Fate of fatty acids at rest and during exercise: regulatory mechanisms

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Abstract
Fatty acids are a major fuel source for humans both at rest and during exercise. Plasma free fatty acids (FFA), although present only in micromolar concentrations, are the major circulating lipid fuel. FFA availability can increase two- to four-fold with moderate intensity exercise. Other potential sources of fatty acids include circulating very low-density lipoprotein (VLDL) triglycerides (TGs) (~1/5 the fuel availability of FFA) and intramyocellular TGs (~2 mmol kg⁻¹ muscle). At rest ~40% of systemic FFA uptake occurs in the splanchnic bed and uptake in legs is ~15–20%. During leg exercise the uptake of FFA in leg tissue increases to 30–60% of systemic uptake and splanchnic uptake decreases to 15%. The fate of VLDL TG fatty acids has not been adequately studied. Intramyocellular TG hydrolysis increases during exercise, but the factors that regulate this response are not clear. The fact that contraction of isolated muscles can stimulate the hydrolysis and oxidation of intramyocellular TGs (in the absence of hormonal or neural input) suggests an intracellular regulation of this process. Additional regulation from changes in catecholamines and insulin may also occur. During moderate intensity exercise circulating FFA and intramyocellular TG provide roughly equal portions of fatty acids for oxidation. In addition to endurance training, dietary factors have been shown to modulate the fatty acid oxidation response to exercise. Much remains to be learned about fatty acid trafficking during exercise. What role do VLDL TG play? How is the oxidation of intramyocellular TGs regulated? Techniques to address these questions in humans are only now becoming available.

Keywords free fatty acids, intramuscular triglyceride, isotopic tracers, lipolysis.

Given that fatty acids are a major oxidative fuel both at rest and during exercise, a better understanding of the regulation of the fate of fatty acids may improve our understanding of human performance. Training is known to increase not only aerobic power, but also increase the proportion of fatty acids that are oxidized at sub-threshold power output. A variety of factors are thought to regulate the fate of fatty acids. Fortunately, techniques are now becoming available that should allow the investigators to better understand how the fatty acid disposal (uptake, esterification and oxidation) are regulated.

Brief overview of fatty acid metabolism
In the post-absorpive state, muscle, heart, liver and renal cortex use fatty acids as their primary fuel. In humans the majority of fatty acids present in the body derives from dietary sources and are stored primarily in adipose tissue. In the post- absorptive state, circulating free fatty acids (FFA) originating almost solely from the lipolysis in adipose tissue provide the vast majority of circulating lipid fuel. Although typically present only in μmol/L quantities in plasma, resting FFA availability generally exceeds resting fatty acid oxidation. The high
rates of FFA uptake and release are accompanied by high clearance rates (the half-life of FFA is ~3–4 min at rest), which explains the large amounts of lipid fuel available in this form despite the low plasma concentrations. On average, FFA release rates in the overnight post-absorptive state average 5–6 μmol kg⁻¹ min⁻¹, whereas whole body fatty acid oxidation rates are only approximately 3–4 μmol kg⁻¹ min⁻¹. Insulin, catecholamines and growth hormone are the primary regulators of adipose tissue lipolysis. Insulin can dramatically inhibit FFA release, which can reduce FFA concentrations by 90% from overnight post-absorptive levels. During exercise the combination of falling insulin concentrations and increasing catecholamine availability can increase FFA release rates by four- to five-fold.

Other sources of fatty acids include very low-density lipoprotein (VLDL) triglyceride (TG) fatty acids. Although present in higher concentrations than plasma FFA, VLDL TG turnover is much slower such that, on average, ~1 μmol kg⁻¹ min⁻¹ of TG fatty acids are available (Sidossis et al. 1998). Another potential source of circulating lipid fuel is chylomicron fatty acids. The amount available in the circulation is entirely dependent upon dietary fat intake and the rate of absorption of fat from the gut. Although not present in the circulation, intramyocellular TGs provide a critical fuel source during exercise, a condition in which it is common for fatty acid oxidation to exceed the availability present in the circulation.

Regulation of fatty acid uptake

In the overnight post-absorptive state, approximately 30–40% of FFA uptake occurs in the splanchnic bed, with 15–20% of FFA uptake occurring in leg tissue (Jensen 1995, Meek et al. 1999). Since most, if not all, of the FFA uptake into leg tissue is in skeletal muscle and leg skeletal muscle represents approximately 40% of total muscle, FFA uptake into whole body skeletal muscle is probably 40–50% of total FFA disposal. Myocardial FFA uptake accounts for approximately 5% of systemic FFA disposal (Wisneski et al. 1987), and the kidney accounts for <4% (Jensen et al. 2001). During exercise, these proportions shift substantially, with the relative contribution of the splanchnic bed dropping to <20% of systemic FFA disposal (Ahlborg et al. 1974), and (with leg exercise) leg fatty acid uptake can now account for 30–60% of total FFA disposal (Ahlborg et al. 1974, Burguera et al. 2000) (Fig. 1).

Tissue FFA uptake varies directly with plasma FFA concentrations (Turcotte et al. 1998). Increasing FFA concentrations, such as occurs during fasting, does not result in a shift in the relative proportions of FFA taken up in the different tissue beds, but results in an overall increase in tissue FFA uptake (Jensen et al. 2001).

Lowering FFA concentrations, such as occurs following meal ingestion (Jensen 1995) or during insulin infusion studies (Meek et al. 1999), does result in a change in the relative proportions of fatty acids that are taken up in different tissue beds. At lower plasma FFA concentrations, the proportion of fatty acids taken up by the splanchnic bed drops to 20–25%, whereas the relative portion taken up in leg tissue increases to 20–30% (Meek et al. 1999).

The fate of chylomicron fatty acids has been studied to some extent (Romanski et al. 2000). Approximately 30% of dietary fatty acids are oxidized in the 24 h following meal ingestion, and the remainder is stored in adipose tissue.

Although it has not been well studied, the working hypothesis is that the fatty acids contained in VLDL TGs are restored in adipose tissue in a type of substrate cycle (Coppack et al. 1990). It is known that a portion of FFA are taken up by the liver, re-esterified, and secreted as VLDL fatty acids. A substantial portion of these fatty acids are believed to be restored in adipose tissue.

The fate of fatty acids taken up in different tissue beds varies considerably. For example, in the overnight post-absorptive state, approximately 20–30% of the fatty acids taken up by the splanchnic bed are converted to ketone bodies (Havel et al. 1970, Wolfe et al. 1976), approximately 20–30% are secreted as VLDL TGs, and the remainder are likely used as an oxidative fuel by liver and other tissues in the splanchnic bed (Havel et al. 1970, Wolfe et al. 1976). In contrast, during extended fasting over 40% of the fatty acids taken up by the splanchnic bed are converted into ketone bodies (Fig. 2) (Wolfe et al. 1976). This shift is largely mediated by decreasing plasma insulin concentrations and increasing plasma glucagon concentrations (Miles et al. 1983).

In muscle, fatty acids taken up can either be oxidized or re-esterified. It appears the major fate of re-esterified fatty acids is into intramyocellular TGs, but other fates include diglycerides and phospholipids. Intramyocellular TGs provide cellular lipid fuel reservoir for
contracting muscle. The factors that determine whether fatty acids taken up into muscle are oxidized or re-esterified in vivo have not been completely worked out.

**Research techniques to study fatty acid metabolism**

A readily available and widely used approach in studying fatty acid metabolism at rest and during exercise is to combine isotopic tracer measurements of FFA kinetics and indirect calorimetry measures of substrate oxidation. Tracer approaches (3H-, 2H-, 13C-, 14C-labelled FFA) are capable of providing accurate measures of the systemic FFA availability (Miles et al. 1987), whereas indirect calorimetry can provide accurate measures of fatty acid oxidation (Frayn 1983). The combined use of these techniques to study humans at rest, during perturbations of fatty acid availability or fatty acid uptake, and during exercise, have allowed investigators to address some of the major issues regarding the fate of fatty acids. Unfortunately, this approach cannot determine which tissues are involved in the processes of fatty acid uptake and oxidation given the systemic nature of the measurements.

More specific assessments of tissue (leg, splanchnic bed, kidney, heart, etc.) bed fatty acid metabolism can be performed using arteriovenous (A-V) balance studies. A common approach is to assess leg balance studies of the compounds of interest at rest and during exercise. Although these studies can be performed using only mass balance (glucose, fatty acids, lactate, amino acids, TGs, oxygen and carbon dioxide), a special problem occurs when attempting to draw conclusions regarding FFA balance. This is because FFA are both taken up and released by leg tissue (Ahlborg et al. 1974). FFA release occurs from adipocytes and FFA uptake can occur into muscle (Dyck et al. 1997, Guo et al. 2000) and possibly fat. In this investigator’s opinion, there is no evidence to indicate that muscle releases FFA (Dyck et al. 1997, Guo et al. 2000). In any case, net uptake of FFA across the leg does not reflect the total uptake. In most circumstances in which the uptake of FFA is measured there are changing and unknown rates of change in the lipolysis of leg adipose tissue (Ahlborg et al. 1974), making it essential to include tracer kinetics. This approach allows the investigators to partition the uptake and release of fatty acids. If carbon-labelled tracers of fatty acids are used the release of 14CO₂ or 13CO₂ into femoral venous blood can allow an estimate of FFA oxidation by working leg muscle. For reasons indicated below we believe the excretion of labelled CO₂ is a rough estimate, not an exact measure, of fatty acid oxidation.

Isotopic measures of FFA oxidation using infusions of 13C- or 14C-labelled fatty acids and measuring 13CO₂ and 14CO₂ production rates have been published (Klein et al. 1994, Kanaley et al. 1995). It is also possible to measure the generation of 13H₂O or 17H₂O from the oxidation of 13H- or 13H-labelled FFA tracers (Guo & Jensen 1995). A problem with using the production of labelled CO₂ from carbon-labelled FFA tracers is the fixation of carbon in substrate cycles (Wolfe & Jahoor 1990). Attempts have been made to account for this carbon fixation by infusing 13C- or 14C-labelled acetate and measuring the recovery in breath and blood CO₂ (Mitteladorfer et al. 1998, Schrauwen et al. 1998). There are some limitations to this approach. For example, tissues that oxidize fatty acids may not oxidize acetate in similar proportions. As different tissues have different carbon fixation cycles (liver – gluconeogenesis, muscle – glutamate/glutamine) the ‘correction factor’ from acetate may not apply to fatty acids under all circumstances. Even if it does apply, carbon fixation changes in response to exercise, meals, etc. and needs to be accounted for separately in different experiments (Sidossis et al. 1995). We believe that the use of acetate correction factors for measuring systemic or regional fatty acid oxidation is a reasonable way to estimate these values, but do not advocate the unquestioning use of correction factors.

The combined use of tracers and A-V balance techniques to study fatty acid metabolism is a potentially powerful approach, but is not without limitations. In this investigator’s experience, measuring the respiratory quotient of leg tissue is extremely difficult at rest due to the small differences between the arterial and venous concentrations of CO₂ even when specific CO₂ analyzers are employed (Jensen et al. 1998). While the differences increase markedly with exercise, numerous blood samples must be taken, handled carefully and processed quickly using precise instruments. Another problem is the lack of good information regarding amino acid oxidation in leg tissue. Differences in amino acid oxidation from those predicted or changes during the course of the study could have significant effects on
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the calculated rates of glucose and fatty acid oxidation. Another weakness of this technique is that A-V differences of some substrates are too small to accurately measure, especially as blood flow can increase dramatically with exercise. This investigator has found this to be the case with TGs. Measuring A-V differences at rest are difficult and somewhat imprecise, and virtually impossible to measure during exercise (Guo et al. 2000). Another concern about relying solely on the A-V balance technique is that substances taken up by leg cannot definitively be partitioned into fat vs. muscle, or (unless carbon-labelled tracers are used) oxidation vs. storage.

Combining tissue biopsies, especially muscle biopsies, with the above-mentioned techniques can add additional information regarding the fate of fatty acids. Measurement of the concentration of fuels in muscle, especially the change in concentration, can give an index of the net loss of fuel (Kiens et al. 1993, Helge et al. 2001). This is commonly the case with muscle glycogen and has been attempted with muscle TG. The measurement of enzyme activities and the quantity of fatty acid binding and transport proteins can be helpful.

Finally, recent approaches have combined tracer techniques with muscle biopsy using the pulse chase approach to determine intramyocellular TG fatty acid turnover (Guo et al. 2000).

A number of approaches to the study of muscle metabolism using *ex vivo* preparations have also been published. The perfused rat hindquarter paradigm allows complete control of the circulating and hormonal milieu, and the ability to study different types of muscle contraction to assess the fate of fatty acids in working muscle (Turcotte et al. 1999). There are also isolated muscle systems, which allow to test the independent effects of substrates, hormones and contractions on the utilization of both extracellular and intracellular fatty acids (Dyck & Bonen 1998).

**Representative results of some *in vivo* studies**

It has long been known that endurance training increases not just the total ability for power output, but the fatty acid oxidation rates at power outputs below the lactate threshold. Previous studies have shown that endurance training increases muscle capilarization (Kiens et al. 1993) increases fatty acid oxidation (Kiens et al. 1993), and may increase the ability of exercising leg to take up VLDL TG (Kiens et al. 1993). In addition to the effects of exercise training, adaptation to a high fat diet appears to further increase the ability of leg to take up and oxidize fatty acids, those present both in the FFA pool and possibly the VLDL TG pool (Helge et al. 2001). These studies (Kiens et al. 1993, Helge et al. 2001), which employ the A-V balance technique, did not define a role for intramyocellular TGs in providing fuels to exercising muscle.

Changes in the amounts and types of fuel available during exercise have some effect on fatty acid metabolism. Increasing glucose and insulin has long been known to suppress fatty acid oxidation, both at rest and during exercise. The converse is not necessarily true, however. Artificially increasing plasma FFA concentrations via an infusion of Intralipid and heparin did not increase fat oxidation during exercise (Hargreaves et al. 1991) despite a reduction in leg glucose uptake. Thus, there may be an upper limit of fatty acid oxidation for working muscle that cannot be exceeded by providing more substrate.

Studies from our laboratory examined the availability and oxidation of fatty acids at rest and during high intensity running. In these trained runners, resting FFA availability (~6–7 μmol kg^-1 min^-1) exceeded resting fatty acid oxidation by indirect calorimetry (~5 μmol kg^-1 min^-1), as expected. During high intensity running, however, fatty acid oxidation peaked at a rate (~53 μmol kg^-1 min^-1) more than double FFA availability as measured by FFA tracers. In addition, we were surprised to find that only 60% of FFA that were taken up were oxidized, indicating that total fatty acid oxidation must have exceeded FFA oxidation by almost threefold. Given that other circulating sources of fatty acids, for example, VLDL TGs, are not thought to increase as dramatically as FFA, our conclusion from that study was that intramyocellular TGs must provide a substantial portion of fatty acid fuels consumed during high intensity exercise.

Contrary to this conclusion, most studies of intramyocellular TG metabolism have not shown depletion of these stores during exercise. Possible explanations for the lack of change in intramuscular TG stores with exercise include inherent variability and regional differences in intramyocellular TG content, difficulty in getting a clean sample of muscle (Guo et al. 2001) (one uncontaminated by adipocytes) or simultaneous depletion and repletion of intramyocellular TG. We have adapted the isotopic fatty acid pulse-chase technique to *in vivo* studies of intramyocellular TG fatty acid metabolism in hopes of better defining some of these issues. The experimental approach is to pre-label the intramyocellular TG fatty acid pools with \([1^{14}C]\)palmitate prior to an exercise study and to turn off the \([1^{14}C]\)palmitate infusion prior to the exercise. During the exercise interval a different tracer – \([^{3}H]\)palmitate – is infused in order to examine (1) the systemic FFA kinetics; (2) the accumulation of circulating (\(^{3}H\) FFA) into intramyocellular TG; and (3) the oxidation of FFA traversing the plasma space. Sequential muscle biopsies are used to determine the loss of
[14C] label from intramyocellular TGs and the accumulation of the [3H] label into the same pool. The appearance rates of 14CO2 in the breath relative to the intramyocellular 14C TG fatty acid specific activity provides an index of the oxidation of intramyocellular TGs. The appearance of 3H2O in body water provides an index of the rate of oxidation of circulating FFA. Our first study using this approach was to examine moderate intensity (45% of peak O2 consumption) bicycle exercise in healthy young men and women. We found that intramyocellular TG fatty acid oxidation accounted for an average of 50% of total fatty acid oxidation during bicycling exercise. Approximately 20% of FFA taken up by leg tissue was found to have been incorporated into intramyocellular TG, providing a partial explanation for the maintenance of intramyocellular TG concentrations. Reassuringly, the sum of plasma FFA oxidation (by 3H2O generation) and intramyocellular TG fatty acid oxidation correlated well with total fatty acid oxidation as measured by indirect calorimetry. This concordance of two independent methods suggests that this approach provides reasonable and accurate data.

In addition to muscle contractile activity a number of factors external to muscle change simultaneously during exercise. Each of these changes could influence fatty acid oxidation. Plasma FFA concentrations increase, muscle blood flow increases, sympathetic nervous system activity (noradrenaline and adrenaline) can increase either slightly or dramatically, plasma insulin concentrations decrease and growth hormone concentrations increase. These factors may modulate the changes induced by muscle contraction on intramyocellular fatty acid metabolism. Currently, in vivo studies have not dissected which of the various components of the exercise response modulate fatty acid uptake, esterification or oxidation. Fortunately, investigators have developed alternative models that allow each of these components to be dissected. These ex vivo systems have been used to study muscle fatty acid metabolism and have provided information as to how these factors independently contribute to the rate of muscle fatty acids.

It has been shown that increasing the extracellular concentrations of FFA increases the uptake of FFA by muscle and the esterification of these fatty acids into intramyocellular TGs (Spriet et al. 1986, Dyck et al. 1997). Likewise, muscle contraction increases extracellular FFA uptake (Spriet et al. 1986, Turcotte et al. 1998), increases the oxidation of both extracellular and intracellular fatty acids (Spriet et al. 1986), and increases the TG synthesis from extracellular fatty acids (Spriet et al. 1986).

The study of muscle obtained from trained animals show further enhancements in the uptake of extracellular fatty acids (Dyck et al. 2000), and these changes seem to correlate with changes in fatty acid binding proteins and fatty acid transport proteins (Turcotte et al. 1999). Exogenous catecholamines increase the breakdown of intramyocellular TGs, but do not increase the oxidation of these TGs in resting muscle (Peters et al. 1998). More recently, this study paradigm has been used to investigate the insulin regulation of muscle fatty acid metabolism. The results indicate that insulin independently stimulates FFA uptake and re-esterification and suppress the hydrolysis of intramyocellular TGs (Dyck et al. 2001). The combination of insulin and exercise enhances FFA uptake during exercise, but partially inhibits the increase in intramyocellular TG hydrolysis that occurs with contraction.

A number of unanswered questions remain with regard to the regulation of fatty acid metabolism at rest and during exercise. There is still little knowledge of the role of VLDL TG fatty acids to provide energy for tissues at rest or during exercise. It does not appear that VLDL turnover has been measured during exercise, nor has the metabolic fate of VLDL TG fatty acids been studied. Given the difficulties our group has experienced with A-V balance technique for measuring regional TG uptake, it seems likely that tracer approaches are more likely to provide eventual answers to these questions.

Another area of investigation that remains to be explored is whether chylomicron TG fatty acids are utilized as fuel sources during exercise. Fortunately, the use of isotopic tracers should readily be able to address these issues. With the availability of techniques to study the regulation of the intramyocellular TG hydrolysis and oxidation, we can begin to understand the fate of intramuscular fatty acids.

References


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