleled by a decrease in plasma TC concentration, demonstrating that a relationship exists between plasma carnitine clearance and muscle carnitine accumulation during hyperinsulinemia (28). With this in mind, the aim of the present study was to further examine the relationship between serum insulin concentration and muscle carnitine accumulation by attempting to identify the serum insulin concentration at which a decrease in plasma carnitine concentration becomes apparent during intravenous L-carnitine infusion.

METHODS

Subjects. Eight healthy, moderately-trained, nonvegetarian men (age 20.3 ± 0.4 yr, body mass 76.2 ± 2.7 kg, and body mass index 23.8 ± 0.9 kg/m\(^2\)) participated in the present study, which was approved by the University of Nottingham Medical School Ethics Committee, in accordance with the Declaration of Helsinki. Before taking part in the study, all subjects underwent routine medical screening and completed a general health questionnaire. All gave their consent to take part in the study and were aware that they were free to withdraw from the experiment at any point.

Study protocol. Subjects reported to the laboratory with a voided bladder following an overnight fast on four occasions, separated by at least a 2-wk “washout” period, having collected urine for the previous 24 h. On arrival, subjects were asked to rest in a supine position on a bed while a cannula was inserted retrogradely into a superficial vein on the dorsal surface of the nondominant hand. This hand was kept in a hand-warming unit (air temperature 55°C) to arterialize the venous outflow from the nondominant forearm for the infusion of insulin and glucose, and a third cannula was inserted into an antecubital vein in the opposite arm for infusion of L-carnitine.

On each experimental visit, a 6-h euglycemic insulin (human Actrapid; Novo Nordisk, Bagsvaerd, Denmark) clamp (12) was performed while a blood glucose concentration of 4.45 ± 0.01 mmol/l was maintained via infusion of a 20% glucose solution. The insulin clamp began at \(t = 0\) and varied between visits, being either 5, 30, 55, or 105 mU·m\(^{-2}\)·min\(^{-1}\) to obtain fasting, fed, or physiologically high serum insulin concentrations as previously achieved by Steenge et al. (27). Following a 1-h equilibration period, a 5-h intravenous infusion of 60 mM L-carnitine (Lonza, Basel, Switzerland) was begun in conjunction with the insulin clamp. First, a bolus dose of 15 mg/kg was administered over 10 min to reach a plasma concentration of ∼550 μmol/l. This was followed by a constant infusion at 10 mg·kg\(^{-1}\)·h\(^{-1}\) for the next 250 min to maintain a supraphysiological steady-state plasma carnitine concentration. At \(t = 6\) h both insulin and L-carnitine infusions were stopped, and subjects were free to leave the laboratory once their blood glucose concentration was stable. Urine was collected in 5-liter bottles, containing 5 ml of 10% thymol/isopropanol preservative (22), for 24 h before and after the commencement of the insulin infusions. Subjects were allowed to eat and drink at liberty over the 48 h prior to each experimental visit and during the urine collection periods but abstained from carnitine-containing foods, alcoholic beverages, and strenuous exercise.

Sample collection and analysis. During each experimental visit, 1 ml of arterialized-venous blood was obtained every 5 min for monitoring blood glucose concentration (YSI 2300 STATPlus; Yellow Springs Instruments, Yellow Springs, OH). In addition, 5 ml of arterialized-venous blood were obtained every hour (and at 80 min) for 6 h. Two milliliters of this blood were collected into lithium heparin containers, and, after centrifugation, the plasma was removed and immediately frozen in liquid nitrogen. These samples were then stored at −80°C and analyzed at a later date for TC concentrations using a radioenzymatic assay described by Cederblad et al. (8). 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 with a radioimmunoassay kit (Coat-a-Count Insulin; Euro-Diagnostic Products). 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 TC concentration using the radioenzymatic method of Cederblad et al. (8).

Statistical analysis. A two-way ANOVA (time and treatment effects, GraphPad Prism version 3; GraphPad Software) was performed to detect differences in plasma TC and serum insulin concentration. If significance was achieved, a repeated-measures one-way ANOVA with Tukey’s post hoc test (GraphPad Prism version 3) was used to locate differences between treatments at each time point. A repeated-measures one-way ANOVA (GraphPad Prism version 3) was also used to compare 24-h urinary TC excretion between treatments. Area under the serum insulin concentration × time curve was calculated using the least squares method (GraphPad Prism version 3). Statistical significance was declared at \(P < 0.05\), and all the values are means ± SE.

RESULTS

Serum insulin. Following the 60-min equilibration period, the four insulin infusion rates of 5, 30, 55, and 105 mU·m\(^{-2}\)·min\(^{-1}\) produced steady-state serum insulin concentrations of 10.1 ± 0.5, 48.8 ± 1.0, 88.9 ± 2.8, and 173.9 ± 6.5 mU/l, respectively (Fig. 1). This equated to area under the serum insulin concentration × time curve values of 3.6 ± 0.3, 16.7 ± 1.7 (\(P < 0.05\) vs. 5 mU·m\(^{-2}\)·min\(^{-1}\) clamp), 29.5 ± 2.3 (\(P < 0.05\) vs. 30 mU·m\(^{-2}\)·min\(^{-1}\) clamp), and 69.0 ± 6.2 (\(P < 0.001\) vs. 55 mU·m\(^{-2}\)·min\(^{-1}\) clamp) U·l\(^{-1}\)·min\(^{-1}\), respectively. The total volumes of 20% glucose solution infused to maintain these circulating insulin concentrations were 208 ± 54, 1,088 ± 80, 1,503 ± 125, and 1,419 ± 70 ml, respectively.

Plasma carnitine. The plasma TC concentration over 5 h of L-carnitine infusion during each of the four insulin clamps is illustrated in Fig. 2. From similar basal plasma TC concentrations of 50.4 ± 2.4, 46.0 ± 3.2, 44.4 ± 2.7, and 50.8 ± 1.7 μmol/l, the bolus 15 mg/kg L-carnitine infusion (indicated by the arrow) produced mean peak plasma TC concentrations (\(t = 80\)) of 541.3 ± 18.0, 550.8 ± 18.4, 548.2 ± 37.4, and 514.7 ± 18.9 μmol/l during insulin infusions at 5, 30, 55, and 105 mU·m\(^{-2}\)·min\(^{-1}\), respectively. Although plasma TC concentration remained elevated above 450 μmol/l throughout all four clamps, plasma TC concentration during the 55 and 105...
mU·m⁻²·min⁻¹ clamps appeared to diverge from the 5 and 30 mU·m⁻²·min⁻¹ clamps from 3 h onward (treatment effect, \( P < 0.001 \)), such that plasma TC concentration during the 105 mU·m⁻²·min⁻¹ clamp was lower than the 30 mU·m⁻²·min⁻¹ clamp at 5 (\( P < 0.05 \)) and 6 h (\( P < 0.01 \)) and lower than the 5 mU·m⁻²·min⁻¹ clamp at 6 h (\( P < 0.01 \)). Similarly, plasma TC concentration during the 55 mU·m⁻²·min⁻¹ clamp was lower than during the 5 (\( P < 0.05 \)) and 30 mU·m⁻²·min⁻¹ (\( P < 0.01 \)) clamps at 6 h.

**Urinary carnitine.** Urinary TC excretion [concentration (mg/l) × urinary volume (liters)] was similar for the 24 h before each visit (62.1 ± 13.8, 63.8 ± 9.8, 67.3 ± 16.9, and 57.4 ± 6.6 mg for the insulin infusions rates of 5, 30, 55, and 105 mU·m⁻²·min⁻¹, respectively) and for the 24 h following each l-carnitine infusion (3.45 ± 0.26, 3.31 ± 0.24, 3.57 ± 0.22, and 3.30 ± 0.24 g for the insulin infusions rates of 5, 30, 55, and 105 mU·m⁻²·min⁻¹, respectively).

**DISCUSSION**

A decrease in steady-state plasma TC concentration was observed when intravenous l-carnitine administration was accompanied by hyperinsulinemia (~160 mU/l) and was matched by a ~15% increase in muscle TC content (28). The aim of the present study was to further examine the relationship between serum insulin concentration and muscle carnitine accumulation by attempting to identify the serum insulin concentration at which a decrease in plasma TC concentration becomes apparent during intravenous l-carnitine infusion. In this respect, the main finding from the present study was that steady-state plasma TC concentration was significantly lower when l-carnitine was administered intravenously in the presence of high (~90 mU/l) and very high (~170 mU/l) serum insulin concentrations compared with when serum insulin concentration was maintained at ~10 mU/l (fasted) or ~50 mU/l during insulin clamp conditions. Collectively, these observations indicated that insulin will not stimulate muscle carnitine retention unless a serum insulin concentration ≥90 mU/l is achieved during hypercarnitinaemia. Our findings are in concordance with the hypothesis that insulin can augment Na⁺-dependent skeletal muscle carnitine transport in humans, secondary to its action of increasing sarcolemmal Na⁺-K⁺ ATPase pump activity (28, 29), but show that it will require a relatively high serum insulin concentration to achieve this effect.

Following the initial l-carnitine bolus, plasma TC concentration peaked at ~550 μmol/l, which is in agreement with previous studies (5, 13, 17, 26, 28) that have administered similar intravenous doses, and was maintained >450 μmol/l for the duration of each euglycemic insulin clamp (Fig. 2). The intravenous l-carnitine infusion rate during each clamp was ~80 μmol/min. If we assume that the plasma TC concentration during the 55 and 105 mU·m⁻²·min⁻¹ insulin clamps was at a steady state throughout the infusion period, then the rate of infusion was equal to the rate of elimination (i.e., 80 μmol/min). The total TC clearance from plasma over the infusion periods can therefore be calculated as the rate of elimination divided by the steady-state plasma concentration (~485 μmol/l during both visits), equating to 165 ml/min. The renal clearance during l-carnitine infusion reported in other studies (5, 17, 26, 28) is ~100 ml/min, equating to a 65 ml/min difference between plasma and renal TC clearance. Since the rate of tissue uptake equals the plasma concentration × plasma clearance, it can be calculated that plasma TC was transported into skeletal muscle at a rate of ~32 μmol/min during the 55 and 105 mU·m⁻²·min⁻¹ insulin clamp visits, which over the 5-h l-carnitine infusion period equates to ~2 g (although this does not take into account the initial 15 mg/kg bolus). Therefore, if we assume that all of the difference between plasma TC clearance and renal TC clearance is due to an insulin-mediated transport of carnitine into skeletal muscle, then we can speculate from the above calculations that skeletal muscle TC content would have been increased by ~10% [assuming a

**Fig. 1.** Serum insulin concentration during 6-h intravenous insulin infusion rates of 5 (●), 30 (■), 55 (△), and 105 (●) mU·m⁻²·min⁻¹ combined with a 5-h intravenous l-carnitine infusion. Arrow indicates the commencement of the l-carnitine infusion. Values are means ± SE (n = 8).

**Fig. 2.** Plasma total carnitine (TC) concentration during 6-h intravenous insulin infusion rates of 5, 30, 55, and 105 mU·m⁻²·min⁻¹ combined with a 5-h intravenous l-carnitine infusion. *P < 0.05, plasma TC concentration during the 105 mU·m⁻²·min⁻¹ insulin infusion significantly lower than during the 30 mU·m⁻²·min⁻¹ insulin infusions at 5 h, and plasma TC concentration during the 55 mU·m⁻²·min⁻¹ insulin infusion significantly lower than during the 5 and 30 mU·m⁻²·min⁻¹ insulin infusions at 6 h. **P < 0.01, plasma TC concentration during the 105 mU·m⁻²·min⁻¹ insulin infusion significantly lower than during the 5 and 30 mU·m⁻²·min⁻¹ insulin infusions at 6 h, and plasma TC concentration during the 55 mU·m⁻²·min⁻¹ insulin infusion significantly lower than during the 30 mU·m⁻²·min⁻¹ insulin infusion at 6 h. The arrow indicates the commencement of the l-carnitine infusion. Values are means ± SE (n = 8).
whole body skeletal muscle carnitine content of 20 g (6, 23), which is in agreement with the increase in muscle carnitine content we have observed in two studies following 5 h of hypercarnitinemia in the presence of a 105 μU·m⁻²·min⁻¹ clamp (28, 29). Furthermore, the present findings suggest that a steady-state serum insulin concentration of ~90 mU/l (achieved during the 55 μU·m⁻²·min⁻¹ clamp) is sufficient to stimulate muscle carnitine transport to a similar degree as that at higher insulin concentrations (28, 29), which could be important, because a circulating insulin concentration of around this magnitude is more practical to achieve in the everyday setting.

Carnitine is a substrate for CPT I and, therefore, plays an essential role in the translocation of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation (14, 15). Another metabolic role of carnitine in skeletal muscle is to regulate the mitochondrial acetyl-CoA/CoASH ratio. In a reaction catalyzed by carnitine acetyltransferase to form acetyl-carnitine, carnitine buffers excess acetyl-CoA production when its rate of condensation with oxaloacetate is less than its rate of formation by pyruvate, i.e., during high-intensity exercise (1, 9, 11, 18). However, this acetylation depletes the free carnitine pool, and it has been hypothesized that the resulting reduction in the availability of free carnitine to CPT I might be limiting to long-chain fatty acid oxidation in human skeletal muscle during exercise. Indeed, research has demonstrated that, as exercise intensity increases to >70% VO₂max, muscle free carnitine content falls to ~30% (5.6 mmol/kg dm) of the TC store and is paralleled by a 35% decrease in the rate of fat oxidation (measured using a [U-¹³C]palmitate tracer (31)). On the basis of this evidence, it was hypothesized that muscle free carnitine availability becomes limiting to fat oxidation at a concentration of ~5 mmol/kg dm (or ~1.52 mM intracellular water). This seems plausible given that the reported Kₘ of CPT I for free carnitine in human skeletal muscle is 0.48 mM (21). Furthermore, the catalytic site of CPT I for carnitine is located within the contact sites of the outer and inner mitochondrial membranes (33), which will limit its exposure to the predominantly cytosolic store of free carnitine. Consequently, the 10% increase in muscle carnitine content predicted in the present study during a steady-state serum insulin concentration of ≥90 mU/l might alleviate the decline in fat oxidation observed during exercise with high glycolytic flux (25) or at high intensities (31), 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 content has been reported to delay fatigue development by 25% in rat soleus muscle strips in vitro (4). Furthermore, our most recent research (29) has demonstrated that a 15% increase in skeletal muscle carnitine content, achieved during conditions identical to the 105 μU·m⁻²·min⁻¹ insulin clamp of the present study, resulted in an inhibition of muscle PDC activity, a decrease in muscle lactate content, and, following an overnight fast, an increase in muscle glycogen and long-chain acyl-CoA content compared with control (saline infusion during euglycaemic hyperinsulinemia) despite identical carbohydrate administration during both visits. Due to carnitine’s role in long-chain fatty acyl group translocation into the mitochondrial matrix, we believe it is entirely plausible that the apparent reduction in glycolytic flux and carbohydrate oxidation (decreased PDC activity and lactate content and increased glycogen accumulation) could have been caused by a carnitine-mediated increase in skeletal muscle long-chain fatty acid oxidation. It is not unreasonable to predict, therefore, that increasing muscle TC content would indeed alleviate the decline in skeletal muscle fat oxidation seen during incremental exercise in healthy individuals. In addition, any mechanism that increases fat oxidation is also relevant to metabolic diseases such as obesity and type 2 diabetes, where an impairment of fat oxidation has been reported during exercise, occurring perhaps as a result of high glycolytic flux (2, 3, 10, 19, 20).

There were no differences in 24-h urinary carnitine excretion across the visits, suggesting that the reduction in plasma TC concentration during the 55 and 105 μU·m⁻²·min⁻¹ insulin clamps was not due to increased urinary carnitine clearance. In healthy humans, >90% of free carnitine filtered at the kidney is reabsorbed when plasma free carnitine concentration is in its normal range (23, 24). However, when plasma carnitine concentration exceeds this range (>70 μmol/l), the excess carnitine is rapidly eliminated and the renal clearance of carnitine is similar to the values obtained for creatinine clearance (i.e., approximating the glomerular filtration rate), suggesting that carnitine is readily filtered through the glomeruli and that the high capacity for tubular reabsorption proposed for free carnitine is saturated. For example, following intravenous administration of l-carnitine, 42% of a 2.3-g dose is retrieved in the urine after 2 h, and 70 and 82% of a 2- and 6-g dose, respectively, is retrieved after 24 h (5, 17, 26), which is in agreement with the 70% mean recovery in this study following an ~5-g dose. A urine collection was not obtained for the 5 h of each l-carnitine infusion during the present study, so it is difficult to calculate renal carnitine clearance during this time. However, because plasma TC concentration was maintained considerably higher (>450 μmol/l) than the normal range for the duration of each insulin clamp, we can predict that plasma carnitine filtration was at a maximum during all clamps and that, assuming carnitine reabsorption was saturated, we would not expect to see any differences between clamps in the 5-h urinary TC recovery. Indeed, in our previous study there was no difference in urinary TC clearance, compared with control, when l-carnitine was infused for 5 h in the presence of hyperinsulinemic clamp (28).

During each insulin clamp, a 20% glucose solution was infused to maintain a fasting blood glucose concentration of 4.5 mmol/l. Therefore, because 1,503 ± 125 and 1,419 ± 70 ml of glucose was infused during the 55 and 105 μU·m⁻²·min⁻¹ insulin clamps, respectively, with 208 ± 54 ml infused during the 5 μU·m⁻²·min⁻¹ insulin clamp, it could be suggested that the lower plasma TC concentration was due to a dilution effect. However, this would seem unlikely since, for example, there was a fivefold difference in the volume of glucose infused between the 5 and 30 μU·m⁻²·min⁻¹ insulin clamps (208 ± 54 vs. 1,088 ± 80 ml, respectively), with no difference in plasma TC concentration (Fig. 2).

In conclusion, the present study demonstrates that insulin can stimulate plasma carnitine clearance, most likely into skeletal muscle, based on the findings of our previous studies (28, 29), but only when present at a physiologically high concentration. This could be of significant relevance to athletes, and in the treatment of obesity and type 2 diabetes, if
l-carnitine is to be used as a tool to modify skeletal muscle energy metabolism.

GRANTS

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