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# Oral *L*-Carnitine Supplementation and Exercise Metabolism

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**Summary.** Oral *L*-carnitine supplementation is frequently reported to have beneficial effects on exercise capacity in clinical populations and has been considered as a potential ergogenic aid for endurance athletes. However, this latter view is largely unsubstantiated possibly due to many experimental studies being poorly controlled or difficult to compare. The potential for oral *L*-carnitine supplementation to influence skeletal muscle carnitine content has been questioned and there are several key factors identified that may explain variations between study outcomes. Recent more well controlled research suggests some potential for *L*-carnitine to act as a key regulator of cellular stress, possibly through an impact on the integration of carbohydrate and lipid metabolism, and this work should be followed up in future by well controlled studies in both athlete and clinical subject groups.

**Keywords.** Carbohydrates; Lipids; Calorimetry; *L*-Carnitine; Substrate metabolism.

## Introduction

Carnitine was first discovered in muscle extracts in 1905 and was later observed to be an essential growth factor in meal worms [1]. Since then the role of carnitine as a nutrient has been examined extensively including its role in infant nutrition [2], athletes [3–5], clinical populations [6, 7], and older adults [2].

Carnitine homeostasis is maintained through a combination of dietary intake as well as endogenous synthesis combined with renal excretion [8]. Carnitine intake in the diet is highly variable and depends largely upon red meat intake. Red meats contain a high carnitine content (venison, beef, and lamb contain approximately 140–190 mg of carnitine per 100 g uncooked weight [9]); but cooking has been shown to significantly reduce the carnitine content of meats [9]. Dietary intake has been estimated to be between 20 and 300 mg per day for healthy non-vegetarian adults [8, 10, 11] and will be in the region of 1–3 mg per day for strict vegetarians [12].

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The endogenous synthesis of carnitine, estimated to be around 10–20 mg per day [2], occurs predominantly in liver from lysine and methionine, in the presence of vitamin $B_6$, vitamin C, niacin, and reduced iron [8]. It has been estimated that the human body pool of carnitine is around 20 g with 98% of this being within cardiac and skeletal muscle pools, 1.4% in liver and kidney, and 0.6% in extracellular fluid (ECF) [13]. Since tissues such as skeletal and cardiac muscle cannot synthesise carnitine, its transport into muscle is of critical importance [8]. The carnitine concentration in skeletal and cardiac muscle is up to 70-fold higher than in plasma [14] and the steady state rate of kinetics of entry of carnitine into human muscle and heart has been determined to be $11.6\,\text{nmol} \cdot \text{h}^{-1} \cdot \text{g tissue}^{-1}$ [14]. More recently, this sodium- and energy-requiring, saturable transporter has been identified as novel organic cation transporter 2 (OCTN2) [15], which is more active in Type I than Type II muscle fibres [16], and has a higher carnitine affinity than the transporter in the liver [1]. However, there also appears to be two $K_m$ values for carnitine uptake, one at plasma levels of 60 $\mu M$ and one at 0.6 m$M$ [17], so whether there are multiple transporters is as yet unknown. Finally, the retention of carnitine in muscle may be promoted by insulin [18].

Within skeletal muscle it has been estimated that ~95% of the carnitine pool is cytosolic. Due to the large pool in skeletal and cardiac muscle there is a slow turnover of carnitine in these pools in comparison to the extracellular fluid pool. Whole body turnover has been estimated to be 65 days with a range between 38 days and 119 days [19]. The combination of a large skeletal muscle pool, slow turnover time, and large concentration gradient between plasma and muscle has led many authors to believe that skeletal muscle carnitine pools cannot be modified by *L*-carnitine supplementation; this will be examined in more detail later in this review.

Absorption of oral doses has previously been considered poor, with bioavailability from a single oral dose of 2 g being estimated at 9–25% and from a single 6 g oral dose being 4–10% [20, 10]. Several factors that may influence oral bioavailability, such as nutritional state (fed *vs*. fasted) or repeated smaller doses throughout a 24 hour period, have not been thoroughly investigated but smaller doses may be more readily absorbed. However, it is clear that oral dosing with large doses results in rises in plasma total carnitine concentration (50–100%) but is unlikely to raise the concentration above the normal range (30–90 $\mu M$ [17]).

## Carnitine Deficiencies and Insufficiency

Primary carnitine deficiency generally occurs due to inborn errors in metabolism such that endogenous carnitine synthesis may be absent or impaired, or due to a genetic defect that results in absence of carnitine transporters [21–23]. Secondary deficiency has been reported due to iatrogenic effects or increased requirements in particular clinical circumstances [21, 22, 24]. There is little evidence to support secondary deficiency due to low dietary intake [25, 26]. In a clinical setting plasma carnitine status is often used in the diagnosis of carnitine deficiency (total plasma carnitine $<20\,\mu M$ [27]).

Carnitine insufficiency, on the other hand, is reported to occur when plasma total carnitine is within the normal range but the plasma acyl carnitine to free carnitine ratio is $>0.40$ (normal value 0.25 [28]). It is interesting to note that

females typically have a higher acyl:free carnitine ratio than males, whereas vegetarians do not seem to suffer from carnitine insufficiency despite a low dietary intake of carnitine and lower plasma total carnitine concentration [29, 2]. To date there is little evidence to suggest that carnitine insufficiency occurs in athletes during training and therefore the rationale for oral supplementation revolves around potential beneficial effects of transiently elevating the plasma carnitine concentration and/or possibly increasing the skeletal muscle carnitine pools above normal following prolonged supplementation.

## Proposed Metabolic Mechanisms of Action

Research between 1955 and 1962 provided evidence of a role of carnitine in the oxidation of long-chain fatty acids in heart and liver preparations [30]. Carnitine plays an obligatory role in the active transport mechanism for long-chain and medium-chain activated fatty acids from the cytosol across the inner mitochondrial membrane [31]. It has been shown *in vitro* that there is a relationship between muscle carnitine content and capacity for fatty acid oxidation within a tissue [32] and that the carnitine concentration seems to be set at a level sufficient for optimal rates of fatty acid oxidation [33].

This role of carnitine in fatty acid transport, and the fact that reliance on fat oxidation can be increased when the availability of fatty acids (FA) is increased [34], has formed the basis of the hypothesis that increasing the carnitine content of skeletal muscle may support enhanced fatty acid oxidation during exercise. An increased reliance on fatty acid oxidation during prolonged exercise may be of benefit to endurance athletes by sparing the potentially limited supply of muscle glycogen and thus increase exercise capacity [35]. In resting healthy subjects, carnitine palmitoyltransferase I (CPT I) is considered to be the rate-limiting enzyme in the transport of FA across the mitochondrial membrane [34]. The activity of CPT I is not limited by the availability of *L*-carnitine under normal circumstances [32]), and plasma carnitine is not rate-limiting for lipid oxidation under resting conditions [36]. Whilst CPT I is inhibited by malonyl-CoA at rest [37], sensitivity of CPT I to malonyl-CoA varies markedly between tissues, and appears to be very low in human muscle [34], especially during exercise [37]. Therefore, unless it can be shown that there is a way of increasing the activity of CPT I, it remains difficult to foresee that the transport and utilisation of FA for fuel in normal humans, either at rest or during exercise, could be manipulated by supplementing with *L*-carnitine.

*Friedman* and *Fraenkel* [38] first confirmed that carnitine could be acetylated and thereby act as an important link with coenzyme A (CoA) in the mitochondrion. *Bieber et al.* [39] suggested that carnitine had an important role in buffering various acyl-CoAs thereby maintaining an optimal CoASH/acyl-CoA ratio. One example is the role carnitine plays in buffering excess acetyl group accumulation within the muscle [40], thereby potentially sustaining glycolytic flux into the tricarboxylic acid (TCA) cycle. If additional free carnitine were available within the mitochondrion it could be considered that this mechanism of action may reduce lactic acid generation during exercise bouts due to more complete oxidation of glucose. Furthermore, excess lactic acid production itself may be an inhibitor of FA

transport into the muscle [34]. At rest, *L*-carnitine infusion prior to a euglycemic insulin clamp has been shown to increase non-oxidative glucose disposal in resting humans, with decreased pyruvate concentration and increased short-chain acyl-carnitine [41]. During exercise, if the rate of acetyl-CoA formation exceeds the rate at which the TCA cycle can recycle reduced CoA, an accumulation of acetyl-CoA will occur which will theoretically inhibit the pyruvate dehydrogenase enzyme complex (PDC). It has been shown that *L*-carnitine maintains a lower acetyl-CoA/free CoA ratio at rest in isolated heart and liver mitochondria [42]. In isolated human muscle mitochondria, there is evidence that pyruvate oxidation nearly doubles in the presence of *L*-carnitine due to direct activation of PDC [43, 44], that acetylcarnitine concentration increases as acetyl-CoA accumulation occurs [45, 46], and that the acetyl-CoA/free CoA ratio correlates with PDC activity at rest [45, 46]. However, doubts have been raised as to whether the acetyl-CoA/free CoA ratio actually regulates PDC activity during exercise, especially in the face of adequate CHO availability [45], as an increase in the acetyl-CoA/free CoA ratio during exercise does not appear to prevent maximal PDC activity, especially during moderate to high intensity exercise (>50% $VO_{2max}$) [40, 47, 46]. Instead, the greater importance may be in maintaining a viable pool of free CoA for the TCA cycle, such as the oxidation of oxoglutarate to succinate [48].

These functions of carnitine have stimulated strong interest in the dual roles carnitine may play in energy supply and reduction of cellular stress during exercise. Some believe that by acylating free carnitine within the muscle, a relative carnitine insufficiency within the muscle may result by reducing the concentration of free carnitine [49, 37]. *Jeukendrup* [34] states that there is no direct evidence that this mechanism is important for regulating fatty acid uptake, but that if it was it would most likely affect high intensity exercise. Others see the buffering role of carnitine to be a benefit as the mitochondria will not be flooded with an excess of acyl-CoA [48, 37], and acetyl units stored as acetylcarnitine during periods of heavy exercise will subsequently be available for utilization during rest periods, or if the exercise intensity decreases [47]. For example, it has also been shown that aerobic carbohydrate oxidation can be increased by elevating acetylcarnitine concentration prior to exercise [50], probably because this reflects an increased acetyl-CoA availability [51]. Furthermore, in moderately active humans after exercise at 55% $VO_{2max}$ the free carnitine content of skeletal muscle decreased by 44% and the acylcarnitine to free carnitine ratio increased from 0.23 at rest to 1.00, indicating a potential free carnitine insufficiency in skeletal muscle even after exercise at moderate intensity [52]. Whether these changes in acylcarnitine to free carnitine ratio within skeletal muscle during exercise are of importance in the regulation and integration of fuel utilization is not yet known. However, there is increasing evidence that these changes may play a key role and could also impact upon branched chain amino acid oxidation as well as carbohydrate and fat metabolism.

More recent research has provided evidence of links between *L*-carnitine and antioxidant status. In rats, *L*-carnitine supplementation has been shown to improve antioxidant status in the brain [53]. Whether the potential antioxidant role of *L*-carnitine supplementation is mediated through reduced lipid peroxidation, reduced ammonia toxicity [2], blood flow changes, increased Vitamin C availability (*i.e.*

less requirement for carnitine biosynthesis [53]), or other mechanisms such as a reduction in cellular stress through better matching of substrate breakdown with metabolic demand, has yet to be established.

Given the wide range of proposed mechanisms of action of *L*-carnitine in relation to exercise metabolism, the study of supplemental *L*-carnitine has become a popular research topic. Early exercise studies investigated the potential for *L*-carnitine to influence maximal aerobic capacity and submaximal work efficiency in healthy subjects [54] as well as potential effects on exercise tolerance in clinical populations such as patients with angina [55] and some positive outcomes were observed.

## Clinically Relevant Studies

A review on oral *L*-carnitine supplementation and exercise metabolism would not be complete without a brief mention of the clinically relevant studies that have often produced positive findings. Clinical research has focused largely, but not exclusively, on the role of *L*-carnitine in treatment or management of ischaemic heart disease, peripheral vascular disease, and end stage renal disease (for review see [3, 7]).

In the ischaemic myocardium, long chain acylcarnitine is produced in excess of physiological levels and is readily bound to the cell membrane, changing membrane integrity. Such changes are responsible, in part, for the development of arrhythmias, decrease in myocardial contractility, and eventual cell death [56]. Several studies have shown that *L*-carnitine (in particular, propionyl-*L*-carnitine) has a protective effect on ischaemic myocardium, by reducing the long-chain acyl-CoA accumulation and increasing mitochondrial ATP and creatine phosphate concentration [57, 58]. *Lopaschuk* [59] and *Broderick et al.* [60] have provided strong evidence for a role of *L*-carnitine in reducing ischaemia/reperfusion injury and suggest that this may be due to a change in substrate use by cardiac muscle in the presence of *L*-carnitine *in vitro*. Control hearts perfused with glucose and palmitate exhibit lower glucose oxidation and higher palmitate oxidation than hearts perfused with the same substrates but in the presence of *L*-carnitine. These authors have explained the increased reliance on glucose oxidation as a carnitine mediated increase in glycolytic flux due to buffering of excess acetyl groups within the cardiac muscle mitochondria.

Alterations in carnitine homeostasis have been reported in patients with peripheral vascular disease [61] and *L*-carnitine supplementation in these patients has been observed to improve walking capacity. *Brevetti et al.* [61] found that propionyl-*L*-carnitine was more effective in improving total walking distance than a similar dose of *L*-carnitine (500 mg), both given by single intravenous infusion. The benefits of *L*-carnitine on walking capacity were assumed to be due to changes in muscle energy metabolism rather than blood flow as no change in haemodynamic variables was noted with supplementation. *Bolognesi et al.* [62] also observed no effect of oral propionyl-*L*-carnitine supplementation on blood flow, as assessed by $^{133}$Xe washout, in patients with peripheral vascular disease. Contrary to these previous observations *Cevese et al.* [63] observed increases in blood flow (130%) in a canine hindlimb preparation during a 2 minute infusion of

propionyl-*L*-carnitine. Whether *L*-carnitine can alter blood flow *in vivo* under physiological conditions has yet to be determined.

During haemodialysis, there is a decline in serum carnitine concentrations, predominantly due to a loss of carnitine in the dialysate fluid [8]. The serum carnitine concentration normally recovers between dialysis sessions but, in some patients, prolonged use of dialysis produces a subsequent chronic carnitine insufficiency and reduction in muscle carnitine [64] with associated decline in functional ability, muscle weakness, and reduced quality of life. The plasma acylcarnitine to free carnitine ratio is elevated in dialysis patients [65] and supplementation with *L*-carnitine has been observed to improve exercise capacity and quality of life in these patients [66].

Therefore, in some clinical situations, *L*-carnitine is observed to improve exercise capacity potentially through a metabolic mechanism of action, although

**Table 1.** Summary of studies examining the effect of acute or chronic *L*-carnitine supplementation on respiratory exchange ratio (RER) during exercise

| Authors | Dose | Time | Training status | Exercise | Change in RER |
|---|---|---|---|---|---|
| *Natali et al.* (1993) | 3 g acute intravenous dose | 40 min before exercise | Active | 40 min @ 60 W and 2 min @ 250 W | ↓ 0.02 |
| *Swart et al.* (1997) | 3 g/day | 42 days | Trained | Maximal exercise | ↓ 0.06 |
| *Gorostiaga et al.* (1989) | 2 g/day | 28 days | Trained | 60 min @ 65% $VO_{2max}$ | ↓ 0.02 |
| *Wyss et al.* (1990) | 2 g/day | 7 days | Active | Maximal exercise | ↓ normoxia 0.02 ↓ hypoxia 0.06 |
| *Colombani et al.* (1996) | 2 g/day | 2 h before and during exercise | Trained | Marathon | No change |
| *Marconi et al.* (1985) | 4 g/day | 14 days | Trained | 120 min @ 65% $VO_{2max}$ | No change |
| *Soop et al.* (1988) | 5 g/day | 6 days | Active | 120 min @ 50% $VO_{2max}$ | No change |
| *Brass et al.* (1994) | 185 $\mu$mol/kg acute intravenous dose | prior to exercise | Untrained | Low intensity and high intensity | No change |
| *Oyono-Enguelle et al.* (1988) | 2 g/day | 28 days | Untrained | 60 min @ 50% $VO_{2max}$ | No change |
| *Greig et al.* (1987) | 2 g/day | 14 and 28 days | Untrained | Maximal and sub maximal | No change |
| *Vukovich et al.* (1994) | 6 g/day | 7 and 14 days | Untrained | 60 min @ 70% $VO_{2max}$ | No change |
| *Decombaz et al.* (1993) | 3 g/day | 7 days | Untrained | 20 min @ 43% and 20 min @ 57% $VO_{2max}$ | No change |

alternative mechanisms of action such as effects on blood flow cannot be ruled out at this stage.

## *L*-Carnitine and Exercise Metabolism

Carnitine uptake into skeletal muscle following external administration was first shown by *Yue* and *Fritz* [67]. They observed that about 50% of injected labeled *L*-carnitine was detected within skeletal muscle of dogs 7 hours after injection. In horses, an increase in muscle carnitine concentration (46%) following 5 weeks of oral *L*-carnitine supplementation has been observed [68]. However, for humans, the evidence is more limited and is the source of some controversy. *Barnett et al.* [69] observed no increase in muscle total carnitine concentration at rest following 14 days supplementation with $4\,g$ *L*-carnitine $\cdot\, d^{-1}$ ($7.3\,\mu mol \cdot g$ $WW^{-1}$ *versus* $7.4\,\mu mol \cdot g$ $WW^{-1}$, for control and *L*-carnitine supplementation, respectively). *Wachter et al.* [70] reported an increase in muscle total carnitine from $4.10 \pm 0.82$ to $4.79 \pm 1.19\,\mu mol \cdot g^{-1}$ in 7 healthy males supplemented with 2 daily doses of 2 g *L*-carnitine for 3 months, but despite the 17% increase, the difference was not statistically significant. The increase in total carnitine was through a large increase in short chain acylcarnitine (72% increase) and a small increase in free carnitine (6%).

**Table 2.** Summary of studies examining the effect of acute and chronic oral administration of *L*-carnitine on blood lactate response to exercise

| Authors | Dose | Time | Training status | Exercise | Lactate response |
|---|---|---|---|---|---|
| *Siliprandi et al.* (1990) | 2 g orally | 90 min before | Trained | Maximal test | ↓ |
| *Vecchiet et al.* (1990) | 2 g orally | 60 min before | Trained | Maximal test | ↓ |
| *Dragan et al.* (1987) | 3 g/day | 21 days | Trained | Evoked muscle potential | ↓ |
| *Marconi et al.* (1985) | 1 g/6 h | 14 days | Trained | 65% $VO_{2max}$ | No change |
| *Trappe et al.* (1994) | 4 g/day | 7 days | Trained | Anaerobic exercise | No change |
| *Ransone et al.* (1994) | 1 g/day | 14 days | Trained | Anaerobic exercise | No change |
| *Kasper et al.* (1994) | 4 g/day | 14 days | Trained | Run | No change |
| *Zapf et al.* (1994) | 4 g/day | 28 days | Trained | $VO_{2max}$ and endurance exercise | No change |
| *Gorostiaga et al.* (1989) | 2 g/day | 28 days | Trained | 60 min @ 65% $VO_{2max}$ | No change |
| *Maasen et al.* (1994) | 2 g/day | 35 days | Trained | $VO_{2max}$ | No change |
| *Wyss et al.* (1990) | 3 g/day | 7 days | Untrained | Maximal exercise | No change |
| *Fink et al.* (1994) | 4 g/day | 14 days | Untrained | Anaerobic exercise | No change |
| *Oyono-Enguelle et al.* (1998) | 2 g/day | 28 days | Untrained | 50% $VO_{2max}$ | No change |
| *Barnett et al.* (1994) | 6 g/day | 14 days | Untrained | High Intensity | No change |
| *Greig et al.* (1987) | 2 g/day | 14 and 28 days | Untrained | Max and submax | No change |

**Table 3.** Summary of studies examining the effect of oral *L*-carnitine suppl ementation on muscle power output and fatigue resistance

| Authors | Dose | Time | Training status | Exercise | Change in muscle power output/fatigue resistance |
|---|---|---|---|---|---|
| *Dragan et al.* (1987) | 4 g | 90 min before | Trained | | ↑ muscular potential |
| *Siliprandi et al.* (1990) | 2 g | 90 min before | Active | Maximal exercise | ↑ total work done |
| *Vecchiet et al.* (1990) | 2 g | 90 min before | Active | Maximal exercise | ↑ total work done |
| *Dragan et al.* (1987) | 3 g/day | 21 days | Trained | Evoked muscular potential | ↑ muscular potential |
| *Maasen et al.* (1994) | 2 g/day | 35 days | Trained | Maximal exercise | No change in power output |
| *Wyss et al.* (1990) | 3 g/day | 21 days | Untrained | Maximal exercise | No change in maximum work done |

Despite the lack of consensus on whether oral *L*-carnitine supplementation can increase the skeletal muscle carnitine pool many studies have been conducted in an attempt to elucidate the potential effects of *L*-carnitine supplementation on exercise metabolism and exercise performance. Several reviews have been published including *Brass* [71] which highlights the lack of evidence for a benefit on exercise performance, although suggesting that there are potential benefits from supplementation in trained individuals, and *Karlic* and *Lohninger* [72] which promotes the positive effects of *L*-carnitine supplementation for athletes.

From closely examining the human exercise studies it is immediately evident that clear, consistent outcomes following oral *L*-carnitine supplementation are not observed. Many studies are supportive of an effect of oral supplementation with reports of: increased maximal aerobic capacity [73, 74]; increases in strength and fatigue resistance and reductions in circulating blood lactate concentration [74–77]; and reduction in respiratory exchange ratio during exercise, reflective of an increased reliance on fat as a fuel source [78, 79, 49]. However, many studies do not observe these positive outcomes, with no change in maximal aerobic capacity [54, 80], strength or fatigue resistance, or blood lactate response to exercise [78, 81, 82] and many unsupportive of an effect on respiratory exchange ratio [83–86]. For an overview of some data from these studies see Tables 1–3.

## Confounding Variables in Human *L*-Carnitine Studies

Upon closer examination of these studies many factors can be identified that could explain the differences in outcomes of the studies reported here. The main factors that were different between studies were: differences in exercise intensity and duration; lack of dietary intake control, or reporting of dietary intake; differences in training status of subjects; different type of supplement, dose, and duration of supplementation used; and differences in gender of participants. To fully understand whether *L*-carnitine supplementation can have a positive impact upon

exercise metabolism, studies that systematically control for these factors need to be undertaken. The various human *L*-carnitine supplementation studies already mentioned in this review are extremely varied with respect to their design features. These discrepancies make comparisons between the study results difficult. By referring back to studies previously mentioned, this section will address the confounding variables that have been identified in the literature and how these could affect study outcomes.

### *Intensity and Duration of Exercise*

Studies examining oral *L*-carnitine supplementation during steady state exercise have examined varied intensities from around 40% $VO_{2max}$ to 70% $VO_{2max}$. Most of these studies focus on workloads below or around lactate threshold. *Lennon et al.* [87], *Hiatt et al.* [88], and *Heinonen et al.* [89] have noted that there are no changes in total carnitine content of muscle at intensities below threshold, however, this does not mean that supplementation is impractical for lower intensity exercise as changes in acylcarnitine to free carnitine ratio within muscle may be important in regulation of substrate utilisation. Furthermore, *Wyss et al.* [78] and *Swart et al.* [49] suggest that *L*-carnitine supplementation may be more effective during sustained exercise of lower intensity, since during incremental exercise, or exercise of high intensity, carbohydrate is the predominant fuel source and this may mask any potential effect of *L*-carnitine on fatty acid metabolism.

### *Dietary Intake Control*

A very important aspect to control when performing a study based on substrate utilisation is dietary intake. Many studies have used respiratory exchange ratio to estimate changes in carbohydrate and fat contribution to metabolism following *L*-carnitine supplementation and it is well known that this ratio is influenced greatly by prior dietary intake [90]. Even when diet has been controlled, in many studies it was not done in a consistent fashion. In some studies the only dietary control introduced was an overnight fast [91, 36]. *Colombani et al.* [86] prescribed a carbohydrate rich pre-exercise dinner and breakfast, and a compulsory 125 mL sugared tea drink to be consumed during exercise (marathon). Other studies only controlled factors on the day of the test, restricting food intake, sugared drink intake, and nicotine intake [76, 54]. Some investigators asked subjects to replicate their diet for 2 days prior to testing [73] or for the whole duration of their study [75] in conjunction with an obligatory overnight fast. *Maasen et al.* [80] set a controlled carbohydrate rich diet to be consumed for 3 days prior to testing. A variety of authors report their subjects as having an 'unchanged' diet throughout their study, the composition of these diets, however, is neither reported, nor controlled [85, 81, 82, 92]. *Wyss et al.* [78] set target calorie levels at 2700–2800 $kcal \cdot day^{-1}$. *Dragan et al.* [93, 74] took it a step further by setting their targets relative to body mass: 60–65 $kcal \cdot kg^{-1} \cdot day^{-1}$ (including 2–3 g protein $\cdot kg^{-1} \cdot day^{-1}$) in one study and 65–70 $kcal \cdot kg^{-1} \cdot day^{-1}$ (including 2.5–3 g protein $\cdot kg^{-1} \cdot day^{-1}$) in another. These targets give subjects a good guideline regarding energy intake but do not define the % of calories or gram intake

derived from carbohydrate and fat which is probably more important in metabolic studies. In addition, *Heinonen et al.* [94] observed a 50% depletion of tissue carnitine concentration after putting subjects on a carnitine free diet which highlights the importance of determining how much carnitine is normally present in each of the subjects' diets, and ensuring that changes in habitual intake of carnitine do not occur during a study.

The most controlled studies with regards to diet were those by *Vukovich et al.* [84] and *Arenas et al.* [95, 96]. *Vukovich et al.* [84] prescribed a diet consisting of $3000\,\text{kcal} \cdot \text{day}^{-1}$ (30% fat, 55% CHO, 15% protein), *Arenas et al.* [95] prescribed $3000–4000\,\text{kcal} \cdot \text{day}^{-1}$ (25–30% fat, 55–62% CHO, 13–15% protein). Their dietary regimens were adapted according to sex and event. Sprinters, both male and female, were constrained to a diet made up of 23.5% fat, 57.5% CHO, and 19% protein. The only difference lying in the fact that males were required to consume between $3500–4000\,\text{kcal} \cdot \text{day}^{-1}$ as opposed to the females at $3000–3500\,\text{kcal} \cdot \text{day}^{-1}$. Endurance athletes, all males, had the same kilocalorie equivalent as the sprinters but were required to consume 27.5% fat, 57.5% CHO, and 14% protein.

If dietary intake is controlled rigorously in the days preceding exercise then it is possible to measure effects of *L*-carnitine supplementation on substrate utilization using the indirect calorimetry method. However, tracer methods are likely to be preferable in examining potential influences on substrate utilization. To date, two tracer studies investigating oxidation of $^{13}C$ palmitic acid at rest have been conducted following *L*-carnitine ingestion [97, 98]. These studies observed an increase in oxidation of labeled palmitic acid at rest following supplementation with *L*-carnitine. No stable isotope studies examining the influence of *L*-carnitine supplementation on the rate of carbohydrate and fat oxidation have been conducted during exercise.

### *Training Status*

Endurance training causes an increase in the contribution of lipids to energy production during moderate to heavy submaximal exercise. Hence choosing subjects that are highly endurance trained allows the effects of oral *L*-carnitine to be studied in subjects with a high capacity for fatty acid oxidation [99]. Another reason for choosing trained individuals is that training may cause a relative shortage of endogenous *L*-carnitine [95, 78].

*Trappe et al.* [100] speculated that intensive training programmes may cause subjects to be already nearing their physiological limits within areas such as optimal ratio of carbohydrate and fat metabolism during exercise, mitochondrial density, mitochondrial enzyme concentrations, and carnitine concentrations. This might explain why *L*-carnitine supplementation may not have demonstrated any significant effects in some studies on highly trained individuals [101, 80, 100, 82, 92, 86]. However, the findings depicted in the tables summarising the effects of *L*-carnitine on respiratory exchange ratio (RER, Table 1), blood lactate (Table 2), and muscle power output (Table 3), suggest that it may be possible that there is a relationship between training status and positive outcomes of supplementation. It is clear from Tables 1–3 that there are no studies showing any positive effects of *L*-carnitine supplementation on these specific outcome variables in untrained

individuals. However, despite some positive outcomes in studies on trained or active subjects there are also many studies showing no effect in trained or moderately active subjects. Future work should focus on differences between groups of subjects with differing training status.

### *Type, Dose and Duration of Supplementation*

There are a number of different *L*-carnitine preparations available. Amongst these the most common forms are: *L*-carnitine *L*-tartrate, acetyl-*L*-carnitine, *L*-carnitine free base, and propionyl-*L*-carnitine. *L*-carnitine *L*-tartrate is composed of 68% *L*-carnitine and 32% tartaric acid, and is the most stable form of *L*-carnitine supplement. It is an odourless, non-hygroscopic salt, and it contains the highest *L*-carnitine concentration of all available salts. It has been marketed specifically to the food industry and as a sports performance enhancing substance, therefore, it has regularly been used in performance studies on athletes [102]. Acetyl-*L*-carnitine is a water-soluble short chain acylcarnitine ester. Research has shown that acetyl-*L*-carnitine may enhance brain function in ageing people and can help reduce deterioration in *Alzheimer*'s patients [103]. Several authors have suggested that propionyl-*L*-carnitine has certain advantages over *L*-carnitine free base or other forms of *L*-carnitine. *Anand et al.* [104] asserts that it is more lipophilic as well as having a greater availability to the heart and peripheral muscle. Findings by *Tassani et al.* [105] suggest that besides stimulating PDC activity, propionyl-*L*-carnitine also has an anaplerotic effect by replenishing the TCA cycle intermediate oxaloacetate and hence increasing the flux through the cycle itself. It has been shown that propionyl-*L*-carnitine therapy can improve skeletal or cardiac muscle performance particularly in hypoxic or ischemic states [106] and in models of heart failure [107]. Research has concluded that this substance can reduce the development of myocardial hypertrophy, and unlike *L*-carnitine free base it has a positive inotropic effect on isolated heart preparations [104]. No studies have directly compared the effects of the two key supplement types (*L*-carnitine *L*-tartrate and propionyl-*L*-carnitine) on exercise metabolism.

The dose of supplement administered has been highlighted by *Hultman et al.* [108] to be of critical importance. These authors questioned the results reported by *Vecchiet et al.* [76] by estimating, based on absorption and pharmacokinetic data, the likely muscle carnitine elevation following an acute administration of an *L*-carnitine dose (2 g). The calculated increase was no more than 1–2%, which would be unlikely to alter FA transport capacity or acyl group buffering. This report could provide an explanation for all the studies that have failed to observe an increase in muscle carnitine following supplementation of doses ranging from 4 to 6 $g \cdot day^{-1}$ [69, 81, 84], but does not explain why positive findings were present in some other studies. Only one study on humans has observed an increase in muscle carnitine following supplementation, coupled with a 17-week training programme [95]. The reported increase in that study may be due to the lengthy supplementation period, or the combination of supplementation and training, and these aspects require further exploration.

*L*-carnitine studies have focused mainly on chronic supplementation with the duration ranging from 6 days [85] to 6 months [95]. A few acute-dose studies have

also been performed, showing mixed results. Both acute and chronic studies vary with regard to dose administered, time elapsed from administration, and in the case of chronic studies, the duration of the supplementation. A study conducted by *Bobyleva-Guarriero et al.* [109] demonstrated that acute and chronic supplementation performed on the same rat had different outcomes on metabolism. The acute injected dose of *L*-carnitine inner salt (*i.p.* 0.5 $cm^3$ of 6% solution for 100 g body mass) had a clear ameliorating effect on respiration of liver mitochondria in rats forced to exercise. A chronic dose administered for a month, however, had no effect on the aerobic metabolism of liver when exposed to acute exercise [109] suggesting greater impact of acute dosing than chronic dosing in this particular model.

A factor that appears to be often overlooked when discussing the administration of *L*-carnitine is the exact time before exercise at which the supplement is being given. Many studies fail to indicate the exact time interval between the last dose and the exercise trial. *Barnett et al.* [69] point out flaws relating to administration time in studies conducted by *Siliprandi et al.* [75] and *Vecchiet et al.* [76]. According to *Barnett et al.* [69] administering the supplement only 60–90 minutes before exercise, as was the case in the above mentioned studies, does not give the supplement enough time to be emptied from the stomach, absorbed by the small intestine, and then transported in the blood to reach skeletal muscle and hence the potential site of action [69]. *Gross* and *Henderson* [110] support this argument through their findings. They traced the absorption of labelled *L*-carnitine following administration and reported that 5 hours following supplementation only 22.5% made it to skeletal muscle, while 45.8% was still residing in the intestinal tissue. The positive outcomes in some studies after short term acute dosing raises the question of possible extramuscular actions of *L*-carnitine that have not yet been fully investigated.

### *Gender*

The vast majority of *L*-carnitine supplementation studies have focussed solely on male subjects. A variety of studies have been conducted in an attempt to identify the mechanisms that may be responsible for a greater reliance on lipid as a fuel source in females [111]. Several explanations for this difference in reliance on fat during exercise have been forwarded including: different fiber type composition and Type I:Type II fiber ratio [112]; and hormonal influences related to menstrual cycle phase [113, 114]. It has been consistently reported among the well-controlled studies, that females in their luteal phase do indeed demonstrate a lower RER (by about 4–5%) during submaximal endurance exercise (60% $VO_{2max}$), but yet demonstrate no differences at rest. This has been shown to be the case both before and after a training programme [111]. Furthermore, there may also be possible differences in endogenous carnitine synthesis between males and females, since endurance trained females could be more prone to exhibit iron deficiency [115].

Of the published *L*-carnitine supplementation studies only a few have studied females as a group of their own. *Dragan et al.* [93] conducted an *L*-carnitine supplementation study that examined the effects of 1 g endovenous *L*-carnitine supplementation on seventeen elite female swimmers. The study was performed

in a double-blind crossover fashion. The subjects were given a controlled training programme to follow and a diet that specified the ingestion of 60–65 kcal · $kg^{-1} \cdot day^{-1}$, including 2–2.3 g of protein · $kg^{-1}$. The test consisted of a 60 second maximal exercise bout on a cycle ergometer and the authors observed a reduced capillary lactate response to exercise following *L*-carnitine administration.

In summary, there are a number of factors that could impact upon the outcome of *L*-carnitine supplementation studies and careful methodological control has not always been implemented in the data published to date. The challenge for future work is to undertake well-controlled systematic investigations into the effects of these potential confounding factors in an attempt to determine what effect *L*-carnitine supplementation may have upon exercise metabolism in sedentary, healthy, or trained subjects.

Work conducted in our own laboratory has begun to systematically address some of these factors in well controlled studies with some interesting observations. In all of our studies we have recruited non-vegetarian subjects and placed strict control on dietary intake and physical activity in the days preceding laboratory visits including prescribed diets/exercise and provision of all food. Supplements are always taken twice daily with meals (morning and evening) and the final supplement ingested with a bolus of carbohydrate in the form of a pre-exercise meal (1 g carbohydrate · $kg^{-1}$ body mass) 3 hours prior to exercise. In one of our

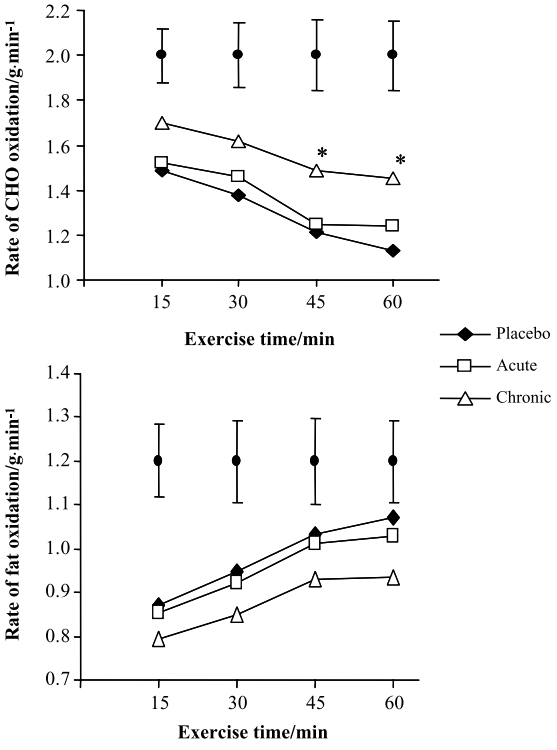


**Fig. 1.** Mean (S.E.M) rate of carbohydrate (CHO) and fat oxidation during 60 minutes of steady state exercise following 14 days of placebo ingestion, 13 days of placebo and 1 day of *L*-carnitine ingestion (Acute), or 14 days of *L*-carnitine ingestion (Chronic); * indicates significant difference ($p < 0.05$) between chronic and placebo trials

initial studies we examined acute *versus* chronic supplementation with *L*-carnitine *L*-tartrate on substrate utilization during 60 minutes of exercise in endurance trained males [116]. We observed no effect of acute supplementation *versus* placebo but chronic *L*-carnitine supplementation resulted in an increased oxidation of carbohydrate during exercise and a tendency for reduction in oxidation of fat (Fig. 1). Although no human studies have reported an increase in carbohydrate oxidation during exercise following *L*-carnitine supplementation no other studies have the combination of rigorous control of dietary intake, physical activity, and use of well-trained endurance athletes. Furthermore, the response we observed has been previously noted in isolated working heart when *L*-carnitine is added to the perfusate [60, 59] and may reflect an effect of *L*-carnitine on PDC activity, assuming that supplemental *L*-carnitine could impact upon the skeletal muscle carnitine pool. Our observations do concur with the previous work of *Huertas et al.* [117] and *Arenas et al.* [96] who observed increases in PDC activity following 4 weeks of *L*-carnitine supplementation in endurance trained runners, and our data may also therefore reflect some support for a buffering role of carnitine that allows better matching of substrate breakdown with demand for *ATP*.

We have subsequently examined longer duration supplementation with *L*-carnitine *L*-tartrate (4 weeks) and observed no effect on carbohydrate or fat oxidation during steady state exercise at 70% of $VO_{2max}$ after supplementation (Figs. 2 and 3 [118]). This does not fit with our previous observations but a possible explanation for the lack of effect after a longer period of supplementation may have been eluded to by *Bobyleva-Guarriero* [109]. Short term dosing may have a different

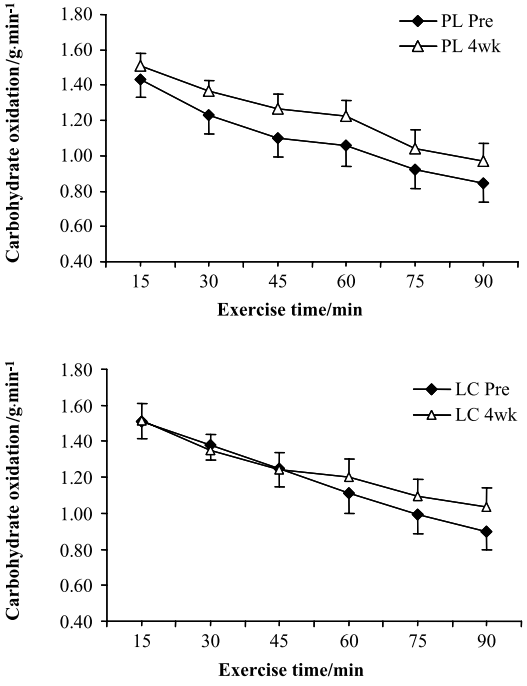


**Fig. 2.** Mean (S.E.M) rate of carbohydrate oxidation during 90 minutes of steady state exercise at baseline (Pre) and following 4 weeks (4 wk) of supplementation with either placebo (PL) or *L*-carnitine *L*-tartrate (LC); $n = 15$

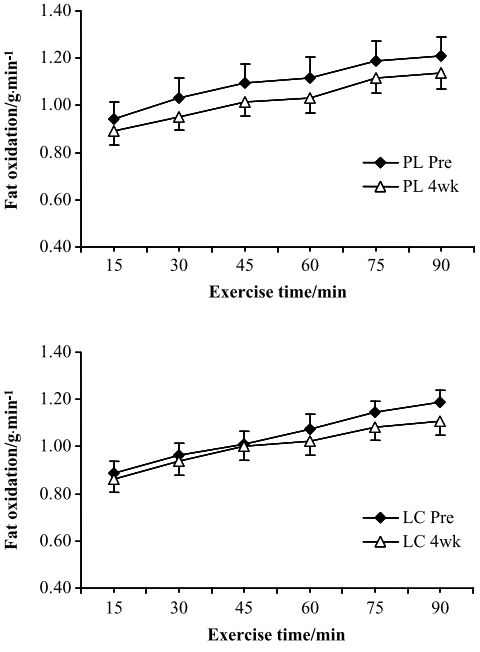


**Fig. 3.** Mean (S.E.M) rate of fat oxidation during 90 minutes of steady state exercise at baseline (Pre) and following 4 weeks (4 wk) of supplementation with either placebo (PL) or *L*-carnitine *L*-tartrate (LC); $n = 15$

response to longer term dosing due to the homeostatic control mechanisms that occur with more prolonged *L*-carnitine administration such as changes in intestinal absorption and/or renal clearance. We also observed in the same study [118] no difference in response to supplementation in subjects varying in training status from moderately trained to well-trained ($VO_{2max}$ ranging between 50 and 85 $mL \cdot kg^{-1} \cdot min^{-1}$, training history ranging from 2 to 20 years, and current training volume ranging from 5 to 13 hours per week) despite significant differences in lipid utilization during exercise between the two subjects groups. We have not yet examined the metabolic response to exercise following supplementation in trained *versus* sedentary individuals who are notable for their lower capacity for lipid oxidation during exercise.

In a recent investigation [119] we also demonstrated that a two week period of *L*-carnitine *L*-tartrate supplementation in trained endurance athletes results in a blunting of ammonia accumulation during exercise compared with placebo. This blunting of ammonia accumulation in exercise ties in with clinical studies examining ammonia toxicity such as valproate induced hyperammonemia and its reversal with *L*-carnitine supplementation [120]. It is not known whether a reduced ammonia accumulation during exercise following *L*-carnitine ingestion reflects reduced *AMP* deamination, increased ammonia clearance, or changes in glutamine/glutamate/alanine production, but the supporting data we have does not suggest any change in amino acid contribution to metabolism. In addition, our ammonia data may be linked with the observations of *Volek et al.* [121] who demonstrated a reduction in plasma markers of purine catabolism with a lower plasma hypoxanthine, plasma xanthine oxidase, and serum uric acid following 3 weeks of

*L*-carnitine *L*-tartrate ingestion in resistance trained subjects. These authors suggest that their observations reflect greater recovery from high-intensity resistance exercise, but their data could also be interpreted as a carnitine mediated reduction in cellular stress during intense exercise. To observe these effects of *L*-carnitine it is likely that a change in the muscle carnitine pool is required. Although a change has not been reliably demonstrated in humans this may be related to the reliability of methods used for determination of muscle carnitine content and/or differences in cytosolic *versus* mitochondrial carnitine content that have not been examined.

Therefore, the impact of supplementation on skeletal muscle carnitine content should be examined further to identify whether differences in response to supplementation occur in trained *versus* sedentary individuals, and in response to oral dosing with and without carbohydrate ingestion. The potential influence of insulin on carnitine transport into skeletal muscle is now being investigated [122] and may give some insight into individual differences in skeletal muscle carnitine uptake. Once these questions have been answered more light may be shed on the key factors that contribute to the equivocal nature of the research on *L*-carnitine and exercise metabolism to date, and may reveal the possible metabolic actions of *L*-carnitine supplementation prior to exercise.

## Future Directions

Given the theoretical influences of *L*-carnitine on skeletal muscle metabolism at rest and during exercise, with a role in transport of fatty acids into mitochondria and/or buffering of acyl CoA within mitochondria, *L*-carnitine could be considered as a potential key factor in regulation of glucose and fat metabolism. *L*-carnitine, and in particular free carnitine content of muscle mitochondria, may act to optimize substrate metabolism with *ATP* requirement, however, evidence for this effect is currently lacking. The conflicting data that are available lend some support to a regulatory role of carnitine with increases in fat oxidation and increases in carbohydrate oxidation both being possibilities dependant upon the training status of subjects and the exercise intensity and duration being examined. However, others would argue that any carnitine actions are likely to be due to effects occurring outwith the muscle, such as the proposed effects of *L*-carnitine on blood flow in patients with peripheral vascular disease.

Given the recent findings of *Volek et al.* [121] and our own data [119] it would be interesting to speculate that through regulating carbohydrate and fat oxidation *L*-carnitine could impact upon the *AMP*:*ATP* ratio in muscle and therefore have an effect on phosphorylation of *AMP* activated protein kinase (*AMPK*). An impact upon phosphorylation of *AMPK* in skeletal muscle could alter glucose transport, glycolysis, fatty acid oxidation, lipolysis, glycogen synthesis, protein synthesis, fatty acid synthesis, and blood flow [123, 124]. Many of the highlighted roles of *AMPK* activation or inactivation have been reported in studies following *L*-carnitine ingestion including: increases in mitochondrial oxidative enzymes [117, 96]; changes in fibre type distribution from slow to fast [68]; and changes in carbohydrate or fat oxidation.

Future work examining the effects of *L*-carnitine supplementation on cellular signaling pathways involved in substrate selection, and adaptations to training, may

provide a better insight into the particular roles/actions of *L*-carnitine during exercise.

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