Free fatty acids and exercise1-3

Bengt Saltin and Per-Olof Åstrand

ABSTRACT Although the great explorers were well aware that eating fat was an efficient way to meet their large energy demand, it was not until some decades into this century that it could be demonstrated that lipids are metabolized directly by contracting skeletal muscles. The 1950s produced the first studies with [14C]-tagged fatty acids (FAs), proving that fat is transported into the cell as FAs. An FA-transporting protein that is present in the sarcolemma and in the cytoplasm has been identified. For FA transport into the mitochondria, carnitine and carnitine transferase are needed. It is still unclear how the use of lipids as an energy source for the muscle during exercise is limited. The supply of free fatty acids (FFAs) far exceeds what is taken up by the muscle. Seldom more than 2–4% of the amount of FFAs delivered to an exercising limb is taken up by the muscles and only part of it is oxidized. Physical training induces changes that enhance the uptake of FAs by the contracting muscles, and a larger fraction of this uptake is oxidized, but it is not yet clear which mechanism is behind this adaptation. What is known is that this uptake occurs despite no elevation in the amount of FA supplied to the limb. Am J Clin Nutr 1993;57(suppl):752S-8S.

KEY WORDS Free fatty acids. energy (demand), exercise, lipid, fat oxidation

Introduction

Of the two main fuels stored in the human body and used for muscular exercise, fat has several characteristics that would make it the substrate of choice. Fat contains more than twice the energy per unit weight than does carbohydrate and is not hydrated when stored in the body, which makes it ideal as a fuel during sustained efforts. This was known long before measurements of respiratory-exchange ratios (RERs) demonstrated that fat was the dominant substrate at rest and the preferred fuel at low-intensity exercise.

The Norwegian polar explorer Nansen was one of the first to realize that fat was a necessary component of the diet under long explorations when food supply was limited and had to be brought along by the explorer (1). The fat was provided by the daily consumption of 350–400 g pemican, a food consisting of ground beef into which fat was melted, which provided 60–70% of the daily energy requirement. Sledge dogs were also fed pemican but theirs contained a higher content of fat. This demonstrates an early understanding that although the muscles of different species have similar basic characteristics and demands, they are still different.

This article takes a historical approach, in part. A brief account is given of some of the early studies based on respiratory quotient (RQ) or RER measurements, including the first studies that used isotope-labeled compounds to study mobilization, transport, and uptake by muscle of free fatty acids (FFAs) and the relative and absolute role of lipid oxidation at various exercise intensities. Thereafter, some more recent critical issues are discussed related to the role of intramuscular lipid stores and to which mechanisms bring about the enhancement of lipid oxidation with endurance training.

The early years

There have been many controversies through the years related to lipid combustion and muscular work. One of the first questions to be resolved was whether fat must be converted to sugar before being used by the muscles, as suggested by Chaveau (2). Zuntz (3) and later Krogh and Lindhard (4) elaborated on this problem and demonstrated that fat was used directly. These studies were followed by detailed analysis of the behavior of RER during exercise. RER was found to become elevated with increasing work intensity, indicating a gradual decline in the relative role of fat for combustion. In absolute terms fat utilization is usually the largest at a relative exercise rate of ≈60–70% of maximal aerobic power. when RER still can be below 0.9 and the rate of oxygen uptake is from 1.5 L/min (untrained) to 4.0 L/min (trained). This amounts to 0.3–0.7 fat g/min to be combusted. Even higher values may be obtained during prolonged exercise, especially when preceded by a dietary regimen with enhanced fat intake. In the early studies by Edwards et al (5), RER decreased to 0.75 during 6 h of exercise at an oxygen uptake of 2.4 L/min, which means that > 1.0 g lipids/min were oxidized (Fig 1). After dietary manipulation with a high fat intake, Christensen and Hansen (6) demonstrated that up to 90% of the oxidation was supported by lipids and 1.5 g fat/min was used. Later studies have confirmed these earlier findings (7).

Of some interest in this context is whether availability of fat can limit exercise capacity, as can so easily be demonstrated to be the case for carbohydrate (8). In a series of experiments Pernow and colleagues (9, 10) varied the availability of substrate of exercising limbs. When muscle and liver glycogen were low, reduced mobilization of lipids from adipocytes in the fat pad (and possibly in the muscle) by intake of nicotinic acid elevated RQ and markedly impaired further work (Fig 2).

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The mobilization of FFAs is primarily a function of elevated sympathetic nervous activity to the fat pad, with both direct nervous action as well as an effect of circulating epinephrine (16). Growth hormone has primarily an enhancing role whereas insulin has a quite strong inhibitory effect (17).

Triglycerides as fuel for muscular work

Suggestions have been made that the triglycerides (TGs) stored in the muscle and those within the blood could serve as energy sources to fuel muscle metabolism during prolonged work. The impetus for this suggestion comes from the observation that the uptake and oxidation of plasma FFA cannot account for all fat-derived energy. Attempts to quantitate the contribution of these TG pools of energy have produced equivocal results. Masoro et al (18) could not demonstrate any role for intramuscular TG in the muscle of monkeys during prolonged electrical stimulation. Conversely, Issekutz and Paul (19) concluded that the energy balance during prolonged exercise is such that intramuscular lipid stores must be used to account for all the substrate combusted.

Most studies with human muscle have used the biopsy technique to obtain tissue samples before and after exercise. Results have been mixed in studies of whether the TG store of skeletal muscle is used during exercise and, if so, whether it is an important energy source during exercise. There are reports of a decline in the TG concentration of human skeletal muscle after exercise varying in duration from 1 to 12 h (20–22). However, several studies have failed to identify any major change in the TG concentration of skeletal muscle after exercise (23, 24). In some of these reports, either no change was found or the magnitude of changes was insufficient to account for the “missing fraction” of the energy consumed during exercise. Without doubt the diversity of these findings is related to the samples of biopsied muscle being unrepresentative of the actual muscle mass and, therefore, of TG content because the assay to determine TG is sensitive and accurate. In contrast to glycogen, TG in muscle is not stored homogeneously in the fiber, and the two major muscle-fiber types have different TG contents (see 25). Fröberg and Mossfeldt (22) attempted to resolve this by using two to three large samples (20–50 mg) for the assay. They consistently observed utilization of the TG stored in muscles of 2.6–15.8 mmol/g after many hours of work. Because total energy turnover in this exercise was 2.4 MJ (6000 kcal) or more, the contribution of muscle TG breakdown was ≈5% of total energy turnover. Even if the muscle TG store had been completely depleted, only 10% of the energy could have been derived from this energy source. Later studies using smaller muscle-tissue samples for the assay of TG have not produced consistent results. An approach taken by some to determine muscle TG utilization during exercise has been to use morphometric methods to determine the size of the fat vacuoles in electron micrographs (26). These studies have demonstrated a definite reduction in the size of the fat vacuoles, indicating use of TG in muscle fibers during prolonged exercise. Hurley et al (27) reported large reductions in the TG content of human skeletal muscle after exercise in trained muscles. However, the TG content of the muscle and the net breakdown were very large (Table 1).
Recently, a third approach has been taken in the attempt to determine the extent of intramuscular TG: quantitative measurement of the release of glycerol from contracting muscles. Because serum TG is probably not utilized to any major degree, the glycerol is assumed to be derived by lipolysis within the muscle. Measurements indicate that with prolonged effort, muscle TG hydrolysis contributes 5% or at most 10% of the energy turnover in the later phases of an hour exercise period (28). A limitation with this approach is that it does not establish whether the TG hydrolysis has occurred within or between the muscle fibers. This problem has no bearing on the question of what is the total contribution of FFAs to the energy turnover of exercise, but it is important in the quantitative assessment of the relative roles of the different fat depots that contribute FFAs to the pool utilized during exercise. Kiens et al (29) have approached this problem by measuring both glycerol released from the muscle and TG depletion in groups of fibers that were dissected free from biopsy samples and cleaned before they were assayed. Only a small and insignificant reduction in muscle-fiber TG concentration was found after 2 h of exercise (Table 2). Because glycerol release demonstrates lipolysis, these data suggest that adipocytes between fibers play a significant role in human energy production during exercise. These findings contrast with those of Hurley et al (27), who also estimated changes in TG stores of human muscle fibers by determining TG concentration in fibers dissected

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
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<tr>
<td>Muscle glycogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before exercise</td>
<td>285</td>
<td>328+</td>
</tr>
<tr>
<td>After exercise</td>
<td>82†</td>
<td>209‡‡</td>
</tr>
<tr>
<td>Muscle triacylglycerol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before exercise</td>
<td>59.2</td>
<td>63.3</td>
</tr>
<tr>
<td>After exercise</td>
<td>46.4</td>
<td>37.2‡</td>
</tr>
</tbody>
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* Data from reference 27.
† Significantly different from untrained group (P < 0.05).
‡‡ Significantly different from before exercise (P < 0.05).
§ The depletion is significantly different from untrained group (P < 0.05).

**FIG 2.** Right panel: respiratory quotient (RQ) (determined on blood from the femoral artery and vein) is elevated when nicotinic acid is given to block mobilization of free fatty acids (FFAs), regardless of whether the muscle contains a little (low) or a normal amount of glycogen. Left panel: the reduction in plasma FFA concentration caused by the nicotinic acid (upper part); the low uptake of FFAs and the short exercise time to exhaustion (middle part); and the exercise intensity for the one-legged exercise (bottom part). (Data from ref 10.) Each symbol represents one value.
from biopsy samples. As indicated earlier, the decline in the TG concentration of the fibers after exercise was larger than necessary to account for the total metabolism of the limb muscles. However, the exercise models used in the two studies were quite different. Hurley et al (27) used ordinary two-legged exercise and Kiens et al (29), one-legged, knee-extendor exercise. In the former type of exercise, sympathetic activity is elevated quite markedly whereas in the latter type, almost no sign of activation is present. Because the sympathetic nervous system has a key role in the activation of lipolysis, this explanation of the difference should not be overlooked (29).

From this description it appears that TGs located within the muscle fibers are used during prolonged exercise by humans but that their quantitative role is small. It has not been firmly established whether lipid droplets in the fibers or the TGs of the adipocytes between fibers, or both, contribute to TG depletion. It would appear that both energy pools are used during some types of exercise. It is hard to envisage a role for the lipid droplets in muscle other than to support metabolism.

Little is known about the regulation of lipolysis within the muscle fibers during exercise. Beta-adrenergic blockade reduces the rate at which glycerol is released from the contracting muscle (30). Infusion of a β2-agonist exaggerates this glycerol release (31). Thus, it is most likely that mechanisms similar to those described above for regulation of the lipolysis in the fat pad operate in the muscle. The suggestion that circulating epinephrine may be more critical than direct nervous activation of lipolysis in the muscle (29) is refuted by the results of studies using nicotinic acid, which demonstrated markedly lowered FFA and glycerol release from the contracting muscles and no TG breakdown (10).

Training and fat utilization during exercise

It is an old observation that reliance on fat as a source of energy during submaximal exercise is greater after endurance training (6). Subsequently, studies were completed in which the RER at a given oxygen uptake was observed to be lower after training than before training. This occurred without any difference in the plasma concentration of FFAs, and possibly with less activation of lipolysis via the sympathetic nervous system than occurs in the untrained state. Mobilization of FFAs from the fat pad must have been exaggerated, as was its uptake by muscle. The former could be a function of elevated sensitivity to stimuli enhancing lipolysis, especially sympathetic activation. Support for this notion comes from investigations that use the microdialysis technique in the subcutaneous tissue, demonstrating that a given concentration of epinephrine produces a larger release of FFA and glycerol in trained compared with untrained subjects (32, 33). Henriksson (34) was the first to more directly demonstrate that endurance training of muscle caused an elevated lipid oxidation in the exercising limb. He used the one-legged training model, and his subjects performed prolonged two-legged exercise after the training period. This allowed for a comparison of the metabolism in a trained and an untrained limb (muscle). The deliveries (blood flow × concentration) of oxygen, substrates, and hormones were identical because the arterial blood flow was the same in the trained and untrained limbs while the two-legged exercise was performed. Under these conditions he found a larger fat combustion in the trained muscles, which could be linked to elevated mitochondrial capacity induced by the endurance training.

The questions of interest regarding this shift in choice of fuel for exercise are 1) what is its importance, and 2) what is the mechanism for producing the change? The importance of a shift to greater reliance on fat as a fuel for muscular exercise can be related to two aspects. First, because fat generally constitutes the single largest available energy pool in the body, an attempt to use this energy store to the greatest possible extent represents an intuitive “wisdom of the body.” As pointed out earlier, fat is the most efficient substrate for energy storage devised by nature, fat having the highest energy yield of the three energy sources (fat, carbohydrate, and protein) stored in the body. Second, it has been repeatedly demonstrated that when work intensity is rather high (above ≈ 50% of VO₂max), there is an absolute requirement that a fraction of the fuel be derived from carbohydrate. Carbohydrate originates primarily from the glycogen stored within the working muscles. When the glycogen reserve of muscle is depleted, the exercise either must stop or its intensity must be reduced (8−10). Thus, any increase in energy production that can occur from use of the lipid reserves of the body will

![FIG 3. The estimated contribution of various substrates to energy metabolism during exercise when the limb is trained or untrained. Note the greater dependence on plasma FFAs for the trained limb (data from ref 39). Very similar results were obtained in studies by Henriksson (34) and Kiens et al (29).](https://academic.oup.com/ajcn/article-abstract/57/5/752S/4715959/FIG3)
conserves the glycogen stores, particularly those of the working muscle, and will increase exercise capacity (Fig 3).

Although the relative importance of a shift to greater reliance on fat as a fuel for submaximal exercise can be succinctly summarized, pinpointing the mechanism that produces the response to endurance training is not as easy. The following important adaptations may occur in trained skeletal muscle:

1) increase in concentration of enzymes for the citric acid cycle, for fatty acid oxidation, and for the electron-transfer system; 2) elevation of carnitine and carnitine transferase as transporters for fatty acids within the muscle fiber; 3) increase in transporters for fatty acids through the sarcolemma; 4) proliferation in capillarization of muscle, with both a greater number of capillaries per muscle fiber and a decrease in the area supplied by a single capillary.

Gollnick and Saltin (35) have speculated that the increased concentration of mitochondria per unit muscle tissue results in greater potential for translocation of cytosolic metabolites into the mitochondria. The original model that was proposed dealt with the response of different concentrations of mitochondria to adenosine diphosphate (ADP). With this model, based on a simple Michaelis-Menten type of kinetic regulation, it was shown that an increase in the concentration of mitochondria allowed the entire system to become more sensitive to metabolic regulators. The net result is maintenance of actively respiring muscle cells, which suppresses the Embden-Meyerhof pathway and production of pyruvate.

For the translocation of FA within the muscle fiber from the cytosol into the mitochondria, carnitine palmitoyl transferase is critical. Thus it was believed that this transporter may become elevated with endurance training, but this does not appear to be the case (36). Rather, the content of carnitine in the muscle may be important (36). There are drugs which, when ingested over a long period, lower the carnitine content of skeletal muscle, which is accompanied by a greater dependence on carbohydrates as substrate during exercise (37). Slow- versus fast-twitch muscle fibers have a higher content of carnitine, and muscle training may induce an elevation in carnitine.

Another limiting step in the transport of fatty acids from the capillary bed to the inside the muscle cell is the sarcolemma. In view of the close relationship between arterial inflow of FFAs and their uptake, it was long believed that this occurred by passive diffusion (12, 38). In recent studies it was demonstrated that these earlier findings still are valid, but that the individual variation was large and that endurance training markedly affected the slope of this relationship, i.e., the more FFA that was delivered to the muscle, the larger was the difference in uptake by trained compared with untrained muscles (Fig 4; 29, 38, 39). Further, it can be shown that saturation of the transport occurs when relating the fatty acid uptake to the unbound amount of FFA in plasma. These findings do suggest that the transport of fatty acids across the sarcolemma into the muscle cell is an active process. A fatty acid-binding protein has been identified, and in rat skeletal muscle, its content has been found to be training dependent (38).

At the level of the individual muscle fiber, the greater the area of capillary that can be exposed to the muscle, the greater is the potential for exchange of intracellular and extracellular materials. From data available on humans, it appears that mean transit time is longer after endurance training both at the same absolute and relative rates of exercise (40). The magnitude of the increase may be 10–30%. This can be visualized by a hypothetical example: 150 mL blood perfuses a single muscle fiber in 1 min before and after training. If the fiber has four capillaries involved in perfusion, the blood flow is 37.5 mL·capillary-1·min-1. With five capillaries per fiber, the flow rate is 30 mL·capillary-1·min-1. Thus, the time that it takes the blood to traverse the capillary bed to produce a constant volume is increased. This longer period of time can be used for the exchange of materials between the blood and in the muscular tissue.

Taken collectively, these adaptations can explain the shift toward greater fat oxidation after compared with endurance training. It is, however, impossible to try to pinpoint the relative role of one or the other, and if one of them is more decisive than any of the others. In this context it may be worth remembering that an elevation in the use of fat as substrate during exercise to the same extent as observed with endurance training can be achieved by dietary manipulation. Three to six days on a diet with ≥ 60% of its energy as fat lowers the RER during exercise and RQ in the exercising leg by ≈ 10% (6, 15). Whether any of the above adaptations described for endurance training are also present with a brief period of elevated fat intake is not known; that is not very likely to occur, although lipoprotein lipase activity can rise. Instead, plasma FFA concentration is increased quite dramatically, and that points to its crucial role, either bound or unbound. The question that remains to be answered is why this mechanism, i.e., larger mobilization of FFAs to elevate their concentration in plasma, is not at work in endurance training.

References

2. Chauveau A. Source et nature du potentiel directement utilise dans la travail musculaire d'apres les exanges respiratoires. chez l'homme en etat d'abstinence. CR Acad Sci (Paris) 1896:122:1163-
Discussion

Michael A. Crawford: I have a question about the amount of fat that has been distributed between muscle fibers and within the muscle fiber itself. You mentioned that the human was the only species that seems to have a lot of between-fiber fat. There is at least one other species that has a lot, and that is carp fish for market. Of course, the fat grains that you see in the meat are in fact fat of quite large amounts that has gone in between the muscle fibers. What sort of level do people have when they start this experiment? To what extent is this intramuscular fat that you are looking at actually an artificial byproduct of the modern high-energy Western diet, where we are half way between a natural wild animal and a finished cow ready for the butcher? It seems to me that there are some questions here about the intramuscular fat.

Bengt Saltin: That is an interesting point. We have, I think, needless to say, subjects who are not, in relative terms, large consumers. They are interested in exercise; otherwise, they would never come for our types of experiments. Thus, they have a
carbohydrate-enriched diet (50–60% of the energy, or more). Our muscle TG may thus be in the low range. It is also known that endurance training does not result in an elevation in muscle TG, unlike muscle glycogen, which always is elevated. If I compare, for example, our data with Fröberg’s data from the 1960s, we have among the lowest [muscle TG values]. This is not a favorable situation in which to find a reduction. My personal feeling is that the deposits between fibers play a role. On the other hand, this store of fat should not be looked upon as any other adipose tissue outside the muscle fiber because it also has the problem of being carried through the sarcolemma into the cell and the mitochondria.