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SYMPOSIUM REVIEW

Regulation and limitations to fatty acid oxidation during exercise

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Abstract Fatty acids (FAs) as fuel for energy utilization during exercise originate from different sources: FAs transported in the circulation either bound to albumin or as triacylglycerol (TG) carried by very low density lipoproteins and FAs from lipolysis of muscle TG stores. Despite a high rate of energy expenditure during high intensity exercise the total FA oxidation is suppressed to below that observed during moderate intensity exercise. Although this has been known for many years, the mechanisms behind this phenomenon are still not fully elucidated. A failure of adipose tissue to deliver sufficient FAs to exercising muscle has been proposed, but evidence is emerging that factors within the muscle might be of more importance. The high rate of glycolysis during high intensity exercise might be the 'driving force' via the increased production of acetyl-CoA, which in turn is trapped by carnitine. This will lead to decreased availability of free carnitine for long chain FA transport into mitochondria. This review summarizes our present view on how FA metabolism is regulated during exercise with a special focus on the limitations in FA oxidation in the transition from moderate to high intensity exercise in humans.

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Abbreviations FA, fatty acid HSL, hormone sensitive lipase IMTG, intramyocellular triacylglycerol LPL, lipoprotein lipase RER, respiratory exchange ratio TG, triacylglycerol VLDL, very low density lipoprotein.

Introduction

The work by Krogh and Lindhard and Christensen and Hansen in the 1920s and 1930s demonstrated, from measurements of the respiratory exchange ratio (RER), that fatty acid (FA) oxidation increased 5- to 10-fold above resting levels during mild to moderate exercise and peaked at exercise intensities around 65% of maximal oxygen uptake ($\dot{V}_{O_2,peak}$) (Krogh & Lindhard, 1920; Christensen & Hansen, 1939). When exercise intensity increased further, FA oxidation progressively decreased. Today a remaining unsolved question is: What are the limitations and regulation of skeletal muscle FA oxidation at high exercise intensities?

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Delivery of FA

Plasma FA to the working muscle is primarily supplied from lipolysis of triacylglycerol (TG) stored in adipose tissue. During low to moderate intensity exercise lipoprotein lipase (LPL) mediated hydrolysis of plasma TG also delivers a minor amount of FA to the total plasma FA concentration (Kiens *et al.* 1993; Morio *et al.* 2004; Sondergaard *et al.* 2011). The contribution of FA derived from plasma TG hydrolysis at higher exercise intensities remains, however, to be elucidated.

When whole body exercise was studied by the use of isotopically labelled long chain FAs in endurance trained men, the results confirmed the classical findings from the 1920s and 1930s that total FA oxidation was higher during moderate exercise at 65% of $\dot{V}_{O_2,peak}$ compared to exercise performed at 25% or 85% of $\dot{V}_{O_2,peak}$ (Romijn *et al.* 1993). Despite the relatively high rate of energy expenditure during exercise at high intensities, rate of disappearance (R_d) of plasma FAs and FA oxidation was decreased to values below those observed during moderate intensity exercise.

The inability of FA oxidation to support the energy demand during high intensity exercise could be reflected in either a failure of adipose tissue lipolysis, and thus insufficient delivery of FA to the exercising muscle, or a limitation in skeletal muscle to oxidize FAs. A failure in adipose tissue to supply the exercising muscle with sufficient FAs could be due to either a lack of stimulus to adipose tissue lipolysis or an inadequate perfusion of the adipose tissue. It has been shown that plasma catecholamine concentration, one of the major regulators of lipolysis in adipose tissue in humans, increases almost exponentially with exercise intensity (Galbo *et al.* 1975; Romijn *et al.* 1993). In the study by Romijn *et al.*, glycerol rate of appearance (R_a), which was used to determine adipose tissue lipolysis (Romijn *et al.* 1993), was not reduced during whole body exercise at 85% of $\dot{V}_{O_2,peak}$ compared to exercise at 65% of $\dot{V}_{O_2,peak}$ (Romijn *et al.* 1993) implying that adipose tissue lipolysis was not reduced at the high exercise intensity. An important point here is that R_a of glycerol reflects both adipose and muscle tissue lipolysis as well as LPL mediated hydrolysis of very low density lipoprotein (VLDL) bound TG. However, even though the net glycerol balance across skeletal muscle points towards glycerol release being substantial at rest (Stallknecht *et al.* 2004; Wallis *et al.* 2007), glycerol is both released and taken up by the leg resulting in low net release during exercise (van Hall *et al.* 2002; Stallknecht *et al.* 2004; Wallis *et al.* 2007). In a study performed by Stallknecht *et al.* (2004), the glycerol concentration in the interstitial space was measured using the microdialysis technique. Here it was demonstrated that the skeletal muscle interstitial glycerol concentration increased during low intensity exercise (25% of $\dot{V}_{O_2,peak}$), indicative of a net release of glycerol from muscle. However, a net release of glycerol did not occur at moderate and high intensity one legged knee extensor exercise (Stallknecht *et al.* 2004). In contrast, subcutaneous adipose tissue interstitial glycerol concentration, which was ∼10-fold higher than in skeletal muscle, increased with increasing intensities up to 85% of maximal leg work capacity (Stallknecht *et al.* 2004) supporting that the contribution from skeletal muscle to the arterial glycerol concentration during moderate and high intensity exercise is relatively small compared to that released from adipose tissue. At higher exercise intensities the high plasma catecholamine concentration can lead to inhibition of adipose tissue lipolysis by α-adrenergic mechanisms (Frayn, 2010). Moreover, the high sympatho-adrenal response during whole body exercise can induce a reduction in adipose tissue blood flow (Bulow & Madsen, 1981). This might explain why a decrease in long chain FA R_a from adipose tissue was observed during high intensity exercise compared to both low and moderate exercise (Romijn *et al.* 1993; van Loon *et al.* 2001). This coincided with a reduction in plasma FA concentration and oxidation. On the other hand, when plasma FA concentrations were increased to 2 mmol l^{-1} by infusion of a lipid emulsion and heparin (increasing the activity of LPL in plasma and thus VLDL-TG hydrolysis) during high intensity exercise, FA oxidation increased only 27% compared to exercise at the same intensity without infusion of intralipid. Importantly, FA oxidation was only partially restored when compared to levels observed at 65% of $\dot{V}_{O_2,peak}$ even though the plasma concentration of FAs was above 2 mmol l^{-1} (Romijn *et al.* 1995). These findings were further extended by van Loon *et al.* (2001), who reported a decrease in both plasma FA oxidation and total FA oxidation during high intensity exercise (72% $\dot{V}_{O_2,peak}$) compared to moderate intensities at 44% and 55% of $\dot{V}_{O_2,peak}$, despite no change in plasma FA availability. In addition, when whole body exercise was performed in healthy male volunteers the plasma concentration of FA decreased by 23% during high intensity workload (90% $\dot{V}_{O_2,peak}$) compared to an exercise workload of 65% $\dot{V}_{O_2,peak}$ (Kiens *et al.* 1999). Concomitantly with the decrease in plasma FA concentration at the high exercise intensity, an accumulation of intramyocellular FA was observed (Kiens *et al.* 1999). Together these findings strongly indicate that limitations in FA oxidation at high exercise intensities are not due to failure of adipose tissue to deliver FAs and that the decrease in FA oxidation during high exercise intensity is due to limitations within the muscle cell. The decline in plasma FA concentrations at the very high exercise intensities may to be coupled with an inability of muscle to use the FA.

Transsarcolemmal FA transport

Within the past years several membrane bound lipid binding proteins have been identified in human skeletal muscle and increasing evidence is emerging that these proteins either individually or in complexes act as regulators of FA transmembrane transport (Fig. 1). However, the mechanism by which this occurs is unknown. The 43 kDa membrane bound fatty acid binding protein (FABPpm) and the 88 kDa fatty acid translocase CD36 (FAT/CD36) proteins are currently the best described lipid binding proteins in human skeletal muscle (for detailed review see Glatz *et al.* 2010). Recent studies have suggested a role for FAT/CD36 in the acute increase in FA uptake in skeletal muscle seen in the transition from rest to exercise (Bonen *et al.* 2000; Jeppesen *et al.* 2011). This idea of FAT/CD36 as a dynamic regulator of FA uptake originates from Bonen *et al.* (2000), who showed that [^{3}H]palmitate transport into giant sarcolemmal vesicles (GSVs) was higher in GSVs from contracted rat muscle compared to resting muscle. Furthermore, this change was correlated with a contraction induced increase in membrane FAT/CD36 protein content (Bonen *et al.* 2000). In further support, it was shown that the contraction induced increase in FA oxidation was greater in isolated soleus muscle from transgenic mice over-expressing FAT/CD36 protein compared to their WT controls (Ibrahimi *et al.* 1999), even though resting FA oxidation was similar. This could indicate that a greater relocation of FAT/CD36 protein from an intracellular compartment to the plasma membrane during muscle contraction had occurred in the transgenic mice. In turn, this might have facilitated the higher flux in FA metabolism compared to WT mice.

The question is whether the transsarcolemmal transport is limiting for FA oxidation at higher exercise intensities. As mentioned above, an accumulation of intramyocellular FAs was observed in human vastus lateralis muscle when exercise intensity was increased from 65% $\dot{V}_{O_2,peak}$ to 90% $\dot{V}_{O_2,peak}$ despite a decrease in plasma FA concentration (Kiens *et al.* 1999), suggesting that the transport across the sarcolemma was not limiting FA oxidation at high exercise intensities. Data from studies in the perfused rat hindlimb model (Raney & Turcotte, 2006) have revealed a relation between FA uptake and oxidation, but only at low to moderate contraction intensities. When increasing to higher intensities, FA uptake was still elevated compared to basal levels, despite FA oxidation being decreased to resting values (Raney & Turcotte, 2006), supporting the

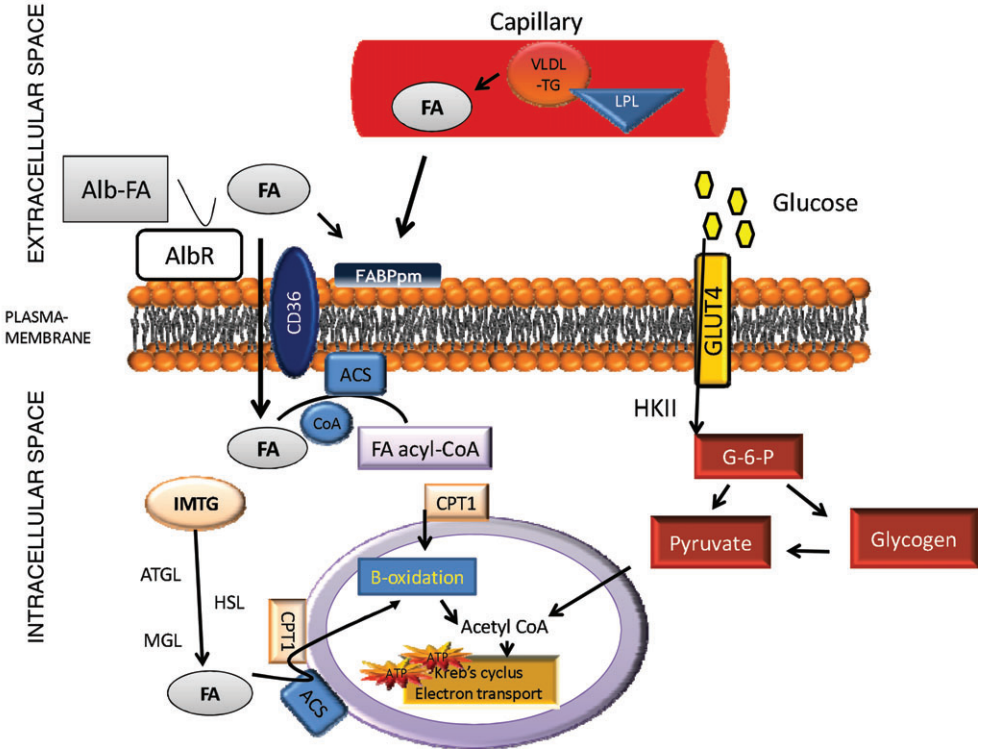

Figure 1. Simplified overview of lipid and glucose metabolic pathways in skeletal muscle
LPL, lipoprotein lipase; VLDL-TG, very low density lipoprotein triacylglycerol; FA, fatty acids; AlbFA, albumin bound fatty acids; AlbR, albumin receptor; FABPpm, plasma membrane fatty acid binding protein; CD 36, fatty acid translocase 36; ACS, acyl-CoA synthase; IMTG, intramyocellular triacylglycerol; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase, MGL, monoglycerol lipase; CPT-1, carnitine palmitoyltransferase; G-6-P, glucose-6-phosphate; HK II, hexokinase II.

notion that the transport of FAs across the membrane is not a limiting factor for FA oxidation when switching to higher exercise intensities.

Intramyocellular TG

Intramyocellular triacylglycerol (IMTG) stored within striated muscle cells represents a large energy source, contributing to FA oxidation. To what extent IMTG is utilized during exercise varies depending on intensity, duration and mode of exercise, dietary status, pre-exercise IMTG levels, training status of the subjects and sex (for review see Kiens, 2006). When applying the ^{1}H-MRS technique to male volunteers running at 60–70% of $\dot{V}_{O_2,peak}$, a decreased IMTG content in both the soleus and tibialis anterior muscles was observed, whereas running at 80–90% of $\dot{V}_{O_2,peak}$ did not cause changes in IMTG content in either muscle (Brechtel *et al.* 2001). Similarly, IMTG breakdown did not occur at high intensity exercise in the knee-extensor model (Stallknecht *et al.* 2004; Helge *et al.* 2007) when different methods for IMTG analysis were applied. However, these observations are all of net breakdown of IMTG. Recent findings from resting conditions in female and male subjects, using pulse–chase methods by intravenous infusions of two distinct isotopically labelled FAs combined with mass spectrometry measurements of intramuscular lipids, revealed that upon uptake by the muscle, plasma FA was not directly converted to long chain acylcarnitine (LCAC) and oxidized, but traversed the IMTG pool prior to oxidation (Kanaley *et al.* 2009). Whether FA taken up by muscle during exercise also undergoes esterification and then subsequent hydrolysis prior to mitochondrial entry is unknown.

Lipolysis of IMTG is regulated by adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoglyceride lipase (MGL) (Fig. 1). Only a few studies have looked at intensity-dependent lipase activity in skeletal muscle. Watt *et al.* (2003) showed that HSL activity, when measured in male subjects at three different exercise intensities (30%, 60% and 90% of $\dot{V}_{O_2,peak}$), were increased in all trials and did not differ between exercise intensities. Furthermore, HSL activation was shown to increase in untrained subjects from rest to exercise at 70% of $\dot{V}_{O_2,peak}$ and remained unchanged when increasing exercise intensity to ∼90% of $\dot{V}_{O_2,peak}$ (Kjaer *et al.* 2000). These observations are supported by our own findings that HSL activity was activated by exercise even at low exercise intensities (30% of $\dot{V}_{O_2,peak}$) with no further increase in activity at 60 and 87% of maximal oxygen uptake (Kiens B. and Alsted TJ., unpublished data). Despite HSL being activated by exercise, no significant hydrolysis of IMTG was detected (Kiens B. and Alsted TJ., unpublished data). This study is not the first to demonstrate dissociation between HSL activation and IMTG hydrolysis. Watt *et al.* (2004) showed that reduced plasma FA availability during exercise, induced by nicotinic acid ingestion, increased IMTG hydrolysis despite no HSL activation. An explanation for a disassociation between lipase activity and IMTG breakdown could be that allosteric regulators of HSL (the influence of which is not measured in the *in vitro* HSL activity assay) override the covalent regulation of HSL by phosphorylation of different serine residues (for review see Watt & Steinberg, 2008). Fatty acyl-CoA, an allosteric inhibitor of HSL, may inhibit the *in vivo* HSL activity especially during exercise at high intensity when intracellular accumulation of FAs has been shown to occur (Kiens *et al.* 1999). In the study by Watt *et al.* (2004), the decline in plasma FA concentration by nicotinic acid may have decreased the intramuscular fatty acyl-CoA concentration thereby relieving the allosteric inhibition of HSL and allowing for increased *in vivo* HSL activity. It is, however, remarkable that nicotinic acid in the study by Watt *et al.* (2004) on the one hand reduced lipolysis in adipose tissue and on the other increased lipolysis in skeletal muscle. These observations may give further support to the view that lipolysis is regulated differently in the two tissues (Watt & Steinberg, 2008). Although from these observations it seem unlikely that IMTG breakdown during high intensity exercise poses limitations for FA oxidation, this warrants further studies.

Mitochondrial metabolism

Long chain FAs taken up into cells are activated in the cytosol by reaction with CoA to yield long chain fatty acyl-CoA, an ATP consuming process catalysed by acyl-CoA synthetase (ACS) (Fig. 1). The active site of ACS has been located to the cytosolic surface of the peroxisomal endoplasmatic reticulum and outer mitochondrial membranes (Coleman *et al.* 2000). It was recently demonstrated in 3T3-L1 adipocytes that long chain ACS is an integral membrane protein also located in the plasma membrane (Gargiulo *et al.* 1999) and it was suggested that incoming long chain FAs are immediately esterified at the plasma membrane. This efficient esterification will maintain a low intracellular long chain FA concentration and contribute to uptake of long chain FAs.

Regulation of long chain FA entry into mitochondria is a highly regulated process, as acyl-CoA derivatives cannot cross the mitochondrial inner membrane directly. This is in contrast to short and medium chain FAs, which passively diffuse across the mitochondrial membranes. Long chain FAs first have to be converted to their acylcarnitine form, a reaction catalysed by carnitine palmitoyltransferase 1 (CPT-1) located at the outer mitochondrial membrane (Fig 2). Mitochondrial CPT-1 exist in two isoforms: the liver-type (L-CPT1) and muscle-type (M-CPT1). In skeletal muscle the M-CPT1

isoform is predominant (McGarry & Brown, 1997). The importance of CPT-1 in long chain FA oxidation was demonstrated when CPT-1 function was blocked by etomoxir resulting in a marked decrease in FA oxidation both *in vivo* and *ex vivo* (Hubinger *et al.* 1992; Dzamko *et al.* 2008). In addition, when the human muscle isoform of CPT-1 protein was electroporated into skeletal muscle of rats, an increase in maximal CPT-1 activity of ∼30% was paralleled by an increase of ∼24% in pamitoyl-CoA oxidation in isolated muscle mitochondria (Bruce *et al.* 2007). Earlier findings demonstrated that CPT-1 was potently regulated by malonyl-CoA (Bird & Saggerson, 1984) and a close relationship between malonyl-CoA concentration in muscle and decreased FA oxidation was observed in both humans and rats under resting conditions (Bavenholm *et al.* 2000; Chien *et al.* 2000). The formation of malonyl-CoA from acetyl CoA in skeletal muscle is catalysed by acetyl-CoA carboxylase (ACC). One type of regulation of ACC involves phosphorylation and inactivation by 5′-AMP-activated protein kinase (AMPK). During exercise AMPK and ACC phosphorylation are increased, which results in AMPK activation and in turn ACC inactivation (Richter & Ruderman, 2009). This will hypothetically lead to decreased muscle malonyl-CoA content during exercise and, in turn, increased CPT-1 activation, resulting in increased long chain FA oxidation. However, FA oxidation measured at rest and during isolated muscle contractions (Dzamko *et al.* 2008), and during whole body exercise (Dzamko *et al.* 2008; Miura *et al.* 2009) was similar in mice with a genetically reduced AMPKα2 activity as in wild-type (WT) mice. These findings were supported by O'Neill *et al.* (2011), who demonstrated that FA oxidation during exercise, evaluated by RER, was higher in mice with abolished AMPK activity (muscle specific β1 and β2 double knockout (KO)) compared to WT mice, indicating that AMPK is not a major regulator of FA oxidation during exercise in skeletal muscle. Less genetic evidence is available on the role of ACC2, the main isoform of ACC in muscle, in regulation of FA oxidation. But ACC2 deletion in mice did not affect malonyl-CoA content in muscle or RER under resting condition (Choi *et al.* 2007; Olson *et al.* 2010), indicating that either overcompensation by ACC1 had occurred or other mechanisms were responsible for regulating FA oxidation in these mice. The relationship between malonyl-CoA and FA oxidation observed at rest is less

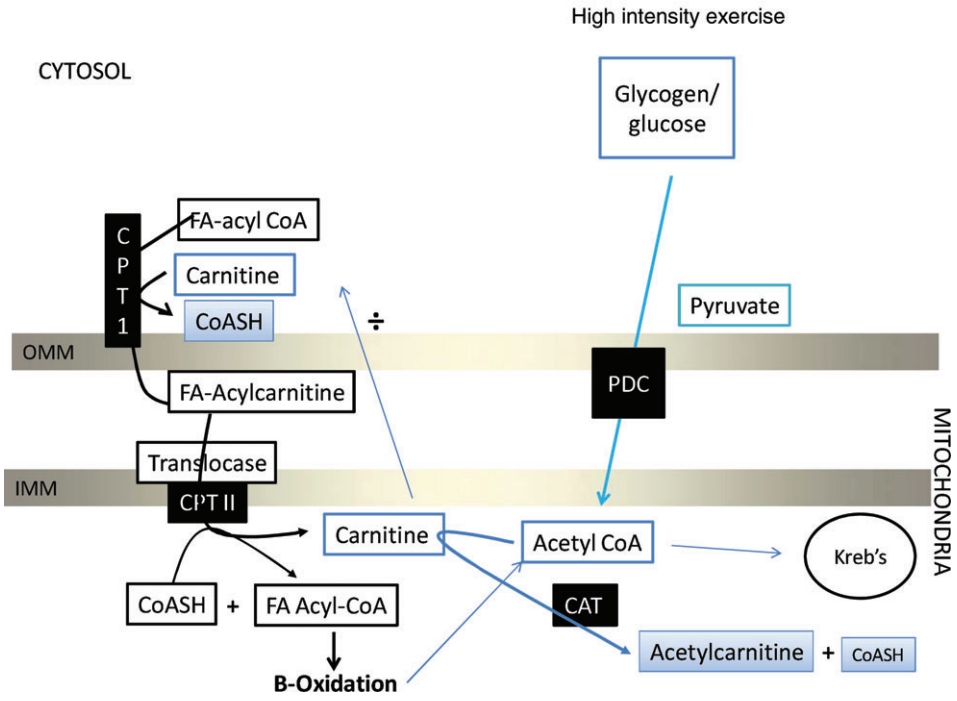

Figure 2. Schematic overview of a proposed interaction between fatty acid metabolism and glycolysis in skeletal muscle during high intensity exercise
At high exercise intensity the high glycolytic rate will cause a production of acetyl CoA which exceeds the rate of the Krebs cycle. Free carnitine acts as an acceptor of the acetyl groups forming acetylcarnitine, mediated by the enzyme canitine acyltransferase. This leaves less free carnitine, substrate for CPT-1, whereby forming of acylcarnitine will be reduced and less FA-acyl will be available for β-oxidation resulting in reduced FA oxidation. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; CPT-1, carnitine palmitoyltransferase; FA, fatty acids: CPT II, carnitine palmitoyltransferase II; PDC, pyruvate dehydrogenase complex; CAT, carnitine acyltransferase.

clear during exercise in humans. In a series of experiments a discrepancy between malonyl-CoA concentrations and FA uptake and FA oxidation in human subjects during exercise has been demonstrated (Odland *et al.* 1996, 1998; Dean *et al.* 2000; Roepstorff *et al.* 2005). When whole body exercise intensity was increased from 65% to 90% of $\dot{V}_{O_2,peak}$ in male subjects, muscle malonyl-CoA content did not change, despite FA oxidation, determined by RER, being markedly decreased (Odland *et al.* 1998). This notion was supported by Dean *et al.* (2000), who showed that increasing knee extensor exercise intensity from 60% to 85% of leg work capacity and further until exhaustion was accompanied by a reduction in muscle malonyl-CoA content, despite RER values concomitantly increasing from 0.84 to 0.99. More recent findings, where pre-exercise muscle glycogen levels were manipulated to induced either high or low FA oxidation during exercise at 65% of $\dot{V}_{O_2,peak}$ (Roepstorff *et al.* 2005), showed marked differences in FA oxidation during exercise without differences in muscle malonyl-CoA content. Taken together, this suggests that malonyl-CoA content is not the major regulator of FA oxidation in working muscle. It should be noted that it is unknown whether local changes in malonyl-CoA concentration in compartments close to mitochondria within the muscle, rather than whole muscle content, have effects on FA oxidation.

Besides the effect of carnitine in mediating FA entry into mitochondria, studies in the 1950s on blowfly muscle revealed that carnitine also serves another important metabolic role. The flight muscle from flies is one of the richest sources of carnitine and at the same time these insects do not oxidize FAs when in flight (Childress *et al.* 1967). When the blowfly flight muscle was studied under flight the concentration of acetylcarnitine increased 4-fold on initiation of flight, which paralleled the increase in pyruvate concentration (Childress *et al.* 1967). From these studies it was proposed that carnitine could act as an acceptor of acetyl groups from acetyl-CoA, by forming acetylcarnitine, a reaction catalysed by the mitochondrial enzyme carnitine acetyltransferase (CAT), when acetyl-CoA was generated faster than utilized by the Krebs cycle. In this way, CoASH can be regenerated permitting glycolysis to proceed to acetyl-CoA. These early findings were later supported by findings in both animal and human skeletal muscle. Indeed, it has been shown in several studies in humans that with increasing exercise intensities, muscle acetylcarnitine content was increased (Sahlin, 1990; Constantin-Teodosiu *et al.* 1991; Odland *et al.* 1998; van Loon *et al.* 2001) concomitantly with a decrease in the free carnitine content (Sahlin, 1990; Constantin-Teodosiu *et al.* 1991; van Loon *et al.* 2001). In the review from Stephens *et al.* (2007), compiled results from four different studies showed that a short bout of exercise (4 min) at different exercise intensities was followed by a gradual decrease in the vastus lateralis muscle free carnitine content from ∼75% of the total muscle carnitine pool at rest to ∼20% at 75–100% $\dot{V}_{O_2,peak}$. In addition, data revealed that acetylcarnitine content accounted for the decrease in free carnitine with high intensity exercise. On the other hand, at low exercise intensities neither free carnitine nor acetylcarnitine content was changed compared to resting values (Stephens *et al.* 2007). These findings further support the notion that carnitine acts as the acceptor for the acetyl groups, by forming acetylcarnitine, when the rate of acetyl-CoA formation from glycolysis at high intensities is in excess of its utilization by the Krebs cycle. On the other hand, since CPT-1 activity is dependent on the presence of carnitine (McGarry *et al.* 1983; Harris *et al.* 1987), a low muscle content of free carnitine is supposed to reduce the activity of CPT-1. Consequently this will lead to a diminished supply of long chain FA CoA to β-oxidation, limiting long chain FA oxidation during high intensity exercise. The importance for carnitine in long chain FA oxidation in skeletal muscle is evident from the findings of an 85% reduced carnitine content and a 75% reduced FA oxidation in skeletal muscle of patients with lipid storage myophathy compared with healthy controls, despite similar levels of CPT-1 and palmityl thiokinase in patients and control subjects (Engel & Angelini, 1973). Thus, an increased availability of pyruvate, acetyl-CoA formation, and 'binding' of the free carnitine during high intensity exercise also provide a potential mechanism whereby FA oxidation is down-regulated (Fig. 2). On the other hand, K_m of CPT-1 for carnitine in isolated mitochondria from human skeletal muscle is ∼0.5 mM (McGarry *et al.* 1983). Thus, with the usual fluctuations in carnitine content in skeletal muscle of healthy humans between 1 and 4 mM it is not expected to influence CPT-1 activity as CPT-1 would be saturated with carnitine at all exercise intensities. However, partitioning of carnitine between the cytosol and the mitochondrial matrix makes it difficult to estimate the absolute carnitine concentration near CPT-1 and furthermore extrapolation of *in vitro* enzyme kinetics to *in vivo* conditions is fraught with assumptions making it difficult to judge the relevance of such measures.

Recently we have shown (Roepstorff *et al.* 2005) that when pre-exercise muscle glycogen stores were high, FA oxidation was reduced by 2.5-fold during 60 min of moderate intensity exercise (65% $\dot{V}_{O_2,peak}$) compared to when pre-exercise glycogen levels were low. This was paralleled by low free carnitine levels in muscle during the high glycogen trial whereas the free carnitine content was high during the low glycogen trial (Roepstorff *et al.* 2005). These findings give support to the notion that a reduction in cellular free carnitine will limit the ability of CPT-1 to transport long chain FAs into the mitochondria, and thus also the rate of long chain FA oxidation at moderate exercise intensities.

In a recent study by Wall *et al.* (2011), 14 healthy male volunteers were given carnitine supplementation (together with carbohydrates) for 24 weeks resulting in an increase in muscle total carnitine by 21%. This increase in total carnitine content was linked to a 55% reduction in muscle glycogen utilisation during exercise at 50% $\dot{V}_{O_2,peak}$ compared with controls not supplemented with carnitine. In addition, the study revealed an 80% greater muscle free carnitine content and a 31% lower activity in the pyruvate dehydrogenase complex (PDC) during exercise after carnitine supplementation compared to control. This suggests an increased FA oxidation during exercise at 50% of $\dot{V}_{O_2,peak}$, but this was unfortunately not measured in the study. When exercise was subsequently increased to 80% $\dot{V}_{O_2,peak}$, no differences were obtained between the groups in glycogen utilisation, but muscle lactate content was ∼44% lower in the carnitine supplemented trial than in the control trial (Wall *et al.* 2011). These findings indicate that at high intensities the formation of acetyl-CoA, probably mostly generated from a high glycolytic flux, is captured by carnitine and thereby prevents a product inhibition of PDC activation, by an increased acetyl-CoA/CoASH ratio (Cooper *et al.* 1975). Support for this are their findings (Wall *et al.* 2011) of a greater activity in PDC (38%) and a greater acetylcarnitine content (16%) in skeletal muscle during exercise at 80% $\dot{V}_{O_2,max}$ in the carnitine supplemented trial than in control. As RER or other measurements of FA oxidation were not performed in the study by Wall *et al.*, it is not possible from these findings to evaluate the influence of carnitine supplementation on FA oxidation either at the moderate or at the high exercise intensities. Thus, it cannot be ruled out that high availability of carnitine might increase FA oxidation during high intensity exercise as well.

A clue to understanding the regulation of FA oxidation during high intensity exercise may be obtained from comparison of metabolism during whole body exercise to exercise with a limited muscle mass like the knee-extensors. Whereas it is well established that FA oxidation during exercise decreases at exercise intensities above ∼65% of $\dot{V}_{O_2,peak}$, as discussed above, different results are obtained with one-legged knee-extensor exercise (Dean *et al.* 2000; Helge *et al.* 2007). Thus, when exercise was allocated to the knee-extensors, plasma FA oxidation, measured by constant infusion of [U-^{13}C]palmitate, increased with increasing exercise intensities from 25% up to 85% of maximal leg work capacity (Helge *et al.* 2007). Furthermore, total FA oxidation increased from rest to exercise and remained unchanged during increasing exercise intensities (Helge *et al.* 2007). In addition, Dean *et al.* (2000) showed (by measuring RER) that FA oxidation was unchanged from 65 to 85% of knee-extensor maximum work capacity but decreased by 34% compared to at 85% when exercise intensity was increased to 100%. Thus when performing exercise with a limited muscle mass it appears that muscle is able to oxidize FA at much higher relative exercise intensities than during bicycle ergometer exercise when more and large muscle groups are involved. How is this explained?

While this cannot be answered conclusively, we offer the following hypothesis. During exercise with a limited muscle mass at 80% of peak leg work capacity there is hardly any increase in plasma catacholamine concentrations compared to rest (Richter *et al.* 1988), whereas substantial increases are observed when heavy exercise is performed with more muscle mass (Galbo *et al.* 1975). The low hormonal response during knee-extensor exercise may limit glycogen breakdown (Richter *et al.* 1982), and thus glycolytic flux, compared to heavy whole body exercise and therefore limit the production of acetylcarnitine. In consequence free carnitine availability and therefore CPT-1 activity may be preserved better than during whole body exercise. In addition, when performing one-legged exercise, muscle blood flow is excessive compared to flow during whole body exercise (Saltin, 1985) and this 'superperfusion' is likely to create conditions in the muscle that favour oxidative ATP production, and thus limit increases in ADP and AMP. This lesser disturbance in energy status of the cell will in turn cause less stimulation of glycolysis. As mentioned above, this again preserves free carnitine in the muscle and therefore creates favourable conditions for FA oxidation. This could explain why FA oxidation is maintained at higher exercise intensities during knee-extensor exercise compared with whole body exercise. In fact the metabolic conditions in the muscle during one-legged exercise may resemble conditions after endurance training where better metabolic control is achieved and decreased glycolytic flux leads to increased FA oxidation at the same absolute work load (Holloszy & Coyle, 1984).

Conclusion

FA oxidation during exercise is subject to multiple possible regulatory steps, ranging from adipose tissue lipolysis to mitochondrial metabolism in skeletal muscle. However, when focusing on limitations of FA oxidation in the transition from moderate to higher intensity exercise, one possibility could be product inhibition from the β-oxidation pathway, but evidence for this is not substantial. It seems that the most attractive regulatory candidate for FA oxidation is the muscle metabolite carnitine, which is essential in CPT-1 regulation and, in turn, FA oxidation. At high intensity exercise the rapid glycolysis provides the mitochondria with excess acetyl-CoA, which is buffered by free carnitine to form acetylcarnitine. Accordingly a fall in muscle concentration of free carnitine may reduce CPT-1 activity, and thus the

ability to transport FA into the mitochondria and therefore also the rate of FA oxidation. In this way, rapid glycogen breakdown and glycolysis are suggested to have a major impact on inhibiting FA oxidation. The absence of any other rigorously identified mechanisms for decreasing FA oxidation during high intensity exercise makes us believe that carnitine is the major direct regulator of FA oxidation in the transition from moderate to higher intensity exercise.

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