# LIMITS TO HUMAN ENDURANCE: CARNITINE AND FAT OXIDATION

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**ABSTRACT**

Fat and carbohydrate are the primary fuel sources for mitochondrial ATP production in human skeletal muscle during endurance exercise. However, fat exhibits a relatively low maximal rate of oxidation *in vivo*, which begins to decline at around 65% of maximal oxygen consumption ($VO_2$max) when muscle glycogen becomes the major fuel. It is thought that if the rate of fat oxidation during endurance exercise could be augmented, then muscle glycogen depletion could be delayed and endurance improved. The purpose of the present review is to outline the role of carnitine in skeletal muscle fat oxidation and how this is influenced by the role of carnitine in muscle carbohydrate oxidation. Specifically, it will propose a novel hypothesis outlining how muscle free carnitine availability is limiting to the rate of fat oxidation. The review will also highlight recent research demonstrating that increasing the muscle carnitine pool in humans can have a significant impact upon both fat and carbohydrate metabolism during endurance exercise which is dependent upon the intensity of exercise performed.

## INTRODUCTION

Fat and carbohydrate are the primary fuel sources for mitochondrial ATP production in human skeletal muscle during endurance exercise. Fat constitutes the largest energy reserve in the body and in terms of the amount available it is not limiting to endurance exercise performance. However, fat exhibits a relatively low maximal rate of oxidation *in vivo*, which begins to decline at around 65% of maximal oxygen consumption ($VO_2$max) when muscle glycogen becomes the major fuel supporting ATP homeostasis [1, 2, 3]. The muscle glycogen stores are limited and it has been well established that muscle glycogen depletion coincides with fatigue during high intensity endurance exercise [4]. As fatigue can be postponed by increasing pre exercise muscle glycogen content [4], it is thought that augmenting the rate of fat oxidation during endurance exercise could delay glycogen depletion and improve endurance exercise performance. Indeed, it has long been known that enhanced fat oxidation is one of the main muscle adaptations to endurance exercise training [5]. For this reason, a large amount of research towards the end of the 20$^{th}$ century was directed towards investigating the effects of L-carnitine supplementation on endurance exercise performance, as carnitine is known to play an essential role in the translocation of fat into the mitochondria, which is considered to be a key rate limiting step in fat oxidation [6]. However, scientific interest in L-carnitine as an ergogenic aid soon declined when it became apparent that L-carnitine feeding did not alter fat oxidation, exercise performance or, more importantly, impact upon the muscle carnitine pool in humans. The purpose of the present review is to outline the role of carnitine in skeletal muscle fat oxidation and how this is influenced by the role of carnitine in carbohydrate oxidation, and to highlight more recent research demonstrating that the muscle carnitine pool can indeed be increased in humans and have a significant impact upon these roles during endurance exercise.

## ROLE OF CARNITINE IN SKELETAL MUSCLE FAT OXIDATION

Irving Fritz and colleagues first established that mitochondria in a variety of tissues are impermeable to fatty acyl-CoA, but not to fatty acylcarnitine, and that carnitine and carnitine palmitoyltransferase

are essential for the translocation of long-chain fatty acids into skeletal muscle mitochondria for β-oxidation e.g. [7]. Since these discoveries it has been established that carnitine palmitoyltransferase 1 (CPT1), situated within the outer mitochondrial membrane, catalyses the reversible esterification of carnitine with long-chain acyl-CoA to form long-chain acylcarnitine. Cytosolic acylcarnitine is then transported into the mitochondrial matrix in a simultaneous 1:1 exchange with intramitochondrial free carnitine via the carnitine acylcarnitine translocase (CACT), which is situated within the mitochondrial inner membrane. Once inside the mitochondrial matrix, acylcarnitine is transesterified back to free carnitine and long-chain acyl-CoA in a reaction catalysed by carnitine palmitoyltransferase 2 (CPT2), which is situated on the matrix side of the inner mitochondrial membrane (see Figure 1). The intramitochondrial long-chain acyl-CoA is then oxidised and cleaved by the β-oxidation pathway. The significance of this "carnitine cycle" to fat oxidation during exercise is highlighted in patients with carnitine, CPT2, or CACT deficiency (CPT1 deficiency appears to be incompatible with life) who typically experience severely reduced rates of fat oxidation during prolonged exercise along with muscle pain and weakness, hypoglycaemia, and hypoketosis. For a detailed review of this fat translocation process see [8].

CPT1 is considered to be the rate-limiting enzyme for long-chain fatty acid entry into the mitochondria and oxidation, but carnitine is not thought to be rate limiting to CPT1. Indeed, the concentration of carnitine in skeletal muscle is around 5 mM intracellular water [9] and far in excess of the *in vitro* Michaelis-Menten constant ($K_m$) of muscle CPT1 for carnitine of approximately 0.5 mM [6]. However, the enzymes of the carnitine cycle co-immunoprecipitate and are mainly located in the specialised contact sites between outer- and inner-mitochondrial membranes in order to allow metabolic channelling [10]. Thus, it is the premise of the present article that the intramitochondrial content of free carnitine determines carnitine availability to CPT1 and, as this is around 10% of the whole muscle carnitine pool [11], that carnitine could be rate limiting to the CPT1 reaction. By way of example, translocation of acylcarnitine from the CPT1 reaction into the mitochondrial matrix in

order to allow continuation of the CPT1 reaction (acylcarnitine is a potent inhibitor of CPT1 flux) is dependent upon exchange with intramitochondrial free carnitine to the CPT1 catalytic site via CACT. It stands to reason, therefore, that any change in the intra-mitochondrial free carnitine pool will likely impact upon CPT1 flux, which may partly explain how the rate of fat oxidation is regulated.

**REGULATION OF THE RATE OF SKELETAL MUSCLE FAT OXIDATION**

While CPT1 is considered a key site in the regulation of the rate of fat oxidation it must be noted that there are several other potential control points that could influence the decline in the rate of fat oxidation in skeletal muscle during high intensity endurance exercise. These other control points include: 1) regulation of lipolysis within adipose tissue and intramuscular triacylglycerol pools; 2) transport and delivery of circulating free fatty acids from adipose tissue stores to working skeletal muscle; 3) transport of fatty acids across the muscle cell membrane and within the cell cytosol to the outer mitochondrial membrane; and 4) intramitochondrial enzymatic reactions distal to CPT1. The contribution of each of these control points to the regulation of fat oxidation within contracting skeletal muscle is not yet fully understood, but it is likely that the role of each of these will vary depending upon the intensity of exercise and will likely all respond to exercise training.

The enzymes in adipose tissue and skeletal muscle that mobilize free fatty acids from triacylglycerol stores are known to be stimulated by epinephrine, norepinephrine, adenosine and some peptides, and are inhibited by insulin, and thus impaired mobilisation would seem unlikely to be a limiting factor at exercise intensities above 65% of $VO_2$max. However, the release of mobilised fatty acids from adipose tissue into the circulation could be limiting if adipose tissue blood flow was compromised at higher exercise intensities. Indeed, increasing plasma fatty acid concentration during high intensity endurance exercise via infusion of a lipid emulsion and heparin increases the rate of plasma fat oxidation [12]. However, the rate of fat oxidation is not fully restored under these conditions and the utilisation of intramuscular triacylglycerol is still inhibited, and so it has not yet

been clearly established whether delivery of fatty acids is a major factor limiting fat oxidation. The regulation of intramuscular triaclyglycerol hydrolysis is being actively investigated at present. In addition, the transport of fatty acids across the muscle cell membrane and within the cell cytosol could be rate limiting. Recently, Smith et al [13] suggested a key regulatory role for fatty acid translocase (FAT/CD36) in the entry of fatty acids into the cell and, more importantly, in delivery of fatty acids to acyl-CoA synthetase located near CPT1 on the outer mitochondrial membrane. However, this regulatory role of FAT/CD36 on fat oxidation has not yet been investigated at higher exercise intensities where rates of fat oxidation are known to decline, leaving no firm conclusions possible at this stage. Reactions distal to CPT1 which involve the limited intramitochondrial free coenzyme A pool (CoASH) such as CPT2 or various stages of the proximal β–oxidation pathway are also likely to be important regulators and require further investigation [14].

Despite the lack of knowledge of these other potential regulatory steps there is increasing evidence to suggest that fat oxidation is indirectly regulated by flux through the pyruvate dehydrogenase complex (PDC) during exercise and that CPT1 is central to this regulation. For example, during exercise at 50% $\dot{V}O_2$max, hyperglycaemia (induced as a result of pre-exercise ingestion of glucose) increased glycolytic flux and reduced long-chain fatty acid ([1-$^{13}$C]palmitate) oxidation [15], whereas medium chain [1-$^{13}$C]octanoate oxidation was unaffected. This suggests an inhibition of fat oxidation at the level of CPT1, as medium-chain fatty acids are oxidized independent of CPT1. Malonyl-CoA is a potent inhibitor of CPT1 activity *in vitro* and is a likely candidate as the intracellular regulator of the rate of long-chain fatty acid oxidation in human skeletal muscle, particularly as changes in muscle malonyl-CoA content have occurred with opposite changes in fat oxidation at rest [16]. However, despite a 122% increase in fat oxidation rates (due to depleted pre-exercise muscle glycogen content) during exercise at 65% $\dot{V}O_2$peak, there were no differences in muscle malonyl-CoA content compared to control [17], suggesting that malonyl-CoA does not regulate CPT1 activity during exercise. Interestingly, a reduction in intracellular pH (from 7.1 to 6.8) *in vitro* reduces CPT1 activity

by 34-40%, independent of any physiological change in malonyl-CoA concentration [18]. It is unlikely that this fall in pH would occur *in vivo* at the exercise intensity where the rate of fat oxidation routinely begins to decline, but it may suggest that changes in intracellular buffering could also play an important role in the regulation of the rate of fat oxidation. It has also been suggested that muscle contraction alters the sensitivity of CPT1 to malonyl-CoA and/or pH, but this would be difficult to test *in vivo*.

**ROLE OF CARNITINE IN THE REGULATION OF FAT OXIDATION**

As carnitine is a major substrate for CPT1 and has been hypothesised that the marked lowering of the muscle free carnitine pool during conditions of high PDC flux may limit CPT1 flux and thus the rate of fat oxidation [2, 19]. For example, during high intensity endurance exercise the rate of acetyl-CoA formation from PDC flux is in excess of its utilisation by the tricarboxylic acid (TCA) cycle leading to its subsequent accumulation. Another role of carnitine in skeletal muscle is to buffer the excess acetyl groups formed, in a reaction catalysed by carnitine acetyltransferase (CAT), to ensure that a viable pool of CoASH is maintained for the continuation of the PDC and TCA cycle reactions [19, 20, 21, 22]. Indeed, following a few minutes of high intensity exercise the increase in acetylcarnitine formation is directly related to an increase in muscle acetyl-CoA [21]. Thus, van Loon *et al*. [2] demonstrated that a 35% decrease in the rate of fat oxidation that occurred at 72% $VO_2$max, was paralleled by a 65% decline in skeletal muscle free carnitine content. Furthermore, the 2.5-fold decrease in the rate of fat oxidation during exercise at 65% $VO_2$max compared to control in the afore mentioned study of Roepstorff *et al*. [17] coincided with a 50% reduction in free carnitine availability.

If we take all of these studies together, it is apparent that there is a dramatic decline in skeletal muscle free carnitine availability when it reaches 50% of the total carnitine pool at around 65% $VO_2$max (Figure 1). This exercise intensity coincides with that routinely reported at which fat oxidation begins to decline in healthy humans during cycling [3] and suggests that free carnitine

availability becomes limiting to the rate of fat oxidation. However, it can also be seen from Figure 1 that free carnitine availability declines at lower exercise intensities where rates of fat oxidation are still increasing. At first glance this may suggest that free carnitine availability is not limiting to CPT1, but if we consider that β-oxidation will be increased several fold at these low exercise intensities then it could be predicted that the rate of acylcarnitine utilisation by CPT2 will be increased, allowing increased CACT flux and intra-mitochondrial carnitine delivery to CPT1. When acetylation of the free carnitine pool reduces it availability to below 50% of the total pool, CACT also becomes limiting to the carnitine cycle flux, which would fit with the 1:1 stoichiometry of the CACT reaction and the fact that predicted intra-mitochondrial carnitine content at rest (equivalent of 0.5 mM intracellular water) is around double that of long-chain acylcarnitine (equivalent of 0.25 mM intracellular water) [23, 24]. Thus, free carnitine availability is limiting to CPT1 at any given exercise intensity and determined by flux through the CACT reaction, which in turn is limited by mitochondrial free carnitine availability above 65% $VO_2$max. With this in mind, increasing muscle total carnitine content could potentially increase the rate of fat oxidation during exercise, spare muscle glycogen, and increase exercise performance. Indeed, it is interesting to note that a significant positive association between total carnitine content and maximal activity of citrate synthase has been observed in skeletal muscle [25]. These observations may highlight an important link between mitochondrial oxidative capacity, intramitochondrial carnitine content, and the capacity for fat oxidation in skeletal muscle cells.

**EFFECT OF INCREASING SKELETAL MUSCLE CARNITINE AVAILABILITY ON THE RATE OF FAT OXIDATION**

The majority of the pertinent studies in healthy humans to date have failed to increase skeletal muscle carnitine content via oral or intravenous L-carnitine administration [26]. For example, neither feeding L-carnitine daily for up 3 months [27], nor intravenously infusing L-carnitine for up to 5 h [28], had an effect on muscle total carnitine content, or indeed net uptake of carnitine across the leg

[29]. Furthermore, feeding 2-5 g/day of L-carnitine for 1 week to 3 months prior to a bout of exercise, had no effect on perceived exertion, exercise performance, $\dot{V}O_2$max, or markers of muscle substrate metabolism such as RER, $\dot{V}O_2$, blood lactate, leg FFA turnover, and post exercise muscle glycogen content [26]. What was apparent from the earlier carnitine supplementation studies was that muscle carnitine content was either not measured or, if it was, not increased. This is likely explained by the finding that carnitine is transported into skeletal muscle against a considerable concentration gradient (>100 fold) which is saturated under normal conditions, and so it is unlikely that simply increasing plasma carnitine availability *per se* will increase muscle carnitine transport and storage [26]. However, insulin appears to stimulate skeletal muscle carnitine transport, and intravenously infusing L-carnitine in the presence of high circulating insulin (>50 mU/l) can increase muscle carnitine content by 15% [23, 28]. Furthermore, ingesting relatively large quantities of carbohydrate in a beverage (>80 g) can stimulate insulin release to a sufficient degree to increase whole body carnitine retention when combined with 3 g of carnitine feeding and, if continued for up to 6 months (80 g carbohydrate + 1.36 g L-carnitine twice daily) can increase the muscle store by 20% compared to carbohydrate feeding alone [24].

Consistent with the hypothesis that free carnitine availability is limiting to CPT1 flux and fat oxidation, the increase in muscle total carnitine content in the study of Wall *et al* [24] equated to an 80% increase in free carnitine availability during 30 min of exercise at 50% $VO_2$max compared to control and resulted in a 55% reduction in muscle glycogen utilisation. Furthermore, this was accompanied by a 30% reduction in PDC activation status during exercise, suggesting that a carnitine mediated increase in fat derived acetyl-CoA inhibited PDC and muscle carbohydrate oxidation. Put another way, L-carnitine supplementation increased muscle free carnitine concentration during exercise at 50% $VO_2$max from 4.4 to 5.9 mmol/l intracellular water, equating to an intra-mitochondrial concentration of 0.44 and 0.59 mmol/l, which is below and above the $K_m$ of CPT1 for carnitine, respectively [6]. The reduction in glycogen utilisation could of course be due to an increase

in plasma glucose disposal and utilisation, which has been observed following carnitine feeding or intravenous infusion over periods which would not be expected to increase muscle total carnitine content [23, 30, 31], albeit under resting conditions. On the other hand, during 30 min of exercise at 80% $VO_2$max with increased muscle total carnitine content the same apparent effects on fat oxidation were not observed [24]. In contrast, there was greater PDC activation (40%) and flux (16% greater acetylcarnitine), resulting in markedly reduced muscle lactate accumulation in the face of similar rates of glycogenolysis compared to control. This suggested that free carnitine availability was limiting to PDC flux during high intensity exercise and that increasing muscle carnitine content resulted in a greater acetyl-CoA buffering capacity and better matching of glycolytic to PDC flux. Furthermore, as free carnitine availability was not significantly greater than control during high intensity exercise in the study of Wall *et al* [24], and was below 50% of the total carnitine pool, it is likely that free carnitine delivery was still limiting to CPT1 and that, quantitatively, the CAT reaction (around 800 µmol/min/kg wm over the first 3 min of exercise) "stole" free carnitine from the CACT/CPT1 reaction (80 µmol/min/kg wm at most).

**EFFECT OF INCREASING MUSCLE CARNITINE CONTENT ON ENDURANCE PERFORMANCE**

The study by Wall *et al* [24] also demonstrated a remarkable improvement in exercise performance in all participants (11% mean increase) during a 30 minute cycle ergometer time trial in the carnitine loaded state. This is consistent with animal studies reporting a delay in fatigue development by 25% during electrical stimulation in rat soleus muscle strips incubated in carnitine *in vitro* [32]. Whether these improvements in endurance performance are due to glycogen sparing as a result of a carnitine mediated increase in fat oxidation, or the reported effect of carnitine on glucose disposal requires further investigation, but due to the nature of the time trial this seems unlikely. In fact, it is the reduced reliance on non-oxidative ATP production from carbohydrate oxidation (increase acetyl group buffering and reduced lactate accumulation) that is the more likely cause of the increased endurance performance observed [33]. Moreover, with the acetyl-group buffering role of carnitine

in mind, increasing muscle carnitine content may also allow a greater stockpiling of acetylcarnitine during a prior ‘warm-up’ exercise, which would then serve as a useful immediate supply of acetyl groups at the onset of a subsequent high-intensity performance task and again reduce the reliance on non-oxidative ATP production [34]. The beneficial effects of carnitine supplementation on glucose disposal may also aid with ‘glycogen loading’, which would likely influence performance during high-intensity endurance exercise [4]. Taken together, the long-held belief that carnitine supplementation can improve endurance performance via augmenting its role in fat oxidation should be revised to place more emphasis on the major role that carnitine plays in carbohydrate metabolism during exercise.

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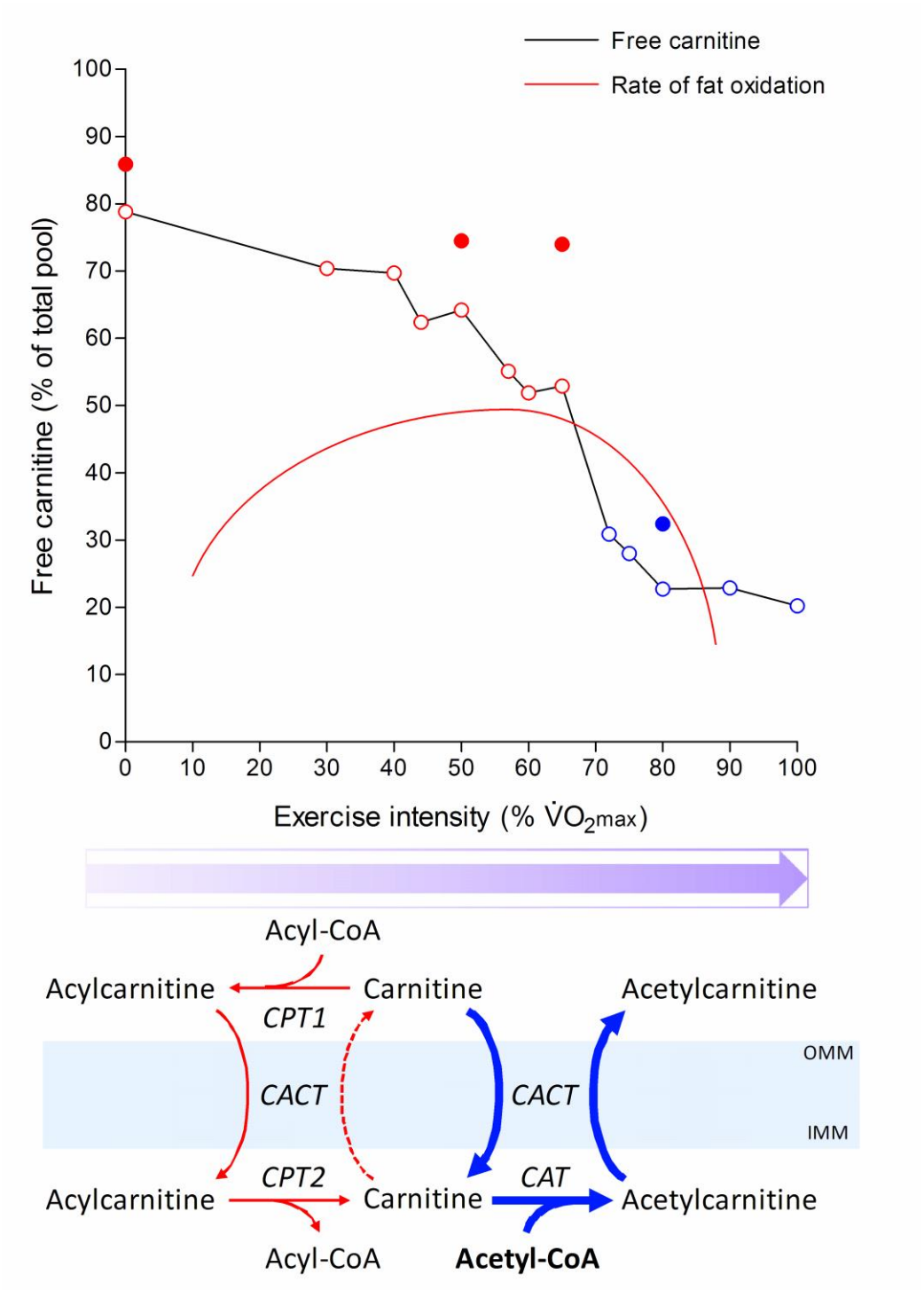
**FIGURE LEGENDS**

**Figure 1**

Above: The fraction of total carnitine (not including acylcarnitine) represented as free carnitine in human *vastus lateralis* muscle at rest and following 4 to 30 min of exercise on a cycle ergometer at various exercise intensities in relation to the rate of fat oxidation. Open circles are a review of results taken from [17, 19, 20, 21, 22, 24] under standard conditions. Closed circles are results from [17, 24] after the muscle free carnitine pool has been manipulated by 6 months of L-carnitine and carbohydrate feeding [24] or at 65% $VO_2$max by reducing glycolytic flux [17] – these interventions shift the rate of fat oxidation curve up, rather than to the right. Below: Schematic diagram of the dual metabolic role of free carnitine within skeletal muscle fat (red arrows) and carbohydrate (blue arrows) oxidation. The small intramitochondrial free carnitine pool is delivered to the catalytic site of CPT1 in a 1:1 exchange with acylcarnitine produced from the CPT1 reaction, and is hypothetically limiting to CPT1. Excess acetyl-CoA from the pyruvate dehydrogenase complex (PDC) reaction is buffered by the large extramitochondrial free carnitine pool via the CAT reaction, which “steals” intramitochondrial free carnitine with increasing exercise intensity (purple arrow). CPT, carnitine palmitoyltransferase; CACT, carnitine acylcarnitine translocase; CAT, carnitine acetyltransferase; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane.

**Figure 1.**


Free carnitine
Rate of fat oxidation
Free carnitine (% of total pool)
Exercise intensity (% V̇O2max)
Acyl-CoA
Acylcarnitine
Carnitine
Acetylcarnitine
CPT1
CACT
CACT
OMM
IMM
CPT2
CAT
Acylcarnitine
Carnitine
Acetylcarnitine
Acyl-CoA
Acetyl-CoA