Metabolic Regulation of Fat Use during Exercise and in Recovery

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Abstract
Fat is an important fuel for exercise but plays a secondary role to carbohydrate (CHO). Increasing fat use during exercise can decrease the reliance on CHO and spare CHO for later in training sessions or competitions that depend on CHO for success. The pathways that metabolize and oxidize fat are activated more slowly than CHO at the onset of exercise and reach a maximum at moderate exercise intensities. As exercise intensity increases to ~75% VO_{2max} and beyond, fat metabolism is inhibited: using CHO will increase the amount of energy produced per liter of oxygen consumed. The capacity for fat use during exercise is increased by aerobic training and the dietary combination of little or no CHO intake and high fat intake. Fat oxidation is very dependent on the mitochondrial volume of muscle but other key sites of regulation include release of fat from storage forms and fat transport across plasma and mitochondrial membranes. This chapter examines the control of fat metabolism during moderate and intense exercise with an emphasis on human findings and the adaptations that occur with aerobic training and other acute nutritional manipulations. Recent work using molecular and cellular compartmentalization techniques have advanced the knowledge in this area.

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Introduction

Work in the early 1900s demonstrated that both fat and carbohydrate (CHO) could be used as fuel for muscular contractions and that the amount of fat and CHO used depended on several factors [1]. The preceding diet was the main factor during short-duration aerobic exercise of low to moderate intensity. As exercise intensity increased, the proportion of fat oxidation decreased, and CHO oxidation increased such that fat use was virtually absent at 100% VO_{2max}. While CHO can provide all the substrate required for exercise at ~100% VO_{2max}, fat can...
only provide substrate at a rate to sustain ~60–75\% VO2max when CHO availability is severely restricted. However, during prolonged moderate-intensity exercise, the relative fat contribution increases due to the increasing availability of plasma free fatty acid (FFA) and decreasing CHO stores. Later studies used more direct experimental approaches to demonstrate the importance of plasma FFA and fat derived from stores inside the muscle (IMTG) as substrates for oxidation during exercise and showed that the absolute amount of fat oxidized was largely a function of the mitochondrial volume of the contracting musculature [2–4].

The pathways that mobilize and deliver fat to the mitochondria are not activated as quickly as CHO metabolism at the onset of exercise, and fat oxidation is inhibited as exercise intensity approaches ~75–85\% VO2max [5, 6] (fig. 1). While important adaptations occur with exercise training, human skeletal muscle is not engineered to maximize fat use during intense aerobic exercise. Maximal fat oxidation occurs at ~60–65\% VO2max, whereas many athletic and sports situations take place at >80\% VO2max.

**Overview of the Regulation of Fat Metabolism**

To understand the potential importance of exercise training and sports nutrition to trigger adaptations in fat metabolism that may lead to improved exercise performance, we need to understand the regulation of fat metabolism. Until recently, knowledge in this area lagged far behind that of CHO metabolism, but new research has revealed many of the sites of control in adipose tissue and skeletal muscle.

Potential control points that regulate fat metabolism in skeletal muscle during aerobic exercise include: (1) adipose tissue lipolysis, FFA release from
adipose tissue, and delivery to the muscle, (2) FFA transport across the muscle membrane, (3) binding and transport of FFA in the cytoplasm, (4) IMTG lipolysis, (5) FFA transport across the mitochondrial membranes, and possibly (6) metabolism in the β-oxidation pathway (fig. 2). Manipulation of the skeletal muscle mitochondrial volume, which determines the overall capacity to oxidize fat, might also be considered an overarching control point.

Long-chain FFA derived from adipose tissue are a major source of fat for the working muscle during exercise. The degradation of FFA from adipose tissue triacylglycerol (TG) and the release and removal of FFA from adipose tissue are regulated processes, and much work has examined the control of the regulatory enzymes, hormone-sensitive lipase (HSL) and the newly discovered adipose tissue triglyceride lipase (ATGL) [7]. A constant supply of albumin in the blood...
perfusing adipose tissue is also required to allow the FFAs to bind to albumin, as transport of FFA in the blood and muscle cells requires a protein chaperone. Bulk transport of FFA to the muscle ([FFA] × blood flow) also plays a role in the uptake of FFA by muscle. A reduction in the ability to move newly released FFA out of the adipose tissue and into the blood appears to be a major reason for the lack of an increase in plasma [FFA] during exercise at ~80% vs. 40–60% VO2max as the lipolytic rate does not decrease at the higher power output [5]. Important questions in this area include how we speed up these processes of fat mobilization at the onset of exercise and maintain an adequate blood flow to this tissue during intense aerobic exercise.

Recent evidence suggests that the majority of the FFAs entering muscle cells are transported or assisted across the muscle membrane by transport proteins, most notably the fatty acid translocase protein (FAT/CD36), the plasma membrane fatty acid-binding protein (FABPpm), and members of the fatty acid transport protein family [8, 9]. FAT/CD36 also translocates to the plasma membrane during 2 h of cycling at 60% VO2max to facilitate FFA movement into the cell [unpubl. obs.]. It is not clear what occurs during more intense exercise, and whether there may actually be a decrease in membrane fat transport proteins. Once inside the muscle, FFAs are bound to cytoplasmic FABP (FABPc). The FFA destined for storage as IMTG or for oxidation in the mitochondria must first be ‘activated’ by coupling to coenzyme A (CoA) through the activity of fatty acyl-CoA synthase.

A second major source of fat is via the release of FFA from IMTG. The first events in regulating muscle lipolysis are activation of the TG lipases, including muscle versions of ATGL and HSL. Additional regulatory steps appear to include movement of the ATGL-HSL complex to the lipid droplet, and penetration of a protective perilipin-like protein layer around the lipid droplet. Lipid droplets are found in close proximity to mitochondria, and it is likely that the regulatory proteins/enzymes involved in activating fat for storage or oxidation, transport into the mitochondria, TG synthesis, and TG degradation are all in close contact [4]. These enzymes appear to be sensitive to both hormonal and contractile stimuli during exercise, and IMTG provides an important energy source during moderate intensity exercise [10, 11]. The activity of HSL actually decreases during intense aerobic exercise, which may be the result of increased AMP kinase (AMPK) activity and contribute to a decrease in IMTG use [11]. However, current understanding of the coordinated regulation of ATGL and HSL during exercise in human skeletal muscle is rudimentary. Lastly, there is also evidence to suggest that increasing plasma FFA levels and uptake of FFA into the cell decreases the reliance on IMTG during moderate exercise lasting 2–4 h [12].

For oxidation to occur, cytoplasmic FABPc-FFA, whether derived from outside the cell (plasma FFA) or inside muscle (IMTG), must be transported to the outer mitochondrial membrane. It is then activated with CoA, if not already
activated, and converted to fatty acyl carnitine by carnitine palmitoyltransferase I (CPT I). This compound is moved across the mitochondrial membranes via a translocase, while carnitine moves in the opposite direction. Recent evidence also suggests that the transfer of fatty acyl carnitine across the membranes is aided in some unidentified manner by the fat transport protein FAT/CD36, and that this protein translocates to the mitochondrial membranes during 2 h of moderate-intensity exercise [13]. Inside the mitochondria, carnitine is removed, and the CoA is rebound to the long-chain fatty acid by the enzyme CPT II.

The level of free carnitine may inhibit mitochondrial FFA uptake during intense exercise as it is a substrate for the CPT I reaction [14]. Muscle carnitine content decreases as a function of increasing exercise intensity, and a low free carnitine level could limit FFA transport into the mitochondria and FFA oxidation during intense exercise when glycolytic flux is high. It is not currently known how much free carnitine is needed in the cytoplasm to maintain CPT I-mediated FFA transport into the mitochondria. However, the highest rates of FFA oxidation occur when the carnitine levels are already substantially lower than resting levels and carnitine is not consumed in the transport process, but recycled back into the cytoplasm. In addition, when fat availability in the blood is artificially increased during intense exercise (~80% VO2max) the muscle oxidizes more fat, suggesting that the level of muscle carnitine is not limiting for fat oxidation at intense power outputs [15]. Collectively, these factors make it unlikely that free carnitine availability limits FFA oxidation during intense exercise, but Stephens et al. [14] have been able to increase muscle carnitine levels by a modest 15% with a 5-hour infusion of carnitine and insulin. This resulted in an apparent increase in fat oxidation, but the results are confounded by the fact that the hyperinsulinemia would increase the content of muscle membrane and mitochondrial fat transport proteins, which may also contribute to increased fat oxidation. In conclusion, no experimental model has been able to conclusively test whether there is a causal relationship between increasing muscle carnitine levels and increased fat oxidation during intense exercise.

A second possible explanation for the decreased reliance on fat at higher power outputs involves the inhibition of CPT I activity. Studies on mitochondria isolated from resting human skeletal muscle reported that decreases in pH from 7 to 6.8, typical of the cellular changes when moving from moderate to intense aerobic exercise, caused large reductions in CPT I activity [16].

Once inside the mitochondria, fatty acyl-CoA molecules are metabolized in the β-oxidation pathway to produce acetyl-CoA and reducing equivalents (NADH, FADH2). There is currently no evidence that the enzymes of the β-oxidation pathway are externally regulated, suggesting that regulation is simply a function of the availability of pathway substrates (fatty acyl-CoA, NAD+, FAD, and free CoA) and products (NADH, FADH2, and acetyl-CoA). The most effective way to change the control or increase the capacity of the β-oxidation pathway is to increase the mitochondrial content, as occurs with exercise.
training. The maximal activity of β-hydroxyacyl-CoA dehydrogenase (β-HAD), a representative enzyme in the β-oxidation pathway, is commonly used to assess the magnitude of the adaptation to training, as discussed below.

Exercise Training Increases Fat Oxidation during Exercise

Holloszy [17] and Holloszy and Coyle [18] in the late 1960s and early 1970s demonstrated the remarkable plasticity of rat skeletal muscle, including the ability to increase the mitochondrial volume and the production of energy from fat and CHO. These landmark studies spawned the field of exercise-induced mitochondrial biogenesis, and additional work quickly confirmed these findings in human skeletal muscle [4, 19]. Work in this area continues to be at the forefront of research today as investigators revisit the early training studies with new and powerful molecular tools in an attempt to understand how skeletal muscle mitochondrial biogenesis results from the cumulative effects of transient increases in mRNA transcripts encoding mitochondrial proteins after successive exercise sessions [20–22]. This process requires the coordinated expression of both nuclear and mitochondrial (mtDNA) genomes through factors dedicated to specific families of genes encoding distinct categories of mitochondrial proteins.

A greater mitochondrial volume following training is believed to include increases in the fat-metabolizing machinery, as representative enzymes of the major fat-metabolizing pathways (e.g. citrate synthase, CS, β-HAD and cytochrome c oxidase IV) are also increased [17, 23]. This provides the means for a greater capacity to produce NADH from fat, and hence more ATP in the electron transport chain. The net result of these changes is a greater reliance on fat as a fuel and improved metabolic control (i.e. matching ATP production to ATP hydrolysis via oxidative mechanisms) during the onset of exercise at moderate and high aerobic exercise intensities. This results in reductions of the signals (free [ADP] and [AMP]) that activate the major enzymes that metabolize CHO (glycogen phosphorylase, phosphofructokinase and pyruvate dehydrogenase) and a decreased reliance on CHO for energy production at any given submaximal power output [17, 24].

The increased capacity for fat oxidation also requires increased provision of fatty acids, and it is not surprising that training increases plasma membrane and mitochondrial FA transport proteins [25]. In addition, endurance training also increases the IMTG store [4, 26] and increases HSL activity in some studies. A recent study has also reported an increase in ATGL activity [27]. Collectively, these adaptations alter the pattern of fuel utilization during submaximal exercise whereby whole body rates of fat oxidation are increased, while the rate of CHO oxidation is decreased, principally through the sparing of muscle glycogen, at both the same absolute and relative work rates as before training.
Recent molecular work has examined the time course of responses of mitochondrial biogenesis, mitochondrial fusion and fission proteins, and selected transcriptional and mitochondrial mRNAs and proteins in human muscle to seven sessions of intense interval training [23]. PGC-1α mRNA was increased \( >10\)-fold 4 h after the first session and returned to control within 24 h (fig. 3). This ‘sawtooth’ pattern continued until the 7th bout, with smaller increases after each bout. PGC-1α protein was increased 24 h after the first session (23%) and plateaued at +30–40% between bouts 3–7. Increases were observed in PPARα and -γ protein (1 session), PPARβ/δ mRNA and protein (5 sessions) and nuclear respiratory factor-2 protein (3 sessions), while no changes occurred in mitochondrial transcription factor A protein (fig. 5). CS and β-HAD mRNA were rapidly increased (1 session), followed by increases in CS and β-HAD activities (session 3), while changes in COX-IV mRNA (session 3) and protein (session 5) were more delayed (fig. 4). Training also increased mitochondrial fission proteins (fission protein-1, \( >2\)-fold; dynamin-related protein-1, 47%) and the fusion protein mitofusin-1 (35%) but not mitofusin-2. These data suggested that the training-induced increases in transcriptional and mitochondrial proteins resulted from the cumulative effects of transient bursts in their mRNAs and that training-induced mitochondrial biogenesis involved remodeling in addition to increased mitochondrial content. Importantly, it appears that the ‘transcriptional capacity’ of human muscle is extremely sensitive, being activated by one training bout. Recent work by Gibala [28] concluded that signaling through AMPK and p38 MAPK to PGC-1α may explain in part the metabolic remodeling induced by intense interval training, including mitochondrial biogenesis and an increased capacity for FFA oxidation.
Strategies to Increase Fat Oxidation in Already Well-Trained Athletes

A key area of interest is to find ways to increase fat oxidation in already well-trained athletes during exercise such that CHO use is curtailed or spared and is available for later in exercise in events where CHO availability is paramount for athletic success. Most of what we know regarding the ability to increase fat use has been derived from studies that used experimental manipulations to increase the plasma FFA availability without affecting many other processes. While many models have been used with varying success, including acute high-fat meals, short- and long-term high-fat diets, caffeine ingestion, fasting, and even prolonged dynamic exercise, the acute infusion of a lipid solution coupled with heparin administration has been most commonly and effectively used. This technique has the advantage of acutely (~30 min) increasing the plasma [FFA] without significant alterations in other substrates, metabolites and hormones [15, 29]. In contrast, dietary attempts to acutely increase the availability of endogenous FFA to the working muscles in humans immediately prior to or during exercise in an attempt to spare CHO have been largely unsuccessful as fat is not digested quickly. As a result, these practices are not used by athletes. Therefore, prolonged high-fat/low-CHO diets are required to alter the exogenous FFA availability and IMTG stores and spare CHO, as discussed in the following chapter. Lipid infusion with heparin injections is also not used by athletes, but these experiments demonstrated that starting moderate to intense exercise with elevated FFA levels (~1.0 vs. 0.3 mM) decreased glycogen use by ~50% in the initial 15 min of exercise and increased fat oxidation by ~15% with no change in exogenous glucose oxidation during 30 min of exercise [15, 29]. However, a decreased plasma FFA level is not the entire explanation for the decrease in fat oxidation with intense exercise. When plasma FFA availability during heavy exercise was increased to levels comparable to those observed during moderate intensity exercise, FFA oxidation was increased, but not fully restored [15]. This suggests that mechanisms within muscle as discussed previously and below also limit fatty acid oxidation at higher exercise intensities. Muscle measurements during exercise with elevated FFA revealed an attenuated rise in free ADP, AMP and P_i. This situation is similar to that found during steady-state exercise following aerobic training, where an increase in mitochondrial content and increased fat oxidation is believed to account for the attenuated insult to the energy state of the cell and reduction in CHO use. However, in the studies where FFA delivery was acutely altered, mitochondrial content was not altered, and therefore a key question is why the acute provision of exogenous fat provides a similar CHO-sparing effect to aerobic training.

Interestingly, caffeine ingestion also increases adipose tissue fat mobilization and skeletal muscle oxidation, but responses to this procedure vary between subjects, limiting what can be concluded on a group basis. When effective, caffeine appears to antagonize the normal inhibitory effects of adenosine on adipose
tissue lipolysis at rest, resulting in measurable increases in plasma FFA concentrations before exercise begins. The increased FFA delivery to and oxidation by the working muscles early in exercise appears to spare the use of muscle glycogen in some individuals [30]. However, the caffeine-induced increase in plasma FFA seen at rest does not increase adipose tissue lipolysis above the exercise effect, and no further glycogen sparing occurs beyond the first 10–20 min of exercise.
Another line of work examined the metabolism of long- and short-chain FFAs during moderate-intensity (~40% VO$_{2\text{max}}$) and heavy-intensity (~80% VO$_{2\text{max}}$) exercise, and while increasing the exercise intensity reduced long-chain FFA uptake and oxidation, the uptake and oxidation of medium-chain fatty acids (MCFA) was unaltered [31]. Since long-chain fatty acids rely on fat transport
proteins to cross membranes and MCFAs are less dependent, this suggested an inhibitory effect of intense exercise and possibly of increased glycolytic flux on the oxidation of long-chain FFA. The location for this regulation likely involves plasma membrane transport and/or mitochondrial fat transport, both of which appear to be highly regulated for long-chain but not medium-chain FFAs.

Therefore, it would seem that the provision of medium-chain triacylglycerols to contracting skeletal muscle should augment the rate of FFA oxidation, as they are rapidly broken down in the stomach and duodenum and the MCFAs empty from the intestine into portal blood, are more soluble in plasma, and cross the muscle and mitochondrial membranes with less dependence on transport proteins. The same could be said for acetate, which freely enters the muscle and mitochondria and can be converted to acetyl-CoA in one step. Ingestion and infusion of these compounds produces accumulations in the blood at rest, and they are oxidized at rest. When exercise begins, these compounds continue to be oxidized in muscle and may account for up to 7–8% of the fuel used [32, 33]. However, they did not decrease the reliance on muscle glycogen to a significant amount to produce measurable glycogen sparing [33–35]. In the case of acetate, most studies have infused this compound as attempts to load the body through oral means are less effective. In the case of MCFA, the ability to ingest and move these fats into the bloodstream is dependent on the GI discomfort, and the ingestion of a tolerable amount (10 g/h) does not spare muscle glycogen or alter the patterns of fuel oxidation in muscle during exercise [34].

Strategies to increase fat oxidation in well-trained athletes at intense aerobic intensities need to elevate plasma FFA levels and target the key sites of fat metabolism and oxidation within the muscle itself. Nutritional schemes that have attempted to provide more FFA in the diet or increase adipose tissue lipolysis have been largely unsuccessful. Attempts to target the muscle and increase fat oxidation at high aerobic power outputs need to overcome what appears to be inherent or native mechanisms that decrease the reliance on fat and increase the reliance on CHO as a substrate.

**Fat Metabolism during Recovery**

As soon as an exercise training session or competition ends, athletes are in recovery mode and preparing for the next training session or competition. It is clear that CHO repletion is of paramount importance, as most training sessions and competitions for endurance and stop-and-go sports require high muscle glycogen stores for fuel. The same can be said regarding the importance of ingesting protein after exercise in order to stimulate muscle protein synthesis and rapidly move the muscle into positive protein balance. There also has been growing evidence that recovery nutrition must consider the amount of fat that is consumed in order to replenish the IMTG stores that have been used during
exercise. Trained athletes often have as much energy stored as IMTG as they do glycogen, and IMTGs are a significant source of fuel during prolonged low and moderate intensity exercise in untrained and trained men and women [10, 26]. Although it is unclear whether beginning an exercise session with a reduced IMTG store will limit the exercise capacity, an inability to replenish this store over repeated training sessions could lead to such a situation.

Several published studies have examined the time course of IMTG recovery following exercise that depleted a portion of the IMTG store. They demonstrate that recovery diets lasting 18–48 h that contain only 10–24% of the total energy intake as fat do not replenish IMTG as quickly as diets that contain higher fat intakes (35–57% of total energy). In fact, two studies reported no IMTG repletion after 18 h with repeated meals containing 21% fat [36] and 48 h where 24% fat was consumed [37]. On the other hand, the recovery of IMTG was complete after only 22 h when a 35% fat diet was consumed after exercise [37]. This study also reported that the exercise-induced decreases in IMTG content and the lack of IMTG replenishment in the low-fat trial was only in the type 1 fibers. It has been estimated that the amount of ingested fat required for IMTG repletion is ~2 g/kg body mass per day [38]. However, a high fat intake right after exercise may compromise the ability to replete muscle glycogen and impair performance, so a common strategy is to emphasize CHO and protein replenishment in the first 6–8 h after exercise, and then increase the fat content beyond this time point [38]. This may be a sound strategy as the replenishment of IMTG in the hours after exercise appears to be delayed by the fact that skeletal muscle oxidizes a large portion of the available FFA as the majority of ingested CHO is stored as glycogen. No changes in muscle IMTG levels were reported at 2, 3 or 6 h of recovery following exercise [36, 39, 40], while glycogen was substantially repleted [36].

Athletes who engage in prolonged exercise every day may have chronically low IMTG content, leaving them in a more CHO-dependent state where their post-exercise diet would require extra CHO. This may not matter as they prepare for an endurance event that requires exercise at a higher power output than training. In other words, is there ever a situation where a greater reliance on fat would benefit an endurance athlete's performance? Also, are there any benefits of training with higher or lower IMTG levels? These questions have not been answered and will be discussed in the following chapter.

**Conclusions**

The regulation of fat metabolism in skeletal muscle is complex and involves many sites of control. Recent work has identified the importance of fat transport across the plasma and mitochondrial membranes and the regulation of IMTG degradation as major sites of control regulating fat metabolism and oxidation during exercise. The oxidation of fat is maximal during moderate intensity
exercise with distinct control mechanisms for downregulation of fat oxidation at intense aerobic intensities associated with high-level sports training and competitions. Exercise training is the most potent stimulus for increasing the muscle mitochondrial volume and the capacity to oxidize fat during exercise. Molecular work has identified that increases in transcriptional and mitochondrial proteins during training result from the cumulative effects of transient bursts in their mRNAs. Nutritional schemes that have attempted to acutely provide more FFA in the diet, increase adipose tissue lipolysis, and augment fat oxidation directly in the muscle have been largely unable to augment fat oxidation enough to substantially spare CHO use during exercise. In addition, the effects of these acute nutritional manipulations on the molecular responses in the muscle have been largely unexplored. Lastly, some work has investigated the importance of the fat content in recovery diets, but little is known about the consequences of these strategies for success in subsequent training sessions and competitions.

References


Discussion

Dr. Hoppeler: You say you can increase fat metabolism during exercise when the fat content in the bloodstream is acutely increased (e.g. by using heparin). To me this suggests that the muscle plasma membrane is a very severe barrier or else the limitation to fat use is within the muscle fiber.

Dr. Spriet: The conclusion is actually the opposite – because simply increasing the free fatty acid concentration restores a good portion of the fat oxidation that was lost when going from 40 to 80% VO\textsubscript{2}\text{max} (plasma FFA level is low at 80% VO\textsubscript{2}\text{max}). The largest barrier to fat oxidation during intense exercise lies in the adipose tissue. However, it doesn’t look like it is adipose lipolysis per se, because measurements of lipolysis suggest that it stays high at high exercise intensities. Instead, it appears that a reduction in blood flow within adipose tissue limits the amount of fat that gets out into the bloodstream and binds to albumin. However, fat oxidation doesn’t get restored all the way back up to the levels seen at lower intensity exercise, suggesting that there are also some limitations either for fat coming into the muscle cell or for fat getting to the mitochondria. Most of the fat that comes into the muscle during exercise goes straight to the mitochondria for oxidation and is not cycled through IMTG. This points the finger at the adipose tissue as well as the muscle in terms of explaining the decreased fat oxidation when moving from a moderate to heavy exercise intensity.

Dr. Hoppeler: Some migratory birds completely rely on fat metabolism during migratory flights when they exercise at a very high intensity. Is it known how this amazing feat is achieved?

Dr. Spriet: McFarlan et al. [1] recently reported that birds have a very good ability to oxidize fat during migration and a very high concentration of fat transport proteins in skeletal muscle. From an evolutionary point of view, fat is a good fuel when you work at a high intensity against gravity as these birds do, because of the amount of energy per gram of stored substrate. They reported a large upregulation of muscle fat transport proteins in the migratory vs. non-migratory seasons. It seems that exercise training in humans does not induce similar changes, and humans are not capable of the high rates of fat oxidation at high exercise intensities that these animals achieve during migration. It would seem that the evolutionary pressure to successfully migrate has driven the changes to maximize fat oxidation.
Dr. Phillips: With respect to your zero change in IMTG content after exercise – you said that there was no net use, but you can’t measure breakdown and synthesis with a static measure of content.

Dr. Spriet: Yes, that is correct. We cannot say whether fat coming into the muscle is first esterified to IMTG and whether some lipolysis is also occurring. However, there is no net change in content during the recovery period, meaning that the fat used is ultimately coming from outside the cell. There has been an argument that recovery requires a net use of IMTG, but we have not seen this [2].

Dr. Phillips: Pulse tracer methods as used by Jensen [3] provide data that seem to be fairly robust; why has nobody followed up on that in human beings?

Dr. Spriet: It has been done in humans, but it's hard to do, and there is some concern about how accurate the pulse chase tracer methodology is. It appears that the fat that is added to IMTG in the pulse phase is the first to break down during the chase phase, rather than fully equilibrating with all IMTG. This would lead to erroneously high values of FFA-IMTG cycling, but we cannot be sure whether or not that's actually the case. Data from Jensen's lab indicate that at rest there is a lot of simultaneous IMTG synthesis and breakdown.

Dr. Phillips: Indeed at 50% VO$_{2\text{max}}$ they show inward and outward flux but no net change in the pool.

Dr. Spriet: Yes, and those results do not match findings from other laboratories where there is net use of IMTG in exercise at ~50% VO$_{2\text{max}}$ that lasts more than 1 h. I suppose that there is some futile cycling, but in their first paper they suggested that 93% of the fat that came into the muscle cell from outside was immediately oxidized during exercise and only 7% was esterified to IMTG [3]. Why it would go through IMTG first and then be oxidized in an exercise situation remains unclear to me. This would make sense during rest as has been shown by a recent paper from their laboratory [4], but during exercise the incoming FFAs need to be oxidized.

Dr. Phillips: The tracer studies suggest that you need to activate lipoprotein lipase to make use of circulating triglycerides. The main regulation of lipoprotein lipases is through insulin, which is very low during exercise. This could be why it is not possible to get too much out of these.

Dr. Spriet: Epinephrine might play a role in some tissues, but it does not appear that this regulation is very powerful in skeletal muscle. We don't know all the regulators of plasma TG degradation, but it seems that skeletal muscle is not designed to make much use of circulating triglycerides, especially as we move to higher exercise intensities. At higher intensities, you don't even have to give an intralipid solution; heparin injections alone will cause an activation of LPL and raise the circulating FFA levels (50–60% of the response you get if you add additional TG from an intralipid infusion). The Scandinavian groups argue that the provision of FFA from circulating TGs does play a role, but of course they don't really measure it, and simply deduce it from their other measurements and assume what they cannot account for is circulating TGs.

Dr. Maughan: You wrote in your manuscript that muscle can function with no carbohydrate. Is it really true that all of the energy can come from fat?

Dr. Spriet: I can't answer that question, but if you do the classic experiment and deplete the muscle of carbohydrate as much as you can, and then put subjects on a high fat diet for two and half days, they have very little glycogen in their muscles. However, there is some carbohydrate and that's where that statement comes from. So, clearly there
is a small amount of carbohydrate, but it's as minimal as you can possibly make it. In the experiment I described above, subjects begin exercise with muscle glycogen in the order of 100–200 mmol per kg dry muscle and they are lasting about 30–35 min during exercise at ~75% VO\textsubscript{2max}. Although some carbohydrate is used at the beginning of exercise, there is little available in the later stages, and they have to rely on what they can get from outside the cell. Invariably, these people have no glycogen in their muscle and are hypoglycemic when they reach volitional exhaustion.

*Dr. Maughan:* You said, the most powerful stimuli to increase fat oxidation are aerobic training and high fat feeding, but what about fasting? Is the high fat feeding really more effective than fasting? Is it not the absence of carbohydrate that is increasing fat oxidation?

*Dr. Spriet:* Yes, you can add fasting too. I don't know who would do it in the real world, but I suppose there are athletes who may try this.

*Dr. Maughan:* But there is more than just a conceptual difference. Is it the fact that carbohydrate is not available that promotes fat use or is there something about fat feeding that promotes fat utilization by muscle?

*Dr. Spriet:* We can argue over whether removing carbohydrate is more important than providing extra fat, but it's virtually impossible to separate the two in an intact person. Taking away carbohydrate suppresses insulin, which is believed to be a signal to downregulate key enzymes in carbohydrate metabolism, like pyruvate dehydrogenase. High fat diets quickly increase free fatty acid levels, which enter the cell and activate transcriptional factors to increase proteins associated with fat metabolism. However, your point is well taken as the body is very sensitive to the lack of carbohydrate. When no carbohydrate has been ingested for a few hours, the activity of pyruvate dehydrogenase in all the tissues will decrease rapidly, and this probably occurs during overnight sleep.

*Dr. Hawley:* Bengt Saltin and John Holloszy say that one of the major factors driving mitochondrial biogenesis is the post-exercise elevation in free fatty acids. You showed very nicely in your study that FFAs are hugely elevated in this situation. Going back to the point of triggers for adaptation – should we withhold carbohydrate after exercise, because as soon as you ingest carbohydrate you decrease fat metabolism. Shouldn't we train intensely with glycogen on board and then proceed with starvation to maintain increased fat availability?

*Dr. Spriet:* With respect to prolonging the increases in the plasma FFAs after exercise, I don't have an answer to that. However, in the rat model that John Holloszy uses, he keeps the FFA levels high for a long period of time and can show mitochondrial biogenesis. However, when working with humans, it does not seem practical to keep the FFA concentration high for 4 h instead of the normal 1–1.5 h, for example.

*Dr. Hawley:* A paper has just come out in rats from Winder's lab [5], where exercise was mimicked by AICAR treatment, and rats were given high-fat diets. It was found that AMPK activation by AICAR and a high-fat diet had an additive effect in some muscle tissue.

*Dr. Spriet:* Yes, I am aware of the use of the so-called 'exercise mimetics' like AICAR. However, we have to keep in mind that the exercise stimulus is very powerful on its own. For example, in the study cited in my talk we used untrained people, and exercised them for 4 min at 90% VO\textsubscript{2max} followed by 2 min of rest, and repeated this procedure 10 times, with workouts every other day. This is indeed a very large stimulus. There is not much you can do to make a person train harder. It may help if you breathe a hyperoxic gas
mixture under these training conditions, as it seems to have a central effect such that you can train at about 25 W greater during your entire training protocol – so 275 instead of 250 W in normoxia. Yet, all the muscle measurements we made at various time points during the training regimen looked identical, suggesting that the extra 25 W didn't add any additional training stimulus, presumably because the insult or training stimulus of the normal training was so great. This might be quite different if you try to further stimulate mitochondrial biogenesis in well-trained athletes; hyperoxia may be more favorable under these conditions.

**Dr. Hawley:** A comment regarding the excellent Perry et al. [6] paper. If you look at the PGC1-α mRNA responses over the 7 days of training, this response is dropping, suggesting that you need more overload to restore the training effect.

**Dr. Spriet:** Yes, you may be correct, but PGC1-β starts to increase after about 5 or 7 days, so PGC1-β might be playing a role later on in training. But you are correct, there could be a downregulation of the PGC1-α response over time, and the same exercise stimulus may not have the same effect as training progresses. This needs to be explored in training protocols.

**Dr. van Loon:** I would like to go back to the possibility that extracellular fat availability might limit fat oxidation during high-intensity exercise. Data published in the past showed that with high intensity exercise there is still an increase in free fatty acids in the cytosol. So, it doesn't seem to be anything extracellular that is limiting, and that goes hand to hand with the rest of your data. If you increase fat provision by intralipid infusion and see an increase in fat oxidation from 40 to 80% maximum work load capacity, that doesn't mean that the fat load is limiting fat oxidation. It just means that if you go from 40 to 60% you would see an increase in fat oxidation, whereas if you go from 60 to 80% you wouldn't see it. Many people have misinterpreted our previous study, looking at the contribution of the different endogenous fat sources during exercise of various exercise intensities [7]. Besides plasma free fatty acid oxidation, we quantified the use of ‘other fat sources’. These other fat sources include everything that is not coming from the plasma and should therefore include both intramuscular triglycerides and plasma lipids. However, the use of plasma lipids is already largely represented by the use of plasma free fatty acids. Due to their high turnover, plasma lipids become rapidly enriched to the same level as the plasma free fatty acids. So, what you consider to be plasma free fatty acid oxidation also includes the use of plasma triglycerides.

**Dr. Spriet:** So, all the so-called ‘other’ sources should be labeled intramuscular? With respect to the importance of free fatty acids inside the cell, the reason I didn't mention these is that the contribution from these and the actual concentration within the cell is pretty controversial. I am not sure that I would hang my hat on that just yet.

**Dr. van Loon:** Following exercise, you see a continuous increase in free fatty acid levels, and the flux continues for 15 min after exercise and then drops. This suggests that plasma free fatty acid provision is quite in line with the amount that the muscle would like to access. So, lipid fuel supply seems to be better organized than what we generally think.

**Dr. Spriet:** Much of the confusion comes from the fact that we are looking for ways to get athletes who work at very high intensities to rely more on fat so they can spare carbohydrate for carbohydrate-dependent aspects later in the competition. However, unlike the migrating bird, the human body is simply not designed to rely heavily on fat oxidation at high exercise intensities.
**Dr. van Loon:** We see direct correlations between the amount of IMTG oxidation, oxidative capacity and performance, so many people, including ourselves, have tried to optimize IMTG storage. But IMTGs might simply be a representation of fluxes in fatty acids because if you reduce the free fatty acid flux you start oxidizing more IMTGs. IMTGs could thus be viewed as a reserve storage depot when the plasma provision of lipids is insufficient to allow maximal fat oxidation rates.

**Dr. Spriet:** Exactly, and that's what we have seen as well. During 4 h of exercise at 55% VO$_{2\text{max}}$, most IMTG is used in the first hour. As soon as the athlete can mobilize free fatty acids to fairly high levels, plasma FFAs become the dominant fat source and the reliance on IMTGs decreases.

**Dr. van Loon:** So, IMTG are an important substrate source but not to allow maximal oxidation.

**Dr. Spriet:** Yes, but if you look at some of the data following 8 h of submaximal exercise, the IMTG concentrations are essentially used up. So, over this prolonged exercise you continue to use some IMTG.

**Dr. Hoppeler:** We showed long ago that running 100 km results in an almost complete loss of IMTGs [8].

**Dr. Baar:** I was going to say the same thing, especially in the birds because what you see in the birds is a huge increase in intramuscular triglyceride as they prepare to migrate, to a point where you actually see a disruption of the myofibrillar lattice to some degree. I think it's because it's just a readily available source.

**Dr. Maughan:** How is the story on the regulation of substrate metabolism in cardiac muscle, is it very different from skeletal muscle?

**Dr. Spriet:** I don't know, as I have not followed the cardiac literature very closely. Obviously the heart relies more heavily on exogenous free fatty acids. There are some big differences between the heart and skeletal muscle in terms of the way it's regulated, but I haven't been following that topic.

**Dr. van Loon:** IMTG levels in heart are high in obesity and in type 2 diabetes, but in athletes they are actually quite low. So, while athletes have high IMTG stores in skeletal muscles, they don't seem to have those in the heart.

**Dr. Spriet:** That fits fairly well with the whole body being under siege in people that are overweight. They simply have too much fat to dispose of, and it simply goes everywhere it can possibly go – in the liver, in the heart, in skeletal muscle, and in adipose tissue.

**Dr. Gibala:** Is the migration of the fat transport protein, FAT/CD36 in the plasma membrane exercise intensity dependent?

**Dr. Spriet:** We don't know that yet. We should repeat some of the studies that we have published and have people exercise at 40, 65 and then 90% of VO$_{2\text{max}}$. Especially if you begin exercise at 65% and then have athletes work at 85% for 30 min or whatever they can handle to see whether CD36 would just sit in the membrane or whether it would actually move back out of the membrane as the reliance on fat decreases. We also don't know anything about the time course of fat transport proteins moving out of the membranes after exercise, which could be interesting.

**Dr. van Loon:** Could you also discuss the experiments in which carnitine content of the muscle was increased?

**Dr. Spriet:** Recent work from Greenhaff’s laboratory suggests that increasing muscle carnitine increases fat oxidation during exercise. However, there are a couple of problems
with those studies. Firstly, insulin infusion is needed along with high levels of plasma carnitine to get additional carnitine into the muscle. Of course, high insulin levels cause many other metabolic changes – one is that it moves more FAT/CD36 to the muscle and mitochondrial membranes. This sets up the alternate hypothesis that it’s not increasing carnitine that augments fat oxidation, but an increase in the transport of free fatty acid into the cell or into the mitochondria to be oxidized. This could be tested of course.

Secondly, other evidence speaks against carnitine being limiting for fat oxidation: carnitine is not consumed in the process of moving fat into the mitochondria, and the highest levels of fat oxidation (~65% VO_{2max}) occur when muscle carnitine levels are already quite low. In addition, if the plasma free fatty acid concentration is artificially raised to high levels during intense exercise, the muscle oxidizes more fat, again suggesting that carnitine is not limiting. However, as with many metabolic processes, all these factors could work together to downregulate fat utilization at high workloads. It makes a lot of sense from a teleological point of view to shift to carbohydrate as a fuel when exercise intensity is close to the limit of your oxygen uptake ability, to get as much energy as possible from each liter of oxygen consumed.

References


