Regulation of Fat Metabolism in Skeletal Muscle

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ABSTRACT: Regulation of carbohydrate and fat utilization by skeletal muscle at rest and during exercise has been the subject of investigation since the early 1960s when Randle *et al.* proposed the so-called glucose–fatty acid cycle to explain the reciprocal relationship between carbohydrate and fat metabolism. The suggested mechanisms were based on the premise that an increase in fatty acid (FA) availability would result in increased fat metabolism and inhibition of carbohydrate metabolism. Briefly, accumulation of acetyl-CoA would result in inhibition of pyruvate dehydrogenase (PDH), accumulation of citrate would inhibit phosphofructokinase (PFK), and accumulation of glucose-6-phosphate (G6P) would reduce hexokinase (HK) activity. Ultimately, this would inhibit carbohydrate metabolism with increasing availability and oxidation of FA. Although there is some evidence for the existence of the glucose-FA cycle at rest and during low-intensity exercise, it cannot explain substrate use at moderate to high exercise intensities. More recently, evidence has accumulated that increases in glycolytic flux may decrease fat metabolism. Potential sites of regulation are the transport of FA into the sarcoplasma, lipolysis of intramuscular triacylglycerol (IMTG) by hormone-sensitive lipase (HSL), and transport of FA across the mitochondrial membrane. There are several potential regulators of fat oxidation: first, malonyl-CoA concentration, which is formed from acetyl-CoA, catalyzed by the enzyme acetyl-CoA carboxylase (ACC), which in turn will inhibit carnitine palmitoyl transferase I (CPT I). Another possible mechanism is accumulation of acetyl-CoA that will result in acetylation of the carnitine pool, reducing the free carnitine concentration. This could theoretically reduce FA transport into the mitochondria. There is also some recent evidence that CPT I is inhibited by small reductions in pH that might be observed during exercise at high intensities. It is also possible that FA entry into the sarcolemma is regulated by translocation of FAT/CD36 in a similar manner to glucose transport by GLUT-4. Studies suggest that the regulatory mechanisms may be different at rest and during exercise and may change as the exercise intensity increases. Regulation of skeletal muscle fat metabolism is clearly multifactorial, and different mechanisms may dominate in different conditions.

KEYWORDS: exercise; fat; pyruvate dehydrogenase; phosphofructokinase; glucose-6-phosphate; carbohydrate metabolism

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INTRODUCTION

At rest and during exercise, skeletal muscle is the main site of oxidation of fatty acid (FA). In resting conditions and especially after fasting, FAs are the predominant fuel used by skeletal muscle. During low-intensity exercise, metabolism is elevated severalfold compared to resting conditions, and fat oxidation is increased. When the exercise intensity increases, fat oxidation increases further, until exercise intensities of about 65% VO_2max, after which a decline in the rate of fat oxidation is observed. In contrast to carbohydrate metabolism, which increases as a function of the aerobic work rate, fat oxidation is reduced at the high exercise intensities (FIG. 1). The changes in fat oxidation as a function of the exercise intensity have recently been described by Achten *et al.*[1] in a group of trained individuals. Given the amount of work done in this area, it is somewhat surprising that this is the first study to report changes in fat oxidation over a wide range of exercise intensities. Although large individual variation was observed, on average, maximal fat oxidation was reported at 64% VO_2max, after which fat oxidation decreased relatively rapidly. It was also concluded that fat oxidation was high over a wide range of intensities, but declined rapidly at high intensities (>80% VO_2max).[1] It is likely that this curve will shift to the right after (endurance) training and shift to the left after detraining. Research is currently being conducted to study the shape of this curve in response to training and other interventions.

This review will discuss potential mechanisms responsible for the upregulation of fat metabolism in the transition from rest to exercise and the downregulation of fat metabolism from low and moderate to high exercise intensities. Although factors

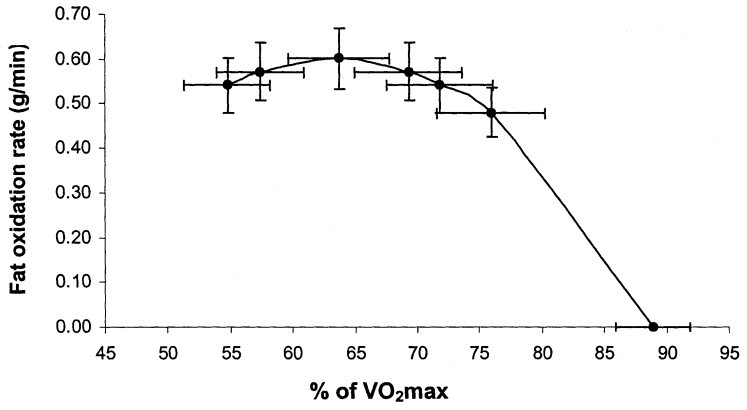

FIGURE 1. Fat oxidation as a function of exercise intensity in trained subjects. From low to moderate intensities, fat oxidation increased, peaked at 63% VO_2max, and decreased at high intensities. (From reference 1, reproduced with permission.)

outside skeletal muscle may influence fat oxidation (i.e., plasma FA concentration), this paper will mainly focus on the factors inside skeletal muscle that turn on skeletal muscle fat oxidation and inhibit fat oxidation. The data presented are based on human skeletal muscle where possible; however, when no information was available in humans, the results of rat studies were used.

FAT UTILIZATION AT REST AND DURING EXERCISE

After an overnight fast, most of the energy requirement at rest is covered by the oxidation of FAs derived from adipose tissue. Lipolysis in adipose tissue is mostly dependent on the concentrations of hormones (epinephrine to stimulate lipolysis and insulin to inhibit lipolysis). When exercise is initiated, the rate of lipolysis and the rate of FA release from adipose tissue are increased. During moderate-intensity exercise, lipolysis increases approximately threefold,[66] mainly because of an increased β-adrenergic stimulation. In addition, during moderate-intensity exercise, the blood flow to adipose tissue is doubled and the rate of reesterification is halved.[48,66] Also, blood flow in skeletal muscle is increased dramatically and therefore the delivery of FAs to the muscle is increased severalfold. During the first 15 min of exercise, plasma FA concentrations usually decrease because the rate of FA uptake by the muscle exceeds the rate of FA appearance from lipolysis. Thereafter, the rate of appearance is in excess of the utilization by muscle, and plasma FA concentrations increase. The rise in plasma FA concentration depends on the exercise intensity and the duration of exercise. During moderate exercise, FA concentrations may reach 1 mmol/L within 60 min of exercise; however, at higher exercise intensities, the rise in plasma FA is very small or may even be absent.

Romijn *et al.*[48] investigated fat metabolism at three different exercise intensities, 25%, 65%, and 85% VO_2max, and used stable isotopic tracers to measure maximum plasma FA oxidation and nonplasma FA oxidation. In their paper, the difference between total fat oxidation (measured by indirect calorimetry) and maximum plasma FA oxidation (determined by a ^{2}H-palmitate tracer) was referred to as intramuscular triacylglycerol (IMTG) oxidation. This assumes that plasma triacylglycerol (TG) and intermuscular fat (adipocytes in between muscle fibers) are not important fuels during exercise. However, some have suggested that these sources may contribute significantly to energy expenditure[23,24] and therefore it would be more correct to refer to this calculated fraction as TG oxidation (intramuscular and plasma) (FIG. 2). It must also be emphasized that the data by Romijn *et al.*[48] are imprecise. The data are based on calculations of the rate of disappearance of FA (Rd FA) and the assumption that all FAs taken up by the muscle are also oxidized. It has been shown that this is not the case,[53] and Rd FA thus overestimates plasma FA oxidation. In addition, all calculations are based on respiratory exchange ratios, and small changes in the ratio can have significant effects on calculated rates of TG oxidation. Furthermore, this study was conducted in well-trained individuals after an overnight fast, which may have forced the muscle to use more fat than would normally be the case. However, it is generally believed that the trends shown in this paper reflect the actual changes in substrate use that occur during exercise (FIG. 2).

The data from Romijn *et al.*[48] suggest that lipolysis increases from rest to exercise and from 25% to 65% VO_2max. However, at 85% VO_2max, there is no further

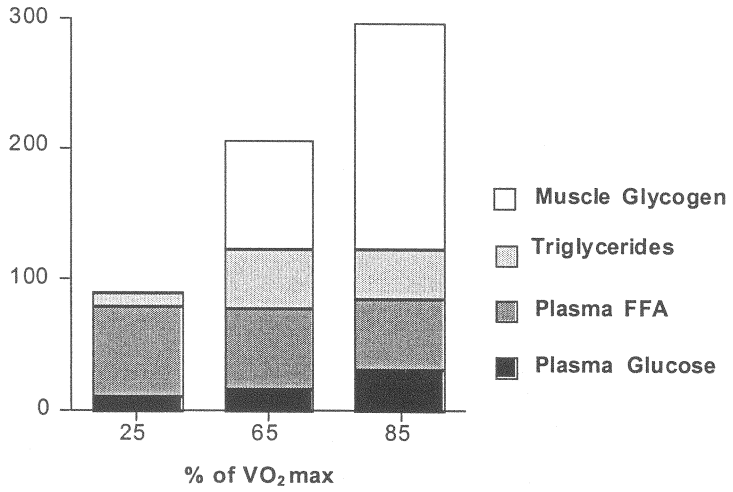

FIGURE 2. Energy expenditure increases with exercise intensity and there are substantial changes in substrate utilization. Fat oxidation increases at moderate intensities and decreases at high intensities. The contribution of plasma FA seems to decrease slightly with increasing intensity, whereas TG provides a more important substrate at 65% VO_2max. Plasma glucose and especially muscle glycogen become more important as the exercise intensity increases. Figure adapted from reference 48.

increase of lipolysis, and lipolytic rates are similar to those at 65% VO_2max. It is likely, however, that the FA availability at 85% VO_2max is reduced, as evidenced by 50% lower plasma FA concentrations after 30 min of exercise.[48] If we assume that blood flow to the working muscle is proportional to the exercise intensity, this must mean that, at 85% VO_2max, fewer FAs are delivered to the muscle, and this could contribute to the observed lower rates of fat oxidation. Indeed, Romijn *et al.*[48] reported a reduced Rd FA at 85% VO_2max.

At low intensities (25% VO_2max), most of the FAs oxidized are plasma-derived and TG oxidation is negligible (FIG. 2). However, when the exercise intensity is increased to 65% VO_2max, the contribution of plasma FAs is similar, but a significant increase in the contribution of TG can be observed that provides about half of the FAs used for total fat oxidation.[48] When the exercise intensity is further increased, fat oxidation decreases even though the rate of lipolysis is still high.[48] The blood flow to the adipose tissue may be decreased (due to sympathetic vasoconstriction) and this may result in a decreased removal of FAs from adipose tissue. It has also been suggested that, during high-intensity exercise, lactate accumulation may decrease lipolysis or increase the rate of reesterification of FAs.[6] However, a recent study using microdialysis probes in adipose tissue concluded that lactate did not impair lipolysis as no differences were found when lactate was infused or saline.[60]

Although the causes are still not entirely clear, plasma FA concentrations are usually low during intense exercise. However, this decreased availability of FA can only partially explain the reduced fat oxidation that is observed in these conditions.

EFFECT OF INCREASED AVAILABILITY OF FATTY ACIDS AT REST AND LOW/MODERATE-INTENSITY EXERCISE

One of the important factors determining substrate utilization at rest and during exercise is the availability of substrates. It has been repeatedly shown that fat oxidation can be increased when the availability of FA is increased.[8,64] It was originally thought that the classical glucose-FA cycle[45] could explain this reciprocal relationship between carbohydrate and fat metabolism (FIG. 3). This theory states that an increase in plasma FA concentration will result in an increased uptake of FAs. These FAs will undergo β-oxidation in the mitochondria where they will be broken down to acetyl-CoA. An increasing concentration of acetyl-CoA (or increased acetyl-CoA/CoA ratio) would inhibit pyruvate dehydrogenase (PDH), which is responsible for the breakdown of pyruvate to acetyl-CoA. Also, increased formation of acetyl-CoA from FAs would increase muscle citrate levels and, after diffusing into the sarcoplasm, these could inhibit phosphofructokinase (PFK), the rate-limiting enzyme in glycolysis. The effect of increased acetyl-CoA and citrate levels will therefore be a reduction in the rate of glycolysis. This, in turn, may cause accumulation of glucose-6-phosphate (G6P) in the muscle, which will inhibit hexokinase (HK) activity and thus reduce muscle glucose uptake.

Most of the evidence for the existence of the glucose-FA cycle is from isolated muscle experiments and *in vitro* studies of diaphragm or heart muscle.[14,44,45] There is relatively little information about skeletal muscle [47] and even less information in human skeletal muscle.

Odland *et al.*[35] investigated the metabolic effects of increasing FA availability by infusing TG (Intralipid®) plus heparin during low- and moderate-intensity exercise (40% and 65% VO_2max, respectively). The elevated FA levels resulted in a 4-fold increase in leg FA uptake and in a 23% reduction in glycogenolysis. The study provides therefore evidence for a shift from carbohydrate toward fat metabolism. However, in this study, the increased FA availability did not affect leg glucose uptake and the authors thus concluded that the regulation must take place at the level of glycogen phosphorylase or PDH (glycogen breakdown). Measurements of the active form of PDH (PDHa) were consistently lower with elevated FA. The PDH is phosphorylated (deactivated) by PDH kinase and dephosphorylated (activated) by PDH phosphatase.[46] The main regulator of the PDH phosphatase is Ca^{2+}, but it is unlikely that this is an important regulator of the transformation to PDHa because increases in Ca^{2+} during exercise would be similar with or without elevated FA concentrations. PDH kinase is inhibited by pyruvate and activated by high ATP, acetyl-CoA, and NADH levels at rest.[42] Odland *et al.*[35] showed that acetyl-CoA and ATP were similar with or without elevated plasma FA concentrations. A reduced pyruvate concentration and a higher NADH concentration in the presence of high FA availability have been suggested to relieve some of the inhibition of PDH kinase, resulting in a reduced activation of PDH.[56]

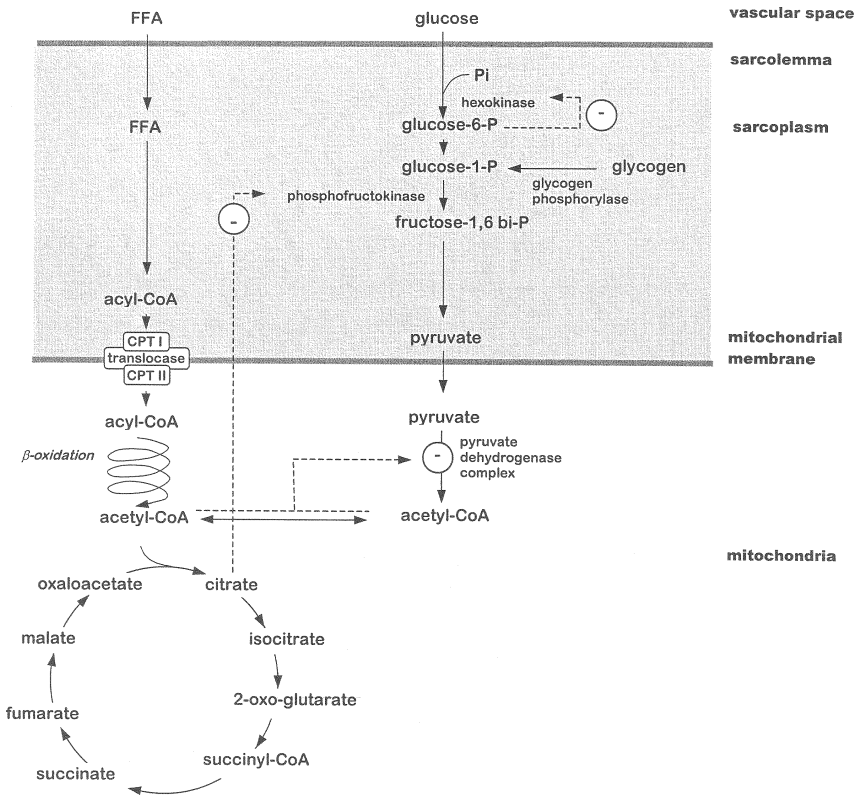

FIGURE 3. The glucose-FA or Randle cycle (adapted from reference 45) describes potential mechanisms involved in the interaction between fat and carbohydrate metabolism. In this model, FA availability seems to be the dominant factor. Briefly, increased FA availability would increase acetyl-CoA concentrations; accumulation of acetyl-CoA would result in inhibition of pyruvate dehydrogenase (PDH); accumulation of citrate would inhibit phosphofructokinase (PFK); and accumulation of glucose-6-phosphate (G6P) would reduce hexokinase (HK) activity. Ultimately, this would inhibit carbohydrate metabolism with increasing availability and oxidation of FA. Although there is some evidence for the existence of the glucose-FA cycle at rest and during low-intensity exercise, it cannot explain substrate use at moderate to high exercise intensities.

Thus, although the increased FA availability seemed to cause a shift in substrate metabolism at low and moderate exercise intensities, this did not seem to be through mechanisms as proposed by the glucose-FA cycle. Although a decreased activation of PDH was observed, this could not be explained by increases in acetyl-CoA concentrations as originally proposed. Increases in muscle citrate concentration were observed, but these may have been too small to significantly affect PFK activity *in vivo*.[41] Finally, no changes in glucose uptake were observed. It is therefore not entirely clear which factors cause the reduction in glycogenolysis in the presence of a high FA concentration at low and moderate intensities.

EFFECT OF INCREASED AVAILABILITY OF FATTY ACIDS DURING HIGH-INTENSITY EXERCISE

Dyck *et al.*[12,13] investigated a similar question at high exercise intensities (80%–85% VO_2max). A 45% reduction of glycogenolysis was observed after 15 min of cycling in the presence of an elevated plasma FA concentration (1.3–1.4 mmol/L). Muscle citrate and acetyl-CoA were unaffected, suggesting that regulation would take place at the level of phosphorylase. It was also observed that, with increased FA availability, there is a reduction of intramuscular free Pi and AMP accumulation during exercise, possibly because of a greater accumulation of mitochondrial NADH. This suggests a better match between ATP production and ATP breakdown. Since Pi and AMP are known to stimulate the enzyme, glycogen phosphorylase, it is possible that the reduction in Pi and AMP levels is at least partially responsible for the reduced muscle glycogen breakdown. No changes were observed in PDH, but glycogen phosphorylase was inhibited with increased FA availability.

In conclusion, at high-intensity exercise, increased FA availability results in a decreased breakdown of muscle glycogen. However, there is no evidence that this process is through the glucose-FA cycle. Allosteric regulators (Pi and AMP) seem to play a major role in the regulation of glycogen breakdown at the level of glycogen phosphorylase.

There is also an alternative explanation for reduced muscle glycogen breakdown after elevation of plasma FA concentrations in some studies.[8,64] When studying the plasma FA concentrations in more detail, it appears that in these studies plasma FAs were significantly elevated (by infusion of TG and injection of heparin) compared to the control condition. The plasma FA concentrations in the control condition, however, were very low (<0.2 mmol/L). It is conceivable that these FA levels are too low to provide the muscle with sufficient fat substrate. As a result of depriving the muscle of fat substrate, muscle glycogen breakdown may have been increased in the control condition. If this is true, the observed "sparing" of glycogen with the high FA concentrations was really caused by an increased breakdown of glycogen in the control condition. It is known that blocking lipolysis and reducing FA availability by giving nicotinic acid or a derivative will increase muscle glycogen breakdown during exercise.[19]

Another situation in which FA availability is reduced is during high-intensity exercise.[12,13,49,54] However, it has been shown that this decreased availability of FAs can only partially explain the reduced fat oxidation that is observed in these conditions. Romijn *et al.*[49] studied endurance-trained cyclists during high-intensity exercise (85% VO_2max). As observed in a previous study,[48] the plasma FA concentration and the rate of appearance of FA (Ra FA) were very low at this intensity.[49] When FA concentrations were restored to levels observed at moderate exercise intensities by infusing Intralipid® and heparin, fat oxidation was only slightly increased, but still lower than at moderate intensities.

This strongly suggests that FA oxidation is at least partly regulated at the muscular level. Further evidence for regulation of FA utilization at the muscular level is derived from tracer studies.[9,54] These studies investigated the oxidation of medium-chain fatty acids (MCFAs) versus long-chain fatty acids (LCFAs) when glycolytic flux is high. In the study by Coyle *et al.*[9] the glycolytic rate was increased by pre-exercise glucose feeding, whereas in the study by Sidossis *et al.*[54] this was achieved

by increasing the exercise intensity from 40% to 80% VO_2max. Sidossis *et al.*[54] infused a ^{14}C-labeled MCFA (octanoate) and a ^{13}C-labeled LCFA (oleate). The percentage of the labeled LCFAs taken up and oxidized decreased at the high exercise intensity. There was, however, no difference in the oxidation of the MCFAs. Since these MCFAs are not as dependent on transport proteins or the specific MCFA transport proteins are not as heavily regulated, this suggests that the transport across membranes is involved in the regulation of FA oxidation and might at least partly explain the reduced fat oxidation at high exercise intensities. Sidossis *et al.*[54] argued that their results provided indirect evidence for regulation at the level of CPT I; however, regulation at the level of transport across the sarcolemma cannot be excluded, as will be discussed below.

Taken together, these studies suggest that FA availability (lipolysis and removal of FAs from the adipose tissue and blood flow to the working muscle) is a major regulatory factor. During low and moderate intensities, there is some evidence for the existence of the glucose-FA cycle. However, most of the regulation seems to be through deactivation of phosphorylase and possibly PDH. During high-intensity exercise, regulation seems to be solely through phosphorylase. Although FA availability plays an important role and fat oxidation may thus be partly regulated at the adipose tissue level, there is also evidence for regulation at the muscular level. The potential regulatory sites inside skeletal muscle will be discussed below in more detail.

POTENTIAL SITES OF REGULATION OF FAT METABOLISM IN SKELETAL MUSCLE

There are a few potential sites for the regulation of fat metabolism inside skeletal muscle (FIG. 4). These include (1) the transport of FAs from the vascular space across the sarcolemma into the sarcoplasm, (2) the release of FAs from IMTG under the influence of a hormone-sensitive lipase, and (3) the transport of FAs across the mitochondrial membrane, involving the enzyme, carnitine palmitoyl transferase I (CPT I). Once in the mitochondrial matrix, the fatty acyl-CoA is subjected to β-oxidation, a series of reactions that splits a two-carbon acetyl-CoA molecule of the multiple-carbon FA chain. This pathway is not believed to be a regulatory site as none of the enzymes seem to be acutely regulated.

TRANSPORT OF FATTY ACIDS INTO THE MUSCLE

For a long time it was believed that the transport of FAs into the muscle cell was a passive process. This was based on early observations that FA uptake increased linearly with FA concentration.[17] However, recently, specific carrier proteins have been identified in various tissues, including skeletal muscle (FIG. 4). In the sarcolemma, two proteins have thus far been identified that are involved in the transport of FAs across the membrane. These proteins are a specific plasma membrane FA-binding protein (FABPpm) and a FA translocase protein (FAT/CD36). A third protein has been identified (FA transport protein, FATP), but its transport role has been questioned because FATP content in the plasma membrane was inversely correlated

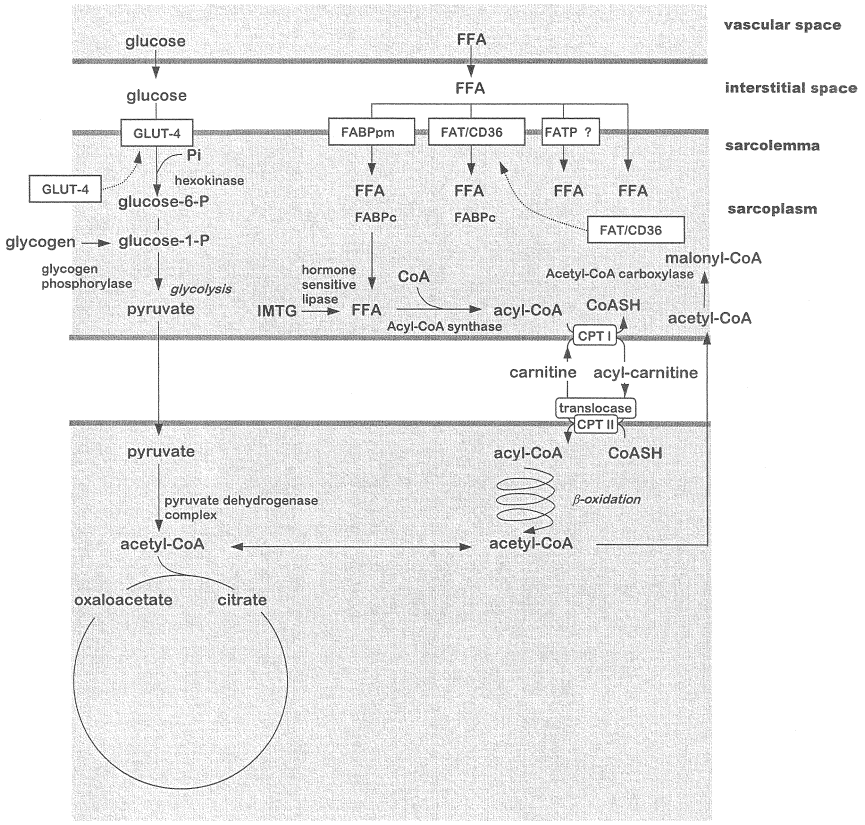

FIGURE 4. Transport of glucose and FAs from the vascular space to the mitochondria and their subsequent oxidation. Although it is possible that some FAs diffuse through the sarcolemma, the majority of the transport of FAs into the mitochondria involves transport proteins. Three candidates have been identified. FAT/CD36 translocates from its intracellular storage space to the membrane in the same manner that GLUT-4 translocates.

with LCFA uptake,[29] whereas positive correlations were found for FABPpm and FAT/CD36.

There is also physiological evidence for the existence of such transporters. In studies with isolated perfused hindlimbs, Turcotte *et al.* [61] showed that a saturation of FA transport occurred with increasing free palmitate concentration in the perfusion medium. Similar data were obtained in giant sarcolemmal vesicles of type I and type II muscle fibers.[4] These data make it highly likely that a large proportion of the LCFAs cross the membrane by transport rather than passive diffusion.

There is also evidence that this transport of FA into the muscle is regulated. After fasting, FABPpm is increased in slow-twitch oxidative muscle.[62] FABPpm also increases after exercise training.[25] After chronic stimulation (24 h/day, 7 days) of skeletal muscles, the FAT/CD36 protein was upregulated and this coincided with increased LCFA transport across the sarcolemma.[3]

More recently, Bonen *et al.*[5] demonstrated that FAT/CD36 can translocate from intracellular vesicles to the cell membrane in a similar manner as the GLUT-4 protein, indicating that FA transport can also be regulated acutely. It was demonstrated that muscle contraction increased plasma membrane FAT/CD36 and decreased the concentration of FAT/CD36 in the sarcoplasma. Along with a higher density of FAT/CD36 at the cell membrane, an increased LCFA transport into the cell was observed. It is currently not known what triggers the translocation of the FAT/CD36 to the cell membrane. However, it is tempting to speculate that similar factors that result in GLUT-4 translocation will also be responsible for the translocation of FAT/CD36. GLUT-4 translocation to the cell surface is stimulated by an AMP-activated protein kinase (AMPK)–dependent signaling pathway. Muscle contraction results in increased cyclic AMP levels, which in turn allosterically activate AMPK. Translocation of FAT/CD36 to the cell membrane is most likely under the control of phosphatidylinositol 3-kinase (PI-3 kinase) and AMPK (J. Luiken and J. Glatz, personal communication).

Although there is now clear evidence for an involvement of a transport mechanism for FA across the sarcolemma, its functional significance is still unclear. For example, it is not known whether there are any physiological conditions in which this transport limits fat oxidation.

In conclusion, FA transporters are likely to be responsible for most of the transport of FA across the sarcolemma, and these transporters can be regulated both acutely and chronically. At present, however, it is not known if there are any physiological situations in which this transport becomes limiting. We also do not know what the triggers are inside the muscle for the up- or downregulation of the transport proteins.

THE BREAKDOWN OF IMTG

IMTG stores, in trained muscle usually located adjacent to the mitochondria as lipid droplets[18](FIG. 5), have been recognized as an important energy source during exercise. However, although some studies reported IMTG breakdown after exercise measured by muscle biopsies, there are also a large number of studies in which no difference was found in muscle TG content before and after exercise.[23,26] However, there is additional evidence that IMTG stores provide an important fuel source. Studies in which muscle samples were investigated under a microscope, for example, revealed that the size of these lipid droplets decreased during exercise.[33] In addition, indirect measures using stable isotope measurements suggest an important role during exercise.[9,22,30,43,48,54,55] Studies using ^{1}H magnetic resonance spectroscopic techniques[2] also found utilization of IMTG during exercise.[10,11] Furthermore, in trained muscle the lipid droplets are located adjacent to the mitochondria,[18] whereas in untrained muscle they may not be associated with the mitochondria. This also suggests a functional role for IMTG.

The breakdown of IMTG is regulated by a lipase similar to that found in adipose tissue. Although lipases and their regulation have been extensively studied in a variety of tissues, little is known about the lipase responsible for the breakdown of TG in skeletal muscle. Langfort *et al.*[28] have now unequivocally shown that hormone-sensitive lipase (HSL), a neutral lipase, also exists in human skeletal muscle. HSL content correlates directly with muscle TG content in different muscle fiber types,

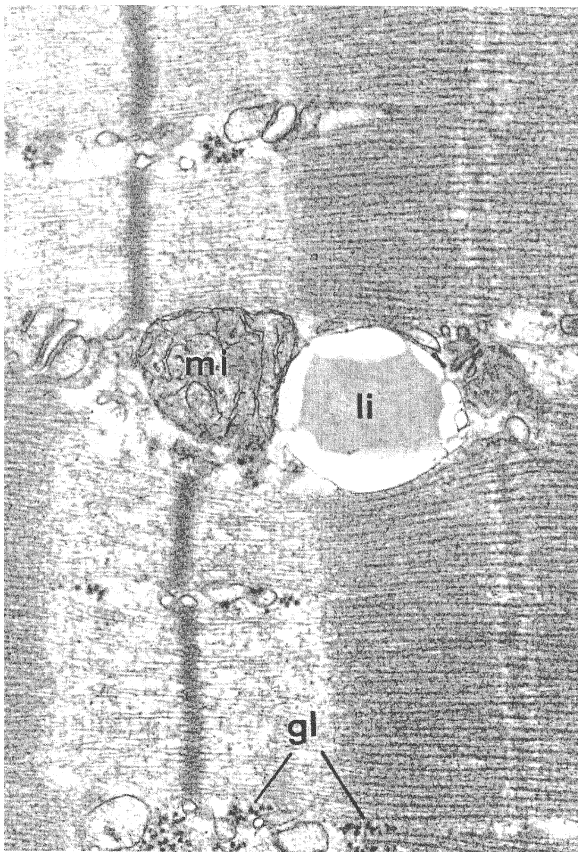

FIGURE 5. Lipid droplets in skeletal muscle. Terms: mi = mitochondria; li = lipid droplet; gl = glycogen. Courtesy of Hans Hoppeler, Bern, Switzerland. (From reference 21, reproduced with permission.)

being highest in slow-twitch oxidative fibers and lowest in fast-twitch glycolytic fibers.[28,40,57,58,63] It has also been shown that this HSL is activated by β-adrenergic stimulation (epinephrine) and that this activation is via the cyclic AMP (cAMP)–activated kinase.[28] In a subsequent study, Langfort *et al.*[27] showed that HSL is activated by muscle contraction independent of adrenergic stimulation. Although the activation of the enzyme probably involves phosphorylation, the mechanisms by which contraction stimulates HSL activity are largely unknown. Studies in rats and humans[27] have demonstrated a simultaneous activation of intramuscular HSL and glycogen phosphorylase during exercise. This suggests that similar triggers stimulate the two enzymes during exercise. It is well known that Ca^{2+} and metabolites related to the energy status of the cell (ADP, AMP, Pi) upregulate glycogen phosphorylase, but there are no studies to show such a relationship with HSL.

It is also important to note that at the very high exercise intensities, despite further increases in circulating epinephrine levels and possible increases in Ca^{2+}, ADP, AMP, and Pi, fat oxidation actually decreases. It is likely that other factors inside the muscle are responsible for reducing the activity of the enzyme at intensities above 70% VO_2max.

TRANSPORT OF FATTY ACIDS INTO THE MITOCHONDRIA

Another site that appears to play a role in the regulation of carbohydrate and fat metabolism in skeletal muscle involves the transport of LCFAs across the mitochondrial membrane. This process is depicted in FIGURE 4. FA in the sarcoplasm may be activated by the enzyme acyl-CoA synthethase or thiokinase to form an acyl-CoA complex (often referred to as an activated FA). This acyl-CoA complex can be used for the synthesis of IMTG or it can be bound to carnitine under the influence of the enzyme carnitine palmitoyl transferase I (CPT I), which is located at the outside of the outer mitochondrial membrane. The binding of carnitine with the activated FA is the first step in the transport of the FA into the mitochondria. As carnitine binds to the acyl-CoA moiety, free CoA is released. The acyl-carnitine complex is transported with a translocase and reconverted into acyl-CoA at the matrix side of the inner mitochondrial membrane by the enzyme carnitine palmitoyl transferase II (CPT II). The carnitine that is released diffuses back across the mitochondrial membrane into the cytoplasm and thus becomes available again for the transport of other FAs. Acyl carnitine crosses the inner membrane in a 1:1 exchange with a molecule of free carnitine.[63] Although it is often believed that short-chain fatty acids (SCFAs) and MCFAs can more freely diffuse into the mitochondrial matrix, carrier proteins with a specific maximum affinity for short- or medium-chain acyl-CoA transport at least some of these FAs.[15,50] It must be noted, however, that SCFAs and MCFAs represent only a small portion of the total FA oxidation.

It is generally believed that CPT I is the rate-limiting enzyme in the transport of FAs across the mitochondria and may be even rate-limiting for FA oxidation. There is substantial evidence that CPT I activity is influenced by numerous regulators. The importance of these regulators will be discussed in subsequent sections in relation to the changes that occur during exercise.

REGULATORS OF CPT I

FA transport into the mitochondria seems to be one of the most regulated steps in the fat oxidation process. Several regulators of CPT I activity have been proposed, including malonyl-CoA concentration, hydrogen ion accumulation in the sarcoplasm, and reduced free carnitine availability. The evidence for each of these mechanisms will be evaluated in the following sections.

Malonyl-CoA

A large number of *in vitro* studies have now established a role for malonyl-CoA in regulating the entry of LCFAs into the mitochondria in a variety of tissues, including

skeletal muscle.[31,32,51,52] Malonyl-CoA is a potent inhibitor of CPT I and is thus a potential candidate for the regulation of fat metabolism. Malonyl-CoA is formed from acetyl-CoA, a reaction catalyzed by the enzyme acetyl-CoA carboxylase (ACC). Malonyl-CoA levels decrease in rodent skeletal muscle from rest to moderate-intensity exercise, when energy production from fat increases.[65] It is believed that the resting concentrations of malonyl-CoA are sufficiently high to inhibit CPT I, and a decrease in the malonyl-CoA concentration would therefore result in a relief of the inhibition of CPT I and increased LCFA transport into the mitochondria. It is also well known that acetyl-CoA concentration in the muscle increases rapidly at the onset of high-intensity exercise, which will stimulate the activity of ACC as it is the primary substrate for this enzyme. The resulting increased concentration of malonyl-CoA could possibly explain a reduced FA uptake into the mitochondria.[51,52]

Initial studies in humans,[34,36,37] though, show little or no evidence that malonyl-CoA is a very important regulator of FA metabolism. In a first *in vivo* study by Odland *et al*.,[36] malonyl-CoA was measured in human vastus lateralis muscle at rest and following 10 min of cycling at 40% VO_2max and 10 and 60 min at 65% VO_2max. Although fat oxidation was increased severalfold from rest to exercise, no significant changes in malonyl-CoA concentration were observed. These data suggest that malonyl-CoA is not an important regulator of fat metabolism in humans. In a follow-up study, Odland *et al*.[37] studied the changes in malonyl-CoA during exercise at different exercise intensities. It was hypothesized that malonyl-CoA would be increased at 90% VO_2max. However, such an increase was not observed despite a significant increase in its substrate, acetyl-CoA. Fat oxidation increased at 35% and 65% VO_2max, despite a lack of decrease in malonyl-CoA levels. This study suggests that a decrease in malonyl-CoA levels is not required in human skeletal muscle in order to increase LCFA uptake and oxidation. Furthermore, malonyl-CoA content does not increase during exercise at high-intensity exercise and does not contribute to the reduced rate of fat oxidation.[37] Although these studies seem to rule out a role for malonyl-CoA in the regulation of fat metabolism, it is interesting to note that the same authors also observed an increased sensitivity of CPT I to malonyl-CoA in trained compared to untrained muscle. This suggests that there may be a role for malonyl-CoA, but this role may not be as simple as suggested by *in vitro* studies.

There are also a few potential methodological limitations that could at least theoretically explain the lack of evidence for an important role of malonyl-CoA in human skeletal muscle. It is possible that the measurement of total muscle malonyl-CoA concentration does not reflect the local concentrations near CPT I. Obviously, we also have to be careful when extrapolating data from *in vitro* studies to *in vivo* situations and from rat skeletal muscle to human skeletal muscle. *In vitro* studies predict that CPT I activity is inhibited 85%–90% at all times, even when malonyl-CoA concentrations are very low. Finally, in humans, resting malonyl-CoA levels are theoretically high enough to completely inhibit CPT I, something that does obviously not occur.

Taken together, this information suggests that regulation of CPT I activity in human skeletal muscle is more complicated than regulation by malonyl-CoA concentration alone. Other factors may be interacting with malonyl-CoA and CPT I in regulating LCFA transport into the mitochondria and oxidation during exercise. One of the possible regulators that has recently been proposed is the accumulation of hydrogen ions.

Hydrogen Ion Accumulation

Another potential regulator of FA oxidation may be hydrogen ion accumulation. Starritt *et al*.[59] studied CPT I activity in isolated mitochondria from resting human skeletal muscle. It was found that small changes in pH from 7.0 to 6.8 inhibited CPT I activity by 50%.[59] Such changes in pH can be observed during exercise at 80% VO_2max in humans. Howlett *et al*.[20] reported muscle lactate levels of 38 and 108 mmol/kg dry muscle after only 10 min of cycling at 65% and 90% VO_2max, respectively. It can be calculated that corresponding pH values must have been 6.9 and 6.6 at these exercise intensities. It is also likely that the activity of the neutral lipase HSL is influenced by the acid environment created during high-intensity exercise.[39] Hydrogen ion accumulation in the muscle may therefore be responsible for a reduction in IMTG hydrolysis as well as a decreased FA uptake into the mitochondria. The relatively large decrease in pH during intense exercise (>80% VO_2max) could be responsible for the sharp decrease in fat oxidation. A small reduction in pH could explain the reduced fat oxidation from moderate- to high-intensity exercise.

It must be noted, though, that this hypothesis is based on *in vitro* studies and these findings have not been confirmed *in vivo*. Further studies are needed to investigate the role of pH on FA transport into the muscle and, ultimately, on fat oxidation.

Carnitine Availability

During low-intensity exercise, the flux through the PDH is lower than the flux through the TCA cycle.[7] This would result in minimal acetylation of the carnitine pool. Relatively low acetylcarnitine concentrations and high free carnitine concentrations have been observed in various studies.[7,16] With increasing exercise intensity, the flux through PDH may increase more rapidly than the flux through the TCA cycle. This would result in an accumulation of acetyl-CoA. In order to free up the CoA, the acetyl units are bound to free carnitine. This acetylation of the carnitine pool will result in a decrease of the free carnitine concentration.

Constantin-Teodosiu *et al*.[7] showed that, at very high intensities (90% VO_2max), a large percentage of the carnitine was bound to acetyl-CoA and the concentration of the free carnitine pool was reduced to very low levels. It is thus possible that the reduced rates of fat oxidation are caused by a reduced transport of FA into the mitochondria because the availability of free carnitine becomes rate-limiting. Although this is an attractive hypothesis, there is currently no direct evidence that this mechanism is important. It could be argued that the low levels of free carnitine observed at high exercise intensities could still support significant rates of FA transport across the mitochondrial membrane, especially since carnitine is recycled, not consumed, in this process. It is also likely that the majority of this carnitine is present in the cytosol[38] where it is needed. If free carnitine availability plays a role in the regulation of fat metabolism, it is likely that this will be limited to high-intensity exercise. At moderate intensities, the free carnitine availability decreases compared to rest and low-intensity exercise, but fat oxidation actually increases. There are currently no studies that have directly addressed this question, and further work is required before a role for free carnitine can be confirmed or ruled out.

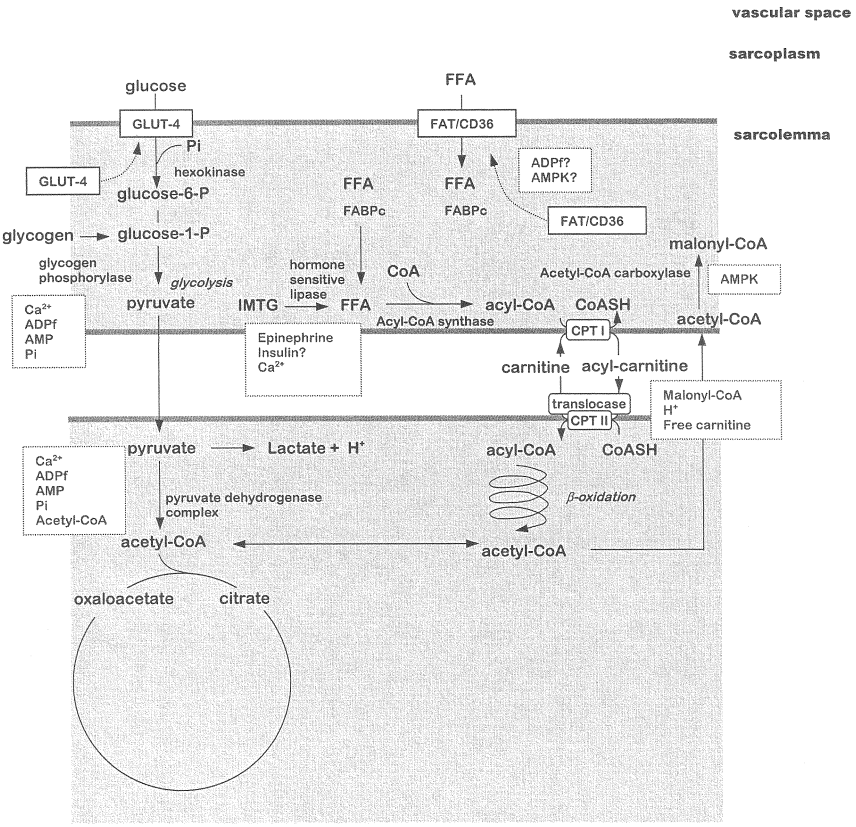

FIGURE 6. Potential regulators of the interaction between carbohydrate and fat metabolism. Three suggested sites of regulation are (1) transport of FA into the muscle controlled by transport proteins (especially the translocation of FA transporter, FAT/CD36), (2) hydrolysis of intramuscular triglycerides (IMTG) controlled by hormone-sensitive lipase (HSL), and (3) transport of FA into the mitochondria controlled by the carnitine palmitoyl transferase I (CPT I) enzyme. Transport of FA across the sarcolemma is regulated by translocation of FAT/CD36, and this translocation is triggered by yet unknown factors. It is possible that AMPK and free ADP play a role. Intramuscular lipolysis is regulated by HSL, which in turn is activated by muscle contraction (Ca^{2+}) and epinephrine. It is also likely that it is inhibited by the presence of insulin. The transport of FA into the mitochondria is regulated by the enzyme CPT I, and malonyl-CoA is known to inhibit this enzyme. It has also been suggested that a decreased pH will inhibit the CPT I, and a reduced free carnitine availability could also reduce FA transport. Regulation of carbohydrate metabolism seems more straightforward since there is a direct relationship between activation of these pathways and the aerobic work rate. Important triggers are related to the energy status of the cell (ADP, AMP, Pi, and AMPK), and the most regulated enzymes seem to be glycogen phosphorylase and pyruvate dehydrogenase (PDH).

SUMMARY

A summary of the potential mechanisms involved in the regulation of carbohydrate and fat metabolism is depicted in FIGURE 6. During low- and moderate-intensity exercise, lipolysis in adipose tissue is increased and this provides an important source of FA for the muscle. At high intensities, the delivery of FA from adipose tissue to the muscle and the use of FA by the muscle are reduced. There is clear evidence that the availability of FA is important for fat oxidation.

However, availability of FA alone cannot explain the changes observed during exercise at different intensities, and mechanisms inside skeletal muscle must be responsible. There are three potential sites of regulation of FA metabolism in skeletal muscle: transport of FA into the muscle, IMTG lipolysis, and transport of FA into the mitochondria. The increased fat oxidation from rest to low-intensity exercise and from low- to moderate-intensity exercise (65% VO_2max) may be mediated by similar factors that are responsible for increases in carbohydrate metabolism (epinephrine, Ca^{2+}, ADP, AMP, Pi, and AMPK). Other mediators may be responsible for the decrease in fat oxidation at high exercise intensities (>70% VO_2max): pH, acetyl-CoA, malonyl-CoA, or decreased free carnitine concentration. Although there have been suggestions that these mediators are involved in the regulation of FA uptake and oxidation, their roles are far from clear and there are many questions yet to be answered.

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