Fat Metabolism During Exercise: A Review

Part II: Regulation of Metabolism and the Effects of Training

Nutrition Research Center, Department of Human Biology, Maastricht University, Maastrict, The Netherlands

This part discusses the complex regulation of fat metabolism. Catecholamines as a stimulator of lipolysis and insulin as a suppressor play very important roles in the regulation of fat oxidation. The interaction of carbohydrate and fat metabolism has been extensively studied in the past decennia but the understanding of this multifactorial regulation is complex and still incompletely understood. In 1963, Randle et al. proposed the glucose-fatty acid cycle as a possible mechanism, and more recently, regulation through malonyl-CoA has been put forward as a possible way to explain shifts in carbohydrate and fat metabolism at rest and during exercise. The exercise intensity affects fat oxidation mainly by increasing lipolysis and fatty acid availability during exercise of low to moderate intensity. At high exercise intensities, both a reduction in fatty acid availability (decreased RfA) and intramuscular factors reduce fat oxidation. These intramuscular factors are largely unknown. The increased mitochondrial density after training and increased oxidative enzymes may partly explain the increased fatty acid oxidation during exercise as observed after training. However, also supply of fatty acids to the mitochondria may be important. The available evidence suggests that the additional fatty acids oxidized after training are primarily derived from intramuscular triacylglycerols and not from adipose tissue derived fatty acids or circulating triacylglycerols.

Key words: Training, glucose-fatty acid cycle, malonyl-CoA, epinephrine, insulin, exercise intensity.

Introduction

In part I of this review “Fatty acid mobilization and muscle metabolism” published in a previous edition of the International Journal of Sports Medicine (70), the importance of fat as a substrate during exercise has been outlined and it was described how fat is mobilized from adipose tissue, transported through the blood, taken up by the muscle and used for oxidation by the muscle. Also the roles of different lipid fuels (plasma fatty acids, fatty acids derived from plasma lipoproteins, fatty acids from intramuscular triacylglycerols) have been discussed. Part II will focus on the interaction between carbohydrate and fat metabolism and the regulation of substrate utilization. In addition the effect of exercise intensity and training will be discussed. In part III “Effect of nutritional interventions”, in a subsequent issue of this journal (71), attention will be paid to the effects of various nutritional manipulations on fatty acid metabolism.

Regulation of Substrate Utilization

Hormonal regulation

Changes in gluconeogenesis, lipolysis and ketogenesis during exercise can at least partly be explained by changes in hormone concentrations. The catabolic hormone profile as observed during intense endurance exercise will be promoted after a low-carbohydrate diet or a negative energy balance (40,41,77,80). Hormones may primarily affect the mobilization of fatty acids (i.e. lipolysis). The insulin concentration decreases while glucagon, epinephrine, norepinephrine but also growth hormone (GH) and cortisol levels increase. GH has a facilitating effect on the catecholamines and thus also on the release of fatty acids into the bloodstream. Catecholamines have a potent stimulating effect on lipolysis while insulin is a strong inhibitor of lipolysis (48); see also part I of this review section on “lipolysis in adipose tissue”. Galbo (40) concluded that the changes in the plasma concentration of these hormones were primarily caused by changes in plasma glucose concentrations. However, a sympathetic stimulation of hormone release could not be excluded. During exercise plasma catecholamines and sympathetic neural activity rise exponentially with increasing exercise intensity. The effect, however, of plasma catecholamine concentrations on lipolysis and fat oxidation during exercise have not been very well described. Romijn et al. (102) showed that during exercise at low intensity (25% VO2max)
fatty acid turnover is increased five times while plasma catecholamine concentration rose only 50% above resting values. When exercise intensity was increased to 65 and 85% VO₂max plasma catecholamine levels increased 3–6 and 17–19 times, respectively, but fatty acid turnover actually decreased. Therefore, other factors such as adipose tissue blood flow (11,12), plasma lactate concentration (67), plasma insulin concentration (63) and increased glycolytic flux (111) may play a major role during exercise at moderate to high exercise intensities. The role of hormones during exercise and their influence on substrate utilization has been extensively reviewed in recent publications (34,38,39,100,120).

The glucose-fatty acid cycle at rest

The interaction between carbohydrate and fat metabolism could partly be explained by the existence of the so-called glucose-fatty acid cycle proposed by Randle in 1963 (94) (Fig.1). An increased plasma fatty acid concentration would lead to an increased fat oxidation. The accelerated flux through the β-oxidation pathway would result in accumulation of acetyl-CoA and NADH, which in turn would inhibit the activity of the enzyme pyruvate dehydrogenase (PDH) and thus inhibit pyruvate oxidation. Inhibition of pyruvate dehydrogenase would lead to a sparing of carbohydrates as once pyruvate has been converted into acetyl-CoA it is committed to oxidation. In addition, an increased acetyl-CoA concentration and in fact an increase in the acetyl-CoA:CoA ratio would result in an increased citrate concentration leading to an inhibition of the enzyme phosphofructokinase (PFK), a key (rate-determining) enzyme in the glycolytic pathway. These effects would result in a decreased rate of glycolysis and glycogenolysis. Lower fatty acid concentration would of course result in the opposite adaptations.

Although evidence in favour of the existence of this glucose-fatty acid cycle has been obtained in rat cardiac muscle (42, 43, 94, 95) and in rat diaphragm (42, 43, 94, 95) initial studies in rat skeletal muscle suggested that availability of fatty acids did not inhibit glucose disposal or oxidation (8, 49, 101). However, results were rather conflicting since other studies reported decreased glucose utilization after adding fatty acids to the perfusion medium in isolated rat skeletal muscle (91,98,99). Rennie et al. (98) found that increasing the fatty acid availability decreased the rate of glycogen degradation in stimulated rat skeletal muscle. Citrate concentrations increased in slow twitch (ST; soleus) and fast twitch oxidative (FTO; deep portion of vastus lateralis) but not in fast twitch glycolytic muscle (FTG; superficial portion of vastus lateralis). So the differences in the above mentioned studies may be in part explained by the different muscle types studied. Another difference between the studies, which may explain part of the conflicting results, could be the time point of the measurement. Zorzano et al. (130) concluded that the glucose-fatty acid cycle does not operate in the resting state in the soleus muscle of a starved rat, but it does operate in the post-exercise period.

Fig. 1 Glucose-fatty acid cycle (Randle cycle). This cycle describes the mechanisms by which fatty acid oxidation may inhibit glucose oxidation. When fatty acids enter the cytosol they may be activated to fatty acyl-CoA and subsequently transported into the mitochondria. Via β-oxidation fatty acyl-CoA units will be broken down to acetyl-CoA. An increased cytosolic acetyl-CoA concentration or acetyl-CoA/CoA ratio will inhibit PDH activity and thus inhibit the decarboxylation of pyruvate to acetyl-CoA. Also increased citrate concentration may lead to inhibition of PFK. Increased cytosolic glucose-6-P concentrations may inhibit glucose transport into the muscle cell. (PDH = pyruvate dehydrogenase; PFK = phosphofructokinase; P = phosphate.)

Although most (5,35,36,115,123,129), but not all (49,130), studies provide data that support the existence of the Randle cycle in resting skeletal muscle, this issue still remains a matter of continuous debate. Ferrannini et al. (36) showed that elevated plasma fatty acid levels inhibited glucose uptake, a finding which was later confirmed by Walker et al. (123). Studies in which glucose tolerance was investigated with high and low plasma fatty acid levels suggest a direct inhibiting effect of fatty acids on glucose transport (35). Wolfe et al. (128), on the other hand, observed that elevated fatty acid levels did not affect plasma glucose oxidation when glucose uptake was kept constant. Instead, elevated fatty acid levels suppressed the oxidation of glycogen. Baron et al. (7) administered Intralipid® and heparin and observed that insulin-mediated whole body glucose utilization at rest was inhibited.

Most studies in resting human subjects support the concept that an increased availability of fatty acids affects the utilization of intracellular or extracellular glucose or both. However, the underlying mechanisms are largely unknown and although there are indications that the Randle cycle is operative in resting human skeletal muscle, there is little support for the cycle in exercising conditions.

The glucose-fatty acid cycle during exercise

In one of the first studies looking at the glucose-fatty acid cycle in man during exercise, it was observed that glucose uptake during exercise was inhibited by an increase in plasma fatty acid concentrations (23). In a classical study Costill et al. (23) fed their subjects a high-fat meal and gave them a heparin infusion to elevate plasma fatty acids. Subjects exercised 30 min on a treadmill at 70% VO₂max. The elevation of plasma fatty acids to approximately 1 mmol·l⁻¹ decreased the rate of mus-
gle glycogen breakdown by 40% as compared to a control trial in which the plasma fatty acid concentration was approximately 0.2 mmol·l⁻¹. Vulovic and colleagues (121) observed similar glycogen sparring effects with fat feeding and Intralipid® infusion in combination with heparin infusion. These findings were further confirmed by Dyck et al. (30) who infused Intralipid® and heparin to elevate plasma fatty acids and observed significant glycogen sparring after 15 min of cycling at 85% VO₂max. However, the latter did not observe any differences in muscle citrate, acetyl-CoA and PDH between the Intralipid® and the control trials, suggesting that another mechanism than the glucose-fatty acid cycle must be responsible for the regulation of fuel utilization. The authors suggest regulation at the level of glycogen phosphorylase (30). Hargove et al. (51) elevated plasma fatty acid concentration by infusion of Intralipid® and heparin. During exercise (knee extensions), arteriovenous differences of different substrates were measured and muscle biopsies were taken. They observed that the uptake of glucose was inhibited, while no differences could be found in the muscle respiratory quotient and glucose-6-phosphate. Elevated fatty acid concentration did not result in a decreased glycogen breakdown. The authors suggested that fatty acids have a direct effect on the uptake of glucose from the vascular space. These results were obtained during low-intensity exercise with no changes in hormone concentrations (51). However, Romijn et al. (103) showed that during intense exercise, glucose uptake was not inhibited by increasing plasma fatty acid concentrations. To further illustrate the contradicting findings Ravussin et al. (96) could not find a change in the relative contribution of fat and carbohydrates to total oxidation during exercise at 44% VO₂max, although plasma fatty acids were significantly elevated by providing a pre-exercise medium-chain triacylglycerol (MCT) containing meal.

The results of several studies are far from consistent, especially during exercise (17, 23, 55, 96, 98, 99). However, some of the differences in the results can be explained by the experimental set-up. Some studies have been performed in situations with very low rates of fatty acid oxidation (79). These low rates of fatty acid oxidation can be found for instance during either very low or very high exercise intensities. Some studies administered only small amounts of exogenous fat, which might have been insufficient to significantly elevate fat oxidation (19, 79). Also other factors may have caused the non-uniformity in the results. In addition, the glucose-fatty acid cycle is probably subjected to influences of several hormones which makes comparison of different investigations difficult. In the literature, the different findings of studies, some of which observed "glycogen sparing" and others which did not, are generally explained by the extent to which plasma fatty acid levels were elevated.

However, more important may be the extent to which plasma fatty acid levels were lowered in the control situation. If the muscle is deprived from plasma fatty acids as a fuel (concentrations below approximately 0.2 mmol·l⁻¹), the energy status of the muscle cell may be decreased leading to accelerated glycogenolysis.

Another way of looking at the seemingly conflicting results is that the glycogen sparing effect seen in some of the studies is not a result of a reduction in glycogen breakdown but rather an accelerated glycogen breakdown in the control trials. Studies in which glycogen sparring was observed with elevated fatty acid levels (23, 30, 121), had very low plasma fatty acid levels in their control groups (below ~0.2 mmol·l⁻¹) which may have deprived the muscle of a plasma substrate. On the other hand, the FFA levels in those studies not able to demonstrate this effect, were somewhat higher (above ~0.4 mmol·l⁻¹) (96). This is analogous to the finding that nicotinic acid, a strong inhibitor of fatty acid mobilization which decreases fatty acid availability usually below ~0.2 mmol·l⁻¹ increased muscle glycogenolysis (9, 92). This view is further supported by observations by Dyck et al. (30) who showed a poor energy status of the muscle (low AMP, PCR) in the control trials in which fatty acid availability was low compared to the Intralipid® trials with very high plasma fatty acid concentrations.

In conclusion, there is no evidence that the glucose-fatty acid cycle as originally proposed is operative in exercising human skeletal muscle. Although several studies showed decreased glucose utilization when fatty acids are elevated, regulation may not be through the classical glucose-fatty acid cycle. The question remains: If the glucose-fatty acid cycle is not the regulating mechanism during exercise then what determines carbohydrate and fat utilization?

Regulation through malonyl-CoA

Another possible mechanism is regulation through malonyl-CoA. Carnitine acyl transferase I (CAT I), the rate-limiting step for the transport of LCFA-CoA into the mitochondria has been suggested to be an important regulating site of fatty acid oxidation (Fig. 2). CAT I activity in turn is regulated by malonyl-CoA, which is formed by acetyl-CoA carboxylase (ACC) in the rate limiting step of fatty acid synthesis. CAT I is very sensitive to changes in the malonyl-CoA concentration (83). In starved
and diabetic rats, hepatic malonyl-CoA levels are decreased and fatty acid oxidation as well as ketosis is increased (85). In situations of glucose paucity such as fasting, ACC is inactivated, and the result is a decreased malonyl-CoA concentration and an increased rate of β-oxidation. After refeeding, the previously starved rats have increased malonyl-CoA levels and decreased fatty acid oxidation (85).

These observations suggest that malonyl-CoA may play an important role in the regulation of fatty acid oxidation (84). Although these effects have been shown in studies of liver (82, 84) and heart (105), there is also evidence for the regulation through malonyl-CoA in skeletal muscle (31, 84, 106–108, 124, 125). Evidence collected in isolated and perfused skeletal muscle suggests that the availability of carbohydrates may be an important factor determining the utilization of fatty acids (107). In liver, heart, and also in muscle, increases in glucose or insulin and especially the two in combination will lead to an increased activity of ACC and an increased formation of malonyl-CoA (28, 107). In addition, acetateacetate (108), exercise (124, 125) and contractions induced by electrical stimulation (28, 107) have been shown to decrease acutely the concentration of malonyl-CoA in rat skeletal muscle. Animal models indicate that when the muscle is supplied with a surplus of fuels other than fatty acids, malonyl-CoA levels increase (28, 107, 124, 125). Conversely, malonyl-CoA levels decrease when the muscle is fuel-deprived or energy use is increased by contraction (28, 107, 124, 125).

However, all these findings are in animal models and no direct measures were made of fatty acid oxidation or any aspect of fat metabolism. Recently, we reported that increased glycolytic flux during exercise, from hyperglycemia and hyperinsulinemia, reduced long-chain plasma fatty acid oxidation but not medium-chain fatty acid oxidation (25). This was interpreted to suggest that increased glycolytic flux activates increases the oxidation of long-chain fatty acids through the mitochondria because the transport of long-chain fatty acids across the mitochondrial membrane appears to be less dependent on CAT I (106). It was suggested that the increased glycolytic flux may have increased the concentration of malonyl-CoA in muscle as shown by Elyan and Winder (31) which may have inhibited CAT I and thereby the entry of long-chain fatty acids into the mitochondria. Similar findings were reported by Sidossis et al. (111) who increased glycolytic flux by increasing the exercise intensity from 40% to 80% VO_{2}max and observed specific inhibition of long-chain fatty acid oxidation compared to medium-chain fatty acid at the high exercise intensity. Again these results suggest that long-chain fatty acid transport into the mitochondria is inhibited at the level of CAT I which may be regulated through malonyl-CoA (111). Although this is an attractive hypothesis, direct evidence for a malonyl-CoA-mediated mechanism could not be provided since muscle malonyl-CoA concentrations were not measured in these studies (25, 111). Odland et al. (90) were the first to measure malonyl-CoA in human skeletal muscle. They reported low levels at rest (compared to the values reported in rat skeletal muscle) and a 20% decline during exercise that failed to be statistically significant (90). So, the first data in humans are not very conclusive and further research is needed to elucidate the role of malonyl-CoA in the regulation of fat oxidation. Another unresolved problem is that the k_i of malonyl-CoA for CAT I measured in vitro is much lower than the concentration measured in rat and human muscle in vivo both at rest and during exercise (84). At the concentration present in vivo full inhibition of CAT I in fact would be expected, but this does not seem to prevent that fatty acids in time become an increasingly important fuel during mild to moderate exercise intensities. A possibility is that the concentration seen by CAT I in the outer mitochondrial membrane in fact is much lower than the malonyl-CoA concentration measured in the whole muscle extract, but it also cannot be excluded that the malonyl-CoA mechanism is not as important as suggested in recent exercise literature. In addition malonyl-CoA concentrations have yet to be directly linked to changes in fat oxidation during exercise in either human or rat muscle (122).

The Effect of Exercise Intensity on Fat Metabolism

The intensity of exercise is the main factor determining the degree of fat or carbohydrate oxidation during exercise. Relatively, fatty acids will be more important during low-intensity exercise. During exercise at 25% VO_{2}max almost all of the energy expenditure was derived from fat (102). During exercise at 65% VO_{2}max fat oxidation accounted for 50% of the energy expenditure but since the energy turnover was so much higher, the absolute fat oxidation rates were greater. The absolute rates of energy provision from fat seem to have an optimum at exercise levels between 50 and 70% VO_{2}max. Romijn et al. (102) investigated plasma fatty acid uptake and estimated IMTG utilization using stable isotopes. They found that during low-intensity exercise (25% VO_{2}max) IMTG contributed minimally to energy provision. Plasma fatty acids and glucose appeared to be the most important substrates at this intensity where fat is by far the predominant fuel. At a moderate exercise intensity (65% VO_{2}max) substrates in the muscle (IMTG and glycogen) became more important (Fig. 3). IMTG were oxidized at high rates at this exercise intensity while plasma fatty acids were used at a slightly lower rate compared to low-intensity exercise (Fig. 3). When the exercise intensity was further increased to 85% VO_{2}max, the contribution of plasma fatty acids became even smaller, while also IMTG oxidation decreased. The decreasing contribution of plasma fatty acids as observed in this study may be caused by a decreased availability of fatty acids which in turn may be caused by lower rates of appearance of fatty acids into the plasma (Raff) (71, 72). This decreased Raff (71) without a simultaneous reduction in lipolysis (102) may indicate that fatty acids are trapped within the adipocyte.

![Fig. 3](image_url) Substrate utilization at different exercise intensities (25% VO_{2}max, 65% VO_{2}max and 80% VO_{2}max). Data adapted from Romijn et al. (102).
(56), maybe as a result of increased lactate concentrations (67) or as a result of vasoconstriction in adipose tissue (12,13,104). However, although fatty acid mobilization from adipose tissue is one of the factors which may be partially responsible for the decreased fatty acid oxidation during exercise at high intensities, this may not be the only factor. When plasma fatty acid levels were elevated to high levels during exercise at 85% \( \text{VO}_{2\max} \) max by Intralipid® and heparin infusion, fat oxidation rates were somewhat increased (103) but still lower than during exercise at 65% \( \text{VO}_{2\max} \) (102). This indicates that other (intramuscular) factors may reduce fatty acid oxidation during high-intensity exercise. A possible mechanism may be that high rates of glycolysis and high rates of acetyl-CoA formation from glucose-6-phosphate at those exercise intensities inhibit long-chain fatty acid transport into the mitochondria at the level of C4AT I by increasing malonyl-CoA concentrations (111).

In addition, during intense exercise, more fast-twitch (FT) fibers will be recruited and less slow-twitch (ST) fibers (110). Since the FT-fibers have a smaller capacity to oxidize fatty acids, fat oxidation will decrease and concomitantly carbohydrate oxidation will increase when more of these fibers will be recruited (110). It has also been suggested that during high-intensity exercise there is an increased competition of pyruvate derived acetyl-CoA with fatty acid derived acetyl-CoA for entry into the TCA-cycle (60).

The contribution of fatty acids can increase markedly when glycogen stores in the muscle become depleted (1). This implies, however, that the "high" exercise intensity cannot be sustained and that exercise has to be continued at a lower level (92) because the rate of ATP production will decrease.

**Training and Fatty Acid Oxidation**

Endurance training affects both substrate utilization and the exercise capacity. A large number of studies, both in animal and man, have established a marked adaptive increase in oxidative potential in response to an increased physical activity (57,58). A notable consequence and probably contributing factor to the enhanced exercise capacity after endurance training is the shift of metabolism to a greater use of fat and concomitant sparing of the glycogen stores (6,18,21,46,47,57 - 59,64,69,109,110). The increased performance and the shift to fat metabolism during submaximal exercise are more pronounced than the change in total-body maximal oxygen uptake (\( \text{VO}_{2\max} \)) (45) and seems unlikely that other training adaptations such as an increased maximal cardiac output or pulmonary function will be a major factor in explaining the shift from carbohydrate to fat metabolism in the trained skeletal muscle (47). The contribution of fat to total energy expenditure increases after training at both the same relative and absolute exercise intensity (23,54,58,64,68,69). This is of utmost importance during prolonged exercise of moderate to high intensity (50-90% \( \text{VO}_{2\max} \)) since carbohydrates are required to maintain those levels of exercise. As soon as glycogen stores become depleted and carbohydrate oxidation falls below a critical level, the exercise intensity has to be reduced since ATP cannot be generated at a sufficient rate (87).

Although the advantages of increased fat oxidation during exercise are obvious, the cellular and molecular mechanisms underlying this beneficial effect of training are incompletely understood. Several adaptations may contribute to a stimulation of fat oxidation in trained subjects: 1) an increase in the number of oxidative enzymes, and mitochondrial content in trained muscle, 2) increased muscle triacylglycerol oxidation, 3) increased fatty acid uptake, 4) alterations in mobilization of fatty acids from adipose tissue. Not only the cause of the increased fatty acid oxidation after training is uncertain, also the source of these fatty acids is still not completely known.

*An increase in the number of oxidative enzymes and mitochondrial content*

Studies on whole muscle homogenates have shown that rat skeletal muscle undergoes adaptive increases in the capacities to oxidize fatty acids (6,86) and ketones (126). Based on enzyme kinetics, Gollnick and Saltin (46) calculated that enhanced activity of enzymes involved in fatty acid oxidation may be the prime cause of the increased fat oxidation after training. Increased levels of enzymes involved in the activation, transport and \( \beta \)-oxidation of long chain fatty acids have been frequently reported (6,15,24,57,86). Increased levels of 3-hydroxyacyl-CoA dehydrogenase have been found in endurance-trained rats (27) and in man (64,68). Other fatty acid handling enzymes of which the activity or content is increased after training include: carnitine palmitoyl transferase I (86), carnitine acyl transferase (86) and fatty acyl-CoA synthetase (86). However, not only the enzymes involved in fatty acid activation, transport and oxidation are increased in trained muscle, but also the enzymes involved in the TCA-cycle and respiratory chain (15,24,54). Since endurance training increases the capacity to oxidize fatty acids as well as pyruvate, the question remains: why are proportionally more fatty acids and less carbohydrates oxidized? This may be explained by the mitochondrial density as discussed by Gollnick and Saltin (46).

Endurance training increases both the size and the number of the mitochondria (58). This increases the surface area where exchange of substrates and ADP can take place and possibly also the number of transport proteins. Gollnick and Saltin (46) proposed that the increased total mitochondrial volume as seen after endurance training, increases the capacity to transport ADP formed by the contractile cycle into the mitochondria. Consequently, free ADP (ADP) levels are lower in muscles of exercise-trained compared to untrained muscles at the same contractile activity (22). The ADP concentration, ATP/ADP ratio and the ATP/(ADP+x Pi) ratio in the cytosol and in the mitochondria have been shown to be key regulatory factors of metabolism (4,29,114). Besides these factors also extra mitochondrial Pi and the supply of hydrogen have been proposed as important regulatory factors of mitochondrial respiration (114). According to the model of Gollnick and Saltin (46) by maintaining lower levels of ADP or increases in the ATP/ADP ratio, a greater entry of acetyl-CoA from fatty acids into the oxidative pathways would be favoured because ADP and ATP/ADP have a stimulating influence on glycolysis (88).

Compared to untrained, trained subjects have a higher mitochondrial content (i.e. higher oxidative capacity) and during exercise at a certain workload less ADP will be formed (along with higher ATP/ADP and ATP/(ADP+x Pi) ratios). These changes will directly control energy metabolism by stimulating glycogen phosphorylase, PK and PDH resulting in an increased glycolytic flux.
Although the enzyme activity and mitochondrial density are increased after training, it remains to be determined whether the enzyme activity is the main limitation in lipid oxidation in the exercising muscle cell. An increase in the oxidative capacity should be accompanied by a quantitatively similar increase in the supply of fatty acids to the mitochondria (119). Evidence to support this view is the observation that runners with similar rates of fat oxidation during a 60 min run at 70% VO2max, displayed considerable differences in carnitine palmitoyl transferase I and succinyl dehydrogenase activity (24).

**Malonyl-CoA and fat utilization after training**

As discussed above malonyl-CoA may be an important regulator of fatty acid oxidation in skeletal muscle during exercise (124,125). Decreased malonyl-CoA levels during exercise may allow more long chain fatty acids to be transported into the mitochondrial matrix, since CAT I is less inhibited. The decreased muscle malonyl-CoA concentrations during exercise parallel a decreased carbohydrate flux. Recently evidence was provided that carbohydrate flux directly regulated fatty acid oxidation during exercise at 50% VO2max (25,112). It is therefore tempting to speculate that training may result in a greater fall in muscle malonyl-CoA concentration during exercise, thereby relieving inhibition of CAT I, and enhancing fatty acid oxidation. However, these mechanisms are rather speculative and additional research is required to test these challenging hypotheses.

Training also decreases glucose uptake during exercise at the same absolute exercise intensity (18,20) even though the number of glucose transporters (GLUT 4) in the muscle may increase (52). This lowering of plasma glucose uptake, in combination with an increased fat oxidation after training has been suggested to be regulated through the classic glucose fatty acid cycle. However, Coggan et al. (20) reported that citrate and glucose-6-phosphate concentrations were lower at the same exercise intensity in the trained than in the untrained state, which is in contrast to the concept of the glucose fatty acid cycle.

**Effect of training on plasma fatty acid utilization**

With fatty acid uptake and oxidation being dependent on their vascular concentration and from the increased oxidative potential of endurance-trained skeletal muscle (2,53,65,73,116), it might be expected that an enhanced rate of lipolysis would accompany training. This could be envisaged as a mechanism to provide more fatty acids to the working muscle to support the increased potential to oxidize fatty acids. However, lower plasma fatty acid concentrations after training (26,64,81,127) suggest that the effect of training on plasma fatty acid oxidation during exercise is either a diminished mobilization from adipose tissue or an increased extraction of fatty acids by the muscle.

A study of Martin et al. (81) using isotopic labeling of palmitate shows that both the rate of appearance (Ra) and the rate of disappearance (Rd) of fatty acids are diminished as a result of training. Henriksson (54) found similar rates of fatty acid uptake between a trained and an untrained leg during 50 min moderate-intensity two-legged exercise and Jansson and Kajser (68) found no effect of training on leg extraction of plasma fatty acids. So, although fat oxidation is markedly increased it is unlikely that plasma fatty acids are the main source of fatty acids that explain this increased fat oxidation. The above mentioned studies (54,64,68,81) support the concept that training does not increase extraction of plasma fatty acids by the muscle. However, Turcotte et al. (117) reported that, above certain plasma fatty acid levels, thigh uptake of plasma fatty acids was limited in untrained compared to trained during the third hour of single-leg knee extension exercise at 60% VO2max. Whereas plasma fatty acid uptake rose linearly with increasing (non-protein bound) plasma fatty acid concentrations in trained subjects, the uptake followed saturation kinetics (i.e. leveled off) in untrained subjects above plasma concentrations of ~700 mmol·l-1. The observation that the uptake of fatty acids is a saturable process was previously shown in a rat model (116). Training may provoke changes in the transport across the membrane (117). It has been suggested that FABP, FATP and FATP may play an important role in the transport across the membrane and inside the cytosol (44,119). In rats, however, training increased FABP content of heart muscle, but not in the EDL and soleus (118). However, as discussed by Coggan (21), the results of studies using knee extension exercise as applied by Turcotte et al. (117) cannot be easily extrapolated to whole body exercise since with this knee extension exercise hormonal changes are much smaller compared to whole body exercise. Since the extraction of fatty acids does not seem to be increased after training during whole body exercise (88,81), the reduction of plasma fatty acid oxidation must be due to reduced lipolysis. The most likely explanation for an effect of training on adipose tissue lipolysis is probably a decrease in the sympathoadrenal response to exercise of the same absolute intensity. The level of sympathoadrenal activity, which is a major determinant of adipose tissue lipolysis in humans, is markedly blunted even after a few weeks of training (127). Winder et al. (127) observed a 55% reduction in the plasma catecholamine concentrations during prolonged exercise (decreased heart rate may be an indicator of decreased sympathoadrenal activity after training). Endurance training also attenuates the exercise-induced increase in plasma concentrations of other lipolytic hormones such as glucagon and growth hormone (127). In addition, Laviole et al. (78) showed that after endurance training the inhibiting effect of insulin on lipolysis is increased, which leads to reduced release (Ra) of fatty acid (78,81) and reduced plasma fatty acid concentrations (18,78,81,127). However, although the reduction of the release of fatty acids after training may mainly be explained by the decreased rate of lipolysis, it cannot be excluded that trained subjects have higher rates of tricacylglycerol-fatty acid cycling. Decreased lipolysis (measured as Ra glycerol) at the same absolute exercise intensity has been reported to be the same (75) or slightly decreased (93) after training while whole body lipolysis at the same relative exercise intensity may be increased (76).

Interestingly, lipolysis in adipose tissue seems to be decreased after training whereas at the same time lipolysis in skeletal muscle seems to be increased (see "Effect of training on IMTG utilisation"). The mechanism behind these disparate effects of training is unclear. There are, however, a few differences in lipolysis in skeletal muscle and adipose tissue that may at least partly explain the different responses to training. First of all, skeletal muscle lipolysis appears to be more sensitive to β-adrenergic blockade than adipose tissue lipolysis (16), impli-
cating that, at lower levels of sympathoadrenal stimulation (such as after training [127]), lipolysis will be stimulated relative more in skeletal muscle. Secondly it has been suggested that there is a tissue specific upregulation of lipolysis in the trained state (81) as evidenced by increased adenylate cyclase activity in skeletal muscle of trained rats compared to untrained rats (10). It also cannot be excluded that exercise leads to allosteric activation of HSL especially in trained muscles or that training leads to an increase in the HSL enzyme concentration in muscle. However, we can only speculate on this interesting difference between trained and untrained individuals as the exact mechanism is not known at this moment in time.

Effect of training on IMTG utilization

Reduced plasma fatty acid oxidation despite higher rates of total fat oxidation after training suggests that the additional fat oxidized during exercise has to be derived from other sources than adipose tissue triacylglycerols, possibly muscle triacylglycerols (53, 64, 66, 81). Some (73, 81), but not all (64) studies reported that trained skeletal muscles have increased intramuscular triacylglycerol concentrations which can serve as a significant energy source during exercise (14, 32, 33, 37, 64, 68, 97, 113). Because lipid droplets are located in close proximity to the mitochondria (61) the transit time of fatty acids from the TG in an intramuscular lipid droplet to the outer mitochondrial membrane will be very short. So, also from a tecteological point of view, the increased intramuscular triacylglycerol stores would have a practical advantage. The observation that training increases the number of lipid droplets (62) is in line with the reported increased intramuscular triacylglycerol oxidation after training (53, 64, 66, 81, 93). Somewhat paradoxically, training reduces the cholesteral drive which decreases lipolysis in adipose tissue (93) but increases intramuscular lipolysis and fatty acid oxidation (see discussion above “Effect of training on plasma fatty acid utilization”). Evidence for a direct effect of training on HSL activity in the muscle is lacking.

In summary, it has been shown fairly consistently that training increases the IMTG pool and IMTG oxidation during exercise at the same absolute intensity. The mechanism by which training increases IMTG utilization is not well understood.

Effect of training on plasma VLDL-TG utilization

It is tempting to believe that increased activity of LPL (3, 74, 89) and a greater capillary endothelial surface area after training (74) will be responsible for increased hydrolysis of plasma triacylglycerols after training. Increases in LPL activity have been shown to be linearly related to increases in capillarization of trained muscle (74). Although extraction rates of circulating TG were 8% in untrained and 15% in trained thigh muscles under resting conditions, differences in VLDL-TG utilization during exercise were relatively small and inconsistent (74). Kiens and Lithell (74) therefore suggested that VLDL-triacylglycerol degradation is probably more important for its potential long-term influence on blood lipid profiles than in contributing to a higher rate of fat oxidation during exercise after training. Moreover, training seems to decrease the availability of circulating VLDL rather than increase it (50) by reducing the production of VLDL by the liver.

Conclusion

In conclusion, the increased mitochondrial density after training and increased oxidative enzymes may partly explain the increased fatty acid oxidation during exercise as observed after training. However, also supply of fatty acids to the mitochondria may be important. The available evidence suggests that the additional fatty acids oxidized after training are primarily derived from intramuscular triacylglycerols and not from adipose tissue derived fatty acids or circulating triacylglycerols.

References


86. Møller PA, Oski LR, Holloszy JO. Increase in levels of palmitoyl CoA synthetase, carnitine palmitoyltransferase and palmitoyl-CoA dehydrogenase, and in the capacity to oxidize fatty acids. J Clin Invest 1971; 50: 2323 – 30


95. Randle PJ, Newsholme EA, Garland PB. Regulation of fatty acids, ketone bodies and pyruvate, and of alloxan diabetes and starvation on the uptake and metabolic rate of glucose in rat heart and diaphragm muscles. Biochem J 1964; 93: 652 – 65


Fat Metabolism During Exercise: A Review


